UNIVERSITY OF KWAZULU-NATAL

Breeding Wheat (*Triticum aestivum* L.) for Drought Tolerance, Improved Yield and Biomass Allocation through Chemical Mutagenesis

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Thesis Abstract

Wheat (*Triticum aestivum* L.; 2n=6x=42, AABBDD) is a key commodity crop globally. Despite its varied economic importance along the value chains, the productivity of wheat has stagnated in sub-Saharan Africa mainly due to unavailability of improved cultivars, recurrent droughts and heat stress presented by global climate change. Breeding and deployment of improved wheat cultivars with improved drought and heat stress tolerance is an important mitigation strategy to enhance wheat production and productivity. Successful breeding is dependent on the availability of adequate genetic variation, however, the genetic diversity in wheat has narrowed down progressively due to selective breeding involving elite parents. Induced mutagenesis has the potential to create genetic variation and novel mutants and to rapidly widen the genetic diversity for wheat breeding programs. Induced mutagenesis and targeted selection will accelerate breeding of superior wheat cultivars with improved drought tolerance, biomass allocation, and enhanced grain yield. The aim of this research was to improve drought tolerance and grain yield, and to enhance biomass allocation in wheat under water-limited conditions through mutation breeding. The specific objectives were: (1) to determine the optimum dosage and treatment conditions of ethyl methanesulphonate (EMS) for effective mutagenesis to induce genetic variation for drought tolerance and enhanced biomass allocation in selected wheat genotypes, (2) to evaluate agro-morphological variation induced through mutagenesis using three pre-determined EMS treatments for a specific wheat genotype to develop breeding populations, (3) to evaluate genetic variation present in the third mutation generation (M_3), and to select families with superior biomass allocation, grain yield and agronomic performance evaluated in the controlled and field environments under non-stressed and drought-stressed conditions, and (4) to induce mutations in a selected wheat genotype using three EMS treatments and develop breeding populations involving M_1 to M_4 generations for enhanced drought tolerance, biomass allocation and agronomic performance. The specific objectives were achieved through four independent studies.

Prior to a large-scale mutagenesis, an ideal dosage and treatment conditions of EMS should be established on selected genotypes. Therefore, seeds of three wheat genotypes (LM29, LM43 and LM75) were treated with three EMS doses (0.1, 0.4 and 0.7% v/v) at three temperatures (25, 30 and 35 °C) for three exposure periods (1hr,

1.5hrs and 2hrs). The ideal treatment conditions for effective mutagenesis were 0.7% EMS for 2 hours at 35 °C for genotypes LM29 and LM43, and 0.4% EMS for 2 hours at 25 °C for LM75. Using linear regression model, the LD_{50} for genotypes LM43, LM29 and LM75 were established to be 0.32, 1.07, and 1.81%v/v EMS, respectively.

From the previous experiment, wheat genotype LM43 was selected and subjected to the above three pre-determined treatment conditions under large-scale mutagenesis to assess agro-morphological variations and estimate the effectiveness and efficiency of the treatments. M_1 plants had significantly ($p< 0.05$) increased number of spikelets per spike (SPS), number of kernels per spike (KPS) and grain yield (GY) while tiller number (TN), KPS and GY significantly increased at M_2. EMS treatment with 0.1% v/v for 1 hour at 30 °C was the most effective and efficient in inducing mutation with the minimum amount of biological damage in this population. Macro-mutations were exhibited as abnormalities in spike, peduncle, awn and flag leaf morphology. Sixty mutants with high biomass and yield potential were selected from each of the treatment conditions.

In the third experiment, seeds harvested from 180 M_2 unique mutant plants were advanced to M_3 generation. Greenhouse and field experiments were carried out under drought-stressed and non-stressed conditions to estimate genetic variation and select superior M_3 wheat families with enhanced biomass allocation to root systems, desirable agronomic traits and high yield potential. Data were collected on days to 50% heading (DTH), days to 90% maturity (DTM), plant height (PH), number of productive tillers (PTN), shoot biomass (SB), root biomass (RB), total biomass (TB), root-shoot ratio (RSR), spike length (SL), SPS, thousand seed weight (TSW) and GY. Mutant families showed significant genotypic ($p<0.05$) variation for yield and biomass traits while genotype × site × water regime interaction effects were significant ($p<0.05$) for DTM, SB, TB, TSW and GY. Superior families designated as 52, 159, 103, 126, 145 were selected for improved drought tolerance and high biomass allocation to roots.

The fourth study focused on developing three mutant populations generated from three pre-determined EMS treatment conditions and, evaluating and selecting

mutants involving M_1 to M_4 generations for drought tolerance, biomass allocation and agronomic performance. Significant ($p<0.001$) differences across generations were observed for all traits while the generation × population interaction effects were significant ($p<0.01$) for SB, TSW and GY. The variation in performance among M_1 to M_4 populations derived from different EMS conditions showed that artificial mutagenesis provided adequate genetic variation for selection across generations.

In summary, the study identified superior mutant populations of wheat and created novel variations in biomass allocation, drought tolerance and agronomic performance. The selected populations are useful genetic resources in developing wheat cultivars with improved biomass allocation, drought tolerance and, improved yield and yield-related traits. This is the first study that reported novel mutants specifically selected for enhanced biomass allocation as a means to improve drought tolerance in wheat.

Declaration

I, **Boluwatife ModupeOluwa OlaOlorun**, declare that:

1. The research reported in this thesis, except where otherwise indicated, 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 

…… ……

Boluwatife ModupeOluwa OlaOlorun

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

……

Prof. Hussein Shimelis

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Dedication

This thesis is dedicated to:

1. every parent that values their girl child and believes in her dreams,
2. everyone that appreciates and supports female scientists,
3. every child of God that believes that all is possible through God.

Abbreviations

%G	Percentage germination
%SS	Percentage seedling survival
AGB	Above-ground biomass
CEF	Controlled Environment Facility
CIMMYT	International Maize and Wheat Improvement Centre
CV	Coefficient of variation
df	Degree of freedom
DMSO	Dimethyl sulfoxide
DTE	Days to 50% emergence
DTH	Days to 50% heading
DTM	Days to 90% maturity
EMS	Ethyl Methanesulphonate
FAO	Food and Agriculture Organization of the United Nations
GM	Grand mean
GY	Grain yield
IAEA	International Atomic Energy Agency
KPS	Kernels per spike
LD_{50}	Lethal dose at 50% reduction in seed germination
LSD	Least significant difference
M Freq	Mutation frequency
M_1	First mutation generation
M_2	Second mutation generation
M_3	Third mutation generation
M_4	Fourth mutation generation

M_5	Fifth mutation generation
Max	Maximum
ME	Mutation effectiveness
Me	Mutation efficiency
Min	Minimum
NS	Non-stress
PC	Principal component
PH	Plant height
PTN	Productive tiller number
RB	Root biomass
RL	Root length
RSR	Root-shoot ratio
SB	Shoot biomass
SE	Standard error
SH	Seedling height
SHL	Shoot length
SL	Spike length
SPS	Spikelets per spike
SVI	Seedling vigour index
TB	Total biomass
TN	Tiller number
TSW	Thousand seed weight
UKZN	University of KwaZulu-Natal
WS	Water stress

Publications Pertaining to This Thesis

Chapter 1

OlaOlorun BM, Shimelis H, Mathew I, Laing M. Progress in mutation breeding in wheat: A review. Under review in South African Journal of Plant and Soil.

Chapter 2

OlaOlorun BM, Shimelis H, Matthew I, Laing M. 2019. Optimizing the dosage of ethyl methanesulphonate mutagenesis in selected wheat genotypes. South African Journal of Plant and Soil. doi: 10.1080/02571862.2019.1610808

Chapter 3

OlaOlorun BM, Shimelis H, Laing M, Mathew I. 2020. Morphological variations of wheat (*Triticum aestivum* L. em. Thell.) under variable ethyl methanesulphonate mutagenesis. Cereal Research Communications. doi: 10.1007/s42976-020-00092-3

Chapter 4

OlaOlorun BM, Shimelis H, Mathew I. 2020. Variability and selection among mutant families of wheat for biomass allocation, yield and yield-related traits under drought-stressed and non-stressed conditions. Journal of Agronomy and Crop Science (Wiley). doi: 10.1111/jac.12459

Chapter 5

OlaOlorun BM, Shimelis H, Mathew I. Development of wheat (*Triticum aestivum* L.) populations for drought tolerance and improved biomass allocation through ethyl methanesulphonate mutagenesis. Under review in Frontiers in Plant Science

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Introduction to thesis

Background

Bread wheat (*Triticum aestivum* L.; 2n=6x=42, AABBDD) is one of the most important food crops in the world contributing up to 20% of the global energy demand (UN, 2017). Global wheat production exceeds 761 million tonnes, while Africa's output is estimated at 25 million tonnes per annum (Nhemachena and Kirsten, 2017; FAO, 2020). South Africa, with estimated production of 1.8 million tonnes per annum, is the second largest producer of wheat in sub-Saharan Africa (SSA) after Ethiopia (DAFF, 2016; Tadesse et al., 2019). However, the country imports more than 1.5 million tonnes of wheat annually to fulfil its domestic consumption requirements (DAFF, 2016). The deficit to meet the national wheat requirements is caused by low production and productivity. In South Africa, the mean wheat yields are 2.5 and 5 tons ha^{-1} under the dryland and irrigation production systems, respectively. The low mean wheat yields in South Africa compared to the global mean of 764 million tons are attributable to various constraints such as poor soils, insect pests, diseases and heat and drought stresses which are related with global climate change (Dube *et al*., 2016; van der Merwe and Cloete, 2018; FAO, 2020).

Climate change is primarily caused by global warming leading to high temperatures and variable and erratic rainfall conditions (Semenov and Stratonovitch, 2013). Industrialization and intensive agricultural activities have contributed immensely to the rise in global temperatures due to the release of greenhouse gases, mainly carbon dioxide (CO_2), into the atmosphere. Wheat is reported to be one of the most vulnerable crops to climate change. It is forested that the current global wheat yields will decline by over 72% due to climate change induced stresses (Adhikari *et al.,* 2015). The impact of climate change on wheat production and productivity threatens food security especially in sub-Sahara Africa where recurrent droughts and crop failures are common. There is a need to develop wheat cultivars with improved tolerance to biotic and abiotic constraints to increase wheat production and productivity in SSA.

Drought is the most important abiotic stress factor with adverse effects on wheat production in South Africa (Esterhuizen, 2018). It is caused by a lack of adequate moisture required for normal plant growth and development. The direct effects of drought stress on wheat include reduced rate of cell division and expansion, leaf size, stem elongation, and root proliferation, and interference with nutrient and water absorption and consequently low potential yields (Francia *et al.,* 2013). Drought stress at the early vegetative stage of growth limits shoot biomass production and photosynthesis. Further, reduced shoot growth has adverse consequences on the development of foliar system, number of tillers per plant, number of spikes, spikelet formation and kernel weight per plant. The above ground biomass is directly related with light interception and photosynthesis that are crucial for grain production. Previous studies on drought tolerance have reported that drought stress increases biomass partitioning to below ground parts (Wasaya *et al.,* 2018; Mathew *et al*., 2019). Plants tend to invest significantly into root biomass during water stress in order to access water and nutrients, which directly influence plant growth potential (Wasaya *et al.,* 2018). Hence, there are indications that plants exhibit phenotypic plasticity by increasing their root to shoot ratios in response to drought stress. The impact of drought stress on plant growth depends on the intensity and duration of the stress, genotype, the developmental stage at which the stress is induced and genotype x environment interaction (Yu *et al.,* 2018). Severe and long duration drought stress induces higher yield losses compared to short duration, or mild stress. Wheat is more sensitive to drought stress during the flowering and grain-filling stages. This is referred to as terminal drought stress and causes higher losses in yield and grain quality (Shamuyarira *et al.,* 2019).

Drought tolerance in crop species including wheat is conditioned by polygenes and their expression is subject to the genotype, environment, and genotype x environment interaction. Improved agronomic practices such as use of minimum tillage and irrigation water have been used to mitigate drought stress in agriculture production. Exploiting the inherent genetic potential of drought adapted genotypes is the most-economic and effective approach to mitigate drought stress. Breeding for drought tolerance and yield gains depends on availability of adequate genetic variation for drought adaptive and constitutive traits (Arterburn *et al.*, 2010). Traits linked to drought tolerance include early flowering and maturity, which enable

genotypes to escape terminal drought stress, tillering capacity, reduced plant height, increased number of spike and kernels and relative allocation of biomass between shoot and roots. Creating and assessing genetic variation based on these traits is important to successfully develop cultivars with enhanced drought tolerance and grain yield.

Genetic variation is harnessed through controlled crosses involving candidate parents selected for their complementary and novel traits. The use of a limited number of germplasm resources as breeding parents in most wheat breeding programs has reduced genetic diversity in wheat (Voss-Fels *et al*., 2015). The narrow genetic diversity presents bottleneck for developing drought adapted cultivars especially for root traits because most breeding programs focus on germplasm selection for above ground or shoot related traits (Govindaraj *et al*., 2015). There is a need to create adequate genetic variation for shoot and root related traits to increase the prospects of developing drought tolerant cultivars.

Genetic variation in wheat can be created through conventional crosses of divergent parental genotypes or through induced mutagenesis. Conventional breeding takes longer period to produce distinct, uniform and stable cultivars. Mutagenesis creates new genetic variation more rapidly and is not constrained by initial divergence in the parental lines compared to the conventional breeding. Mutation breeding provides an opportunity to widen genetic diversity in agronomic traits such as earliness to flowering and maturity, plant height and tillering capacity, which are traditionally targeted for breeding for drought tolerance, and biomass allocation to roots. Mutation breeding has successfully developed mutant wheat varieties, which have significantly contributed to food security in the last three decades (Raina *et al.,* 2017). Mutagenesis can be induced using physical methods such as gamma irradiation, ion beams, UV irradiation, cosmic radiation, or chemical methods such as sodium azide, ethidium bromide and ethyl methanesulphonate. Ethyl methanesulphonate (EMS) is one of the most widely used chemical mutagens in inducing genetic variation in different crops including wheat (Jiang and Dunn, 2016).

Successful mutation breeding is directly related to the extent of genetic variation exhibited in the mutant populations. Kodym and Afza (2003) pinpointed that a large

population size is required during the first mutation generation (M_1) and second mutation generation (M_2) to increase the probability of selection of agronomically desired mutants. Mutation events are dependent on the dose of the mutagen agent and the treatment conditions. These are directly linked to the effectiveness and efficiency of the mutagen agent that need to be known prior to embarking on large-scale mutation breeding (Liamngee *et al.,* 2017). Induced mutagenesis using EMS is highly favored for its high efficiency and effectiveness in inducing point mutations. EMS has relatively low human health and environmental hazards (Espina *et al.,* 2018). Mutation events obtained in crops after exposure to EMS are random and some may not be useful in developing fit-for-purpose varieties. Therefore, there is need to develop various populations and to select superior mutant genotypes after effective mutagenesis. The selected genotypes can serve as parental lines for developing breeding populations or released as mutant varieties.

Despite the importance of roots in nutrient cycling, water extraction, carbon retention to soil, studies on biomass allocation to roots has been neglected in wheat breeding programs. Assessing the genetic diversity present in the above and below ground traits among selected mutant genotypes and evaluating trait associations will assist in devising appropriate selection strategies to develop improved wheat cultivars. Early generation selection in mutant generations is important and can be adopted to advance desirable above and below ground traits. Furthermore, understanding trait associations during early generation selection can enable indirect selection for optimal biomass allocation between above and below ground parts. This will enable selection of elite lines with superior agronomic performance and with drought tolerance and high grain yield production. Figure 0.1 illustrates the field performance of wheat mutant populations under water stress and non-stress conditions during the fourth selection generation in the present study.

Figure 0.1: Field performance of wheat mutant populations during the fourth mutation generation (M_4) under water stressed and non-stressed conditions at Ukulinga Research Station of the University of KwaZulu-Natal

Rationale of the study

Breeding for drought tolerance in wheat has been limited by a number of factors including lack of genetic variation, suitable facilities and test environments among others. Intensive selection within a narrow range of elite germplasm has significantly contributed to genetic erosion. The ever changing environment requires rapid breeding approaches, and mutation breeding offers opportunity to develop improved cultivars within short periods of time. In the past, breeding for drought tolerance in wheat has focused on above ground traits while neglecting the role of roots in increasing water and nutrient extraction capacity. It is important to increase the capacity of wheat cultivars to be adaptive to explore for water and nutrients in deeper soil horizons. In addition, increased root biomass increases the ability of wheat cultivars to deposit carbon into the soil, which is an integral component for maintaining soil structure and water holding capacity.

Aim of research

The aim of this research was to improve drought tolerance and grain yield, and to enhance biomass allocation in wheat under water-limited conditions through mutation breeding.

Specific objectives

The specific objectives of the study included:

1. To determine the optimum dosage and treatment conditions of EMS for effective mutagenesis to induce genetic variation for drought tolerance and enhanced biomass allocation in selected wheat genotypes.
2. To evaluate agro-morphological variation induced through mutagenesis using three pre-determined EMS treatments for a specific wheat genotype to develop breeding populations.
3. To evaluate genetic variation present in the M_3 mutant generation, and to select families with superior biomass allocation, grain yield and agronomic performance evaluated in the controlled and field environments under non-stressed and drought-stressed conditions.
4. To induce mutations in a selected wheat genotype using three EMS treatments and develop mutant populations involving M_1 to M_4 generations for enhanced drought tolerance, biomass allocation and agronomic performance

Research hypothesis

This study was conducted to test the following hypotheses:

1. Mutagenesis using EMS provides variable mutants with different EMS doses, treatment conditions and genotypes.
2. Exposure of wheat genotype LM43 to EMS under three pre-determined EMS treatments conditions will induce genetic variation.
3. The M_3 wheat families developed from EMS mutagenesis will exhibit genetic variation under multiple testing environments.
4. EMS mutagenesis creates distinct breeding populations with desirable genetic variation for drought tolerance, biomass allocation and grain yield for early generation selection, genetic advancement and cultivar release.

Thesis outline

This thesis consists of six chapters in accordance with a number of activities related to the outlined objectives (Table 0.1). Chapters 2-5 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, some 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 of mutation breeding in wheat. Chapter 2 focuses on optimizing the dose of EMS mutagenesis in selected wheat genotypes and was published in South African Journal of Plant and Soil (doi: 10.1080/02571862.2019.1610808). Chapter 3 emphases on the agro-morphological variations of wheat under variable ethyl methanesulphonate mutagenesis and was published in Journal of Cereal Research Communications (doi: 10.1007/s42976-020-00092-3). Chapter 4 presents the study on variability and selection among mutant families of wheat for biomass allocation, yield and yield-related traits under drought-stressed and non-stressed conditions and was published in Journal of Agronomy and Crop Science (Wiley). doi: 10.1111/jac.12459. The core findings and recommendations from the study are presented in Chapter 6. The reference style used in the thesis is based on the format of Euphytica International Journal of Plant Breeding.

Table 0.1: Outline of thesis with chapters and title

Chapter	Title
--	Introduction to thesis
1	Progress in mutation breeding in wheat: A review
2	Optimizing the dose of ethyl methanesulphonate mutagenesis in selected wheat genotypes
3	Agro-morphological variations of wheat (*Triticum aestivum* L.) under variable ethyl methanesulphonate mutagenesis
4	Variability and selection among mutant families of wheat for biomass allocation, yield and yield-related traits under drought-stressed and non-stressed conditions
5	Development of wheat (*Triticum aestivum* L.) populations for drought tolerance and improved biomass allocation through ethyl methanesuphonate mutagenesis
6	An overview of research findings and implications for breeding

References

Adhikari U, Nejadhashemi AP, Woznicki SA (2015) Climate change and eastern Africa: a review of impact on major crops. Food Energy Secur 4(2):110–132

Arterburn MA, Jones SS, Kidwell KK (2010) Soils, plant growth and crop production. In: Verheye WH (ed) Plant breeding and genetics. Encyclopedia of life support systems. Washburn University, USA, pp 184-211.

DAFF (Department of Agriculture, Forestry and Fisheries) (2016) Wheat production guideline. DAFF, Pretoria. https://www.daff.gov.za/Daffweb3/Portals/0/Brochures%20and%20Production%20guidelines/Wheat%20-%20Production%20Guideline.pdf (Accessed June 2019).

Dube E, Mare-Patose R, Kilian W, Barnard A, Tsilo TJ (2016) Identifying high-yielding dryland wheat cultivars for the summer rainfall area of South Africa. S Afr J Plant Soil 33:77-81

Espina MJ, Ahmed CMS, Bernardini A, Adeleke E, Yadegari Z, Arelli P, Pantalone V, Taheri A (2018) Development and phenotypic screening of an ethyl methane

sulfonate mutant population in soybean. Front Plant Sci 9:394. doi: 10.3389/fpls.2018.00394

Esterhuizen D (2018) Grain and feed annual: focus on the supply and demand for grain and feed in South Africa. United States Department of Agriculture Grain Report, pp 1-17

FAO (Food and Agricultural Organization) (2020) Tobacco, land and water, food database. Wheat yield statistics. http://www.fao.org/land-water/databases-and-software/crop-information/wheat/en. (Accessed 17 July 2020)

Francia E, Tondelli A, Rizza F, Badeck FW, Thomas WTB, van Eeuwijk Romagosa I, Stanca AM, Pecchioni N (2013) Determinants of barley grain yield in drought-prone Mediterranean environments. Ital J Agron 8(1):1

Govindaraj M, Vetriventhan M, Srinivasan M (2015) Importance of genetic diversity assessment in crop plants and its recent advances: an overview of its analytical perspectives. Genet Res Int 2015:1-14. doi.org/10.1155/2015/431487

Jiang L, Dunn BL (2016) Ethyl methanesulfonate and caffeine mutagenetic treatment to four ornamental silene species. J Environ Hortic 34(4):95–100.

Kodym A, Afza R (2003) Physical and chemical mutagenesis. In: Grotewold E (ed) Methods in molecular biology. Plant functional genomics: methods and protocols. Humana Press, Springer Nature, Switzerland, pp.189-204

Liamngee SM, Ogah JJ, Amagu KT, Kwon-Ndung EH, Lorkor D, Tervershima JE (2017) Mutagenic action of sodium azide on germination and emergence in landraces of *Phaseolus vulgaris* L. on the Jos Plateau agro-ecological zone. J Agri Vet Sci 10(2):64-70

Mathew I, Shimelis H, Mutema M, Clulow A, Zengeni R, Mbava N, Chaplot V (2019) Selection of wheat genotypes for biomass allocation to improve drought tolerance and carbon sequestration into soils. J Agron Crop Sci 205:385-400. doi:10.1111/jac.12332

Nhemachena CR, Kirsten J (2017) A historical assessment of sources and uses of wheat varietal innovations in South Africa. S Afr J Sci 113:1-8

Raina A, Laskar RA, Khursheed S, Khan S, Parveen K, Amin R, Khan S (2017) Induced physical and chemical mutagenesis for improvement of yield attributing traits and their correlation analysis in chickpea. Int Lett Nat Sci 61:14-22. doi: 10.18052/www.scipress.com/ILNS.61.14

Semenov MA, Stratonovitch P (2013) Designing high-yielding wheat ideotypes for a changing climate. Food Energy Secur 2(3):185-196. doi: org/10.1002/fes3.34

Shamuyarira KW, Shimelis H, Tapera T (2019) Genetic advancement of newly developed wheat populations under drought-stressed and non-stressed conditions. Crop Sci Biotechnol 22:169-176. doi: 10.1007/s12892-018-0262-0

Tadesse W, Bishaw Z, Assefa S (2019) Wheat production and breeding in Sub-Saharan Africa: Challenges and opportunities in the face of climate change. Int J Clim Chang Strateg Manag 11(5):696-715. doi: 10.1108/IJCCSM-02-2018-0015

United Nations, Department of Economic and Social Affairs, Population Division (2017) World population prospects: The 2017 revision, key findings and advance tables. Working Paper No. ESA/P/WP/248. https://esa.un.org/unpd/wpp/Publications/Files/WPP2017_KeyFindings.pdf (Accessed May 2019).

van der Merwe JD, Cloete PC (2018) Financial impact of wheat quality standards on South African wheat producers: a dynamic linear programming (DLP) approach. Dev South Afr 35:53-69

Voss-Fels K, Frisch M, Qian L, Kontowski S, Friedt W, Gottwald S, Snowdon RJ (2015) Subgenomic diversity patterns caused by directional selection in bread wheat gene pools. Plant Genome 8(2). doi:10.3835/plantgenome2015.03.0013

Wasaya A, Zhang X, Fang Q, Yan Z (2018) Root phenotyping for drought tolerance: a review. Agronomy 8:241. doi:10.3390/agronomy8110241

Yu H, Zhang Q, Sun P, Song C (2018) Impact of droughts on winter wheat yield in different growth stages during 2001–2016 in Eastern China. Int J Disast Risk Sci. 9:376-391. doi: 10.1007/s13753-018-0187-4

Chapter 1

Progress in Mutation Breeding in Wheat: A Review

Abstract

Globally, wheat production and productivity are affected by a combination of biotic and abiotic stresses. Hence there is need to develop improved wheat cultivars with high yield potential and quality attributes to warrant the current and future demands for food and industrial uses. Genetic variation is a prerequisite to develop highly productive and climate resilient wheat cultivars. Targeted crosses and induced muatgeneis are key in developing genetically diverse and complementary breeding parents to create superior cultivars. Induced mutagenesis has the potential to widen the genetic diversity by creating heritable changes in crop species including wheat. The use of physical or chemical mutagens has contributed to crop improvement programs and global food security, with 113 wheat mutant varieties having been released in the last two decades. These varieties have been successfully bred for yield improvement, early flowering and maturity, reduced plant height, pest and disease resistance and tolerance to drought and heat stresses. However, developing countries are still lagging in exploring mutation breeding techniques due to financial, technical and other resource constraints. The objectives of this review were to present the current information on mutation breeding of wheat as well as to highlight the prospects of integrating mutagenesis, genomics and conventional breeding for improving drought tolerance and biomass accumulation in wheat for climate change resilience and enhanced productivity. The paper concludes that the complementary use of mutagenesis and genomic tools opens up opportunities for the integration of quantitative trait loci (QTL) in cultivar development programs. Creating genetic variation, breaking unfavourably linked genes and identifying genes for important traits for crop improvement are added benefits in plant breeding and genetic analysis.

Keywords: biomass allocation, crop improvement, drought tolerance, genetic variation, integrated mutation breeding, wheat

1.1 Introduction

Global production of wheat (*Triticum aestivum* L.; 2n=6x=42, AABBDD) is consistently facing multiple biotic and abiotic challenges that are exacerbated by climate change. Due to escalating incidences of biotic and abiotic stresses, there is unprecedented pressure to develop superior crop cultivars to sustain crop production and to meet global food demand for a rapidly growing human population. However, the development of superior cultivars has been curtailed by narrow genetic variation and progressive erosion of genetic diversity, which are critical bottlenecks to crop improvement.

Genetic diversity within a crop species can be lost due to selective breeding, monoculture or environmental changes, among other factors (Govindaraj *et al*., 2015). Selective breeding and replacement of broadly-adapted landraces with modern cultivars has resulted in significant loss of genetic variation in commodity crops (van de Wouw *et al.,* 2010). Modern plant breeding has led to improved food security and continues to impact agriculture. Nevertheless, directional breeding has increased crop uniformity across large areas of production minimizing genetic diversity and leading to genetic resources vulnerable to biotic and abiotic stresses (Keneni *et al*., 2012). Furthermore, modern breeding programs routinely involve crossing of elite germplasm within a narrow range of genetic resources followed by directional selection pressure that further reduces the genetic diversity present in crop germplasm (Voss-Fels *et al*., 2015).

The number of traditional varieties in crop plants such as wheat that are subjected to intensive national and international breeding has dramatically narrowed down genetic diversity. For instance, until year 2000, 86% of the spring bread wheat grown in all developing countries, was derived from varieties with at least one common parent developed by the International Maize and Wheat Improvement Centre (CIMMYT) (Smale *et al*., 2002). This suggests that genetic diversity is dwindling gradually. The stagnating yields and reduced stress tolerance levels reported for bread wheat in many parts of the world could be partially attributed to the narrowing genetic diversity (Voss-Fels *et al*., 2015). The progressive erosion of genetic diversity compels breeders to search for innovative techniques to create new genetic variation for successful crop improvement (Sikora *et al.,* 2011).

Genetic variation can be created via conventional approaches (e.g. sexual recombinations following crosses) and biotechnological techniques (Tadesse *et al*., 2012). In conventional breeding, genetic variation is harnessed through crosses of genotypes with divergent and complementary genetic background. These crosses may involve breeding parents such as cultivated varieties, landraces, distantly related species, and wild species. Crosses between cultivated oat (*Avena sativa* L.) and its weedy relative wild oat (*A. fatua* L.) and, bread wheat and its relative durum wheat (*Triticum turgidum* L.) are prime examples of inter-specific crosses. Conventional breeding takes a longer time (> 12 years) before genetically distinct, uniform and stable varieties are developed and released (UPOV, 2002), which creates a critical bottleneck for cultivar development under a rapidly changing environment (Shivakumar *et al.,* 2018). Genetic variation in crop species can be increased through mutagenesis. Recent advances in induced mutation breeding technology have revolutionized plant breeding by reducing the amount of time taken to create genetic variation and develop a new variety (Shu *et al*., 2012).

Mutagenesis is applicable on self-pollinating species such as wheat, oats and sorghum, which normally show narrow variation for desirable agronomic traits due to continuous self-pollination. Inducing mutations on crops is comparably cheaper and simple allowing a large number of individuals to be tested and novel mutants to be selected. Chemical mutagenesis has been used successfully to develop herbicide resistance in maize (Rizwan *et al*., 2015), improve maturity and agro-morphological traits in sorghum (FAO/IAEA, 2018) and wheat (Singh and Balyan, 2009), and improve the starch and protein contents of sorghum (FAO/IAEA, 2018). However, mutations may occur at small frequencies or randomly and may not be manifested phenotypically, which confounds the identification and selection of mutants. Thus, an integrated approach incorporating conventional breeding with mutagenesis, biotechnology or molecular breeding methodologies has higher potential to create genetic variation and, eventually, develop cultivars that have improved tolerance to the drastically changing crop production environment (Jain, 2010).

Conventional breeding creates genetic variation by exploiting naturally available variation through designed and controlled mating of divergent parental lines. The extent of genetic variation in the resultant progeny is limited by the initial variation in

the breeding population, which may not be adequate for rapidly improving crop response to changing environmental conditions. Mutation breeding can circumvent these challenges by creating mutants, which widen genetic variation. However, mutagenesis only identifies mutants that have distinct phenotype but does not elucidate the genomic loci that has been mutated. The genomic regions responsible for the observed phenotype in mutants can be identified by incorporating molecular markers into mutation breeding and applying techniques such as genome-wide association mapping. Paiva *et al.* (1998) used Restriction Fragment Length Polymorphism (RFLP) markers to identify aluminium tolerance genes in mutant maize, and this has contributed to the understanding of genetic control of aluminium tolerance while also creating new genetic variation to improve maize productivity under acidic soils. Molecular markers can also be used for genetic characterization of mutant germplasm. Genetic characterization is an important preliminary step for crop improvement programs. Incorporating markers into mutation breeding would immensely improve selection efficiency. Recessive alleles may not be expressed if there is strong linkage with a dominant loci, which makes recessive phenotypes to be difficult to identify in natural populations. By using a combination of mutation breeding and molecular methods, unfavourable linkages in natural populations can be broken and the recessive alleles can be identified. For instance, Atanassov *et al*. (1998) used Random Amplified Polymorphic (RAPD) Deoxyribonucleic Acid (DNA) markers to identify soma-clonal and mutagen induced variation in barley. In other instances, mutation breeding can be used to generate mapping populations for developing markers to optimize models for predicting genomic estimated breeding value (GEBV) (Kristensen *et al.,* 2018). Thus, the integration of conventional, mutation and molecular breeding holds great prospects for crop improvement, especially for wheat, whose diversity has narrowed over the years. Hence the objectives of this review were to: 1) present the current information on mutation breeding of wheat as well as to highlight the prospects of integrating mutagenesis, genomics and conventional breeding for improving drought tolerance and biomass accumulation in wheat, 2) highlight the complementary use of mutagenesis and genomic tools for the integration of quantitative trait loci (QTL) in cultivar development programs and 3) discuss on the benefits of induced mutagenesis in creating genetic variation, breaking unfavorably linked genes and identifying genes for important traits for crop improvement and genetic analysis.

1.2 Genetic variation

Genetic variation refers to the variable frequency of genes within a population or among populations of a species over space and time (Yasmin *et al*., 2019). There are several forms in which genetic variation can manifest in a crop species depending on the size of the DNA that is affected. For instance, variation in individuals can occur at gene or nucleotide level or over large sections of their DNA (FAO/IAEA, 2018). At gene level, individuals may have a different sequence resulting in a different protein coding. The most common form of variation is the single nucleotide polymorphism, which shows that individuals may differ at one nucleotide in a particular gene (FAO/IAEA, 2018). Such variation is critical in biochemical process and can influence variation in biomass and yield production or growth habit. The success of any breeding program hinges on the availability of sufficient genetic variation in a trait of economic importance.

1.2.1 Sources of genetic variation

Most of the genetic variation in plant species is primarily derived from three sources; genetic recombination during sexual reproduction, gene transfer and natural or spontaneous mutation (Griffiths *et al*., 2000). Natural or spontaneous mutations occur at relatively low frequency (10^{-5} to 10^{-8} per locus) and may not be useful to develop cultivars with desirable traits for diverse human uses (Jain, 2010; Penna and Jain, 2017). The other proportion is contributed by recombination during reproduction and also genetic drift over time (Aguilar *et al.,* 2008). Crop improvement through recombination is possible when parental lines with wide genetic variation are identified and used in hybridization programs. Often, the required genetic variation for crop improvement is obtained from landraces, elite breeding lines, wild relatives or mutants (Shu *et al.,* 2012). Elite breeding lines represent the most readily available genetic resources because developing economically important cultivars from landraces and wild relatives can take a considerable amount of time. However, continuous use of a limited number of elite lines can lead to genetic erosion. Thus, there is need to widen the genetic variation in the elite germplasm. The use of mutation breeding has gradually increased since the 1900s following the realization that mutants provide an important pool of genetic variation that cannot be obtained in nature or that natural genetic variation has been lost due to evolution or deliberate breeding (Novak and Brunner, 1992; Porbeni *et al.,* 2016).

1.2.2 Mutation breeding

The process of inducing mutations to change the genetic constitution of plants is referred to as mutagenesis (Alemu, 2016) and its deliberate use in crop improvement is termed mutation breeding. Mutation breeding offers an opportunity to create genetic variation where there is a high possibility of genetic drift from continuous hybridization and introgression of genes from related parental lines using conventional breeding methods (Singh and Kole, 2005). The elite lines can be subjected to mutagenesis to induce random mutations that produce a number of mutants with different traits for crop improvement programs. Mutants resulting from induced mutagenesis are new genetic materials exhibiting novel traits (IAEA, 2011). Mutations can occur as inversions, translocations, duplications, deletion, frameshift, or insertion of genes and changes in the chromosome number, which may or may not be expressed phenotypically. Mutations can also be classified as micro-mutations when they result in invisible phenotypic changes or macro-mutations when they cause distinct morphological changes in the individual. Mutation breeding has been used successfully to develop distinct cultivars with novel traits.

Different methods have been developed to reduce over reliance on natural mutations that are unpredictable or insignificant. These methods entail the exposure of plants or seeds to physical agents (e.g., ultraviolet (UV), gamma or X-ray radiation), aerospace (use of cosmic radiation) or chemical agents (e.g., ethyl methanesulphonate) that cause heritable changes in the deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) sequence of a plant (Pierce, 2005; Hu *et al.,* 2010). Exposure of plants or their seeds to mutagenic agents can induce an unlimited amount of mutations in different possible combinations resulting in wide genetic variation compared to conventional methods whose resultant genetic variation can be predictable and within a narrow range (Singh and Kole, 2005).

During mutation breeding, the objective is to obtain a variable number of mutants to increase probability of identifying mutants with superior traits. The probability of obtaining the requisite number of mutants depends on the ability to induce the maximum mutagenic effects with minimal mortality (Shu *et al.,* 2012). However, exposure to mutagens can result in the variable forms of mutation depending on whether the change in DNA occurred at a point, structural, chromosomal, nuclear or

extra-nuclear level (Okagaki *et al*., 1991; Pierce, 2005). In some instances, mutation can result in the substitution of genes and changes in chromosome numbers. Deleterious mutations are usually not useful, and this necessitates the need to develop protocols that increase the occurrence of functional mutations.

1.3 Mutation breeding techniques in wheat

Artificial mutagenesis enhances genetic variation that would otherwise occur in nature at very low frequencies to be fully exploited for breeding purposes (Jain, 2010). Physical or chemical mutagenesis can be used to increase the frequency of mutations which depends on the nature of mutagen used or plant part mutated (Alemu, 2016). Each method has been used in numerous instances with relative success. There is variable information on mutation treatment conditions for many crop species and, even for those crop species such as wheat, which have been widely investigated. The treatment conditions still need to be optimized to increase mutation frequency and reduce biological loss (Pathirana, 2011; OlaOlorun *et al*., 2019; 2020a). The success rates and treatment conditions reported by different researchers show that the resultant mutations are unpredictable and are specific to the prescribed conditions. Thus, there is need to determine what would be the best method between physical and chemical mutagenesis in line with available facilities and the objectives of the breeding program. Both physical and chemical mutagens have been used successfully to create variation and develop wheat cultivars with improved traits such as improved yield, early flowering, shorter plant height and disease tolerance (Maluszynksi, 2001).

1.3.1 Physical mutagenesis

Physical mutagenesis involves the exposure of biological materials to radiation that causes sudden changes in the genetic make-up (Kodym and Afza, 2003). The use of physical mutagenesis is well documented with ionization mutagens such as alpha, gamma and X rays being the most commonly used (Mba *et al*., 2010; Wani *et al*., 2014; Raina *et al.,* 2016). The FAO reported that 1352 mutant cultivars derived from physical mutation breeding were released until 2015 (FAO, 2015). Physical mutation is the most widely used form of mutagenesis compared to chemical mutation.

During physical mutagenesis, an accurate history of the doses that lead to 50% lethality are commonly used (Oldach, 2011) and can be recorded allowing repeatability for large-scale trials (Jain, 2005). As a result, physical mutagenesis accounts for 81% of released mutant varieties (IAEA, 2019). However, the success of physical mutation breeding depends on the properties of the physical agent, the species and the plant part used (Alemu, 2016). There are many reports on physical mutation of wheat using gamma irradiation (Ahmed *et al.*, 2017), ion beams (Khazaei *et al.,* 2018) and UV irradiation (Alexieva *et al.,* 2001). However, the use of physical mutagens especially fast neutron bombardment (Lee *et al.,* 2002) is still challenged by lack of information and high costs associated with installation of requisite facilities. Facilities for conducting physical mutagenesis are not readily available in developing countries. Physical mutation using irradiation requires suitably equipped laboratories that can produce adequate number of neutrons but also be able to prevent environmental and health hazards (Kodym and Afza, 2003). This has limited its effective use in sub-Sahara Africa compared to developed countries such as USA, Germany or Sweden. Although the value of creating new genetic variation is critical, the cost associated with physical mutagenesis are prohibitive for countries with limited resources to invest in long term projects. There is therefore, a need to invest in appropriate and affordable technologies to carry out mutagenesis via physical mutation.

1.3.2 Chemical mutagenesis

Alternative to physical mutagenesis, mutations can be induced through chemical mutagens. Chemical mutagenesis entails exposure of biological material to a chemical agent that interferes with biological processes, such as DNA replication and translation, resulting in sudden changes in the DNA sequence of the organism (Hingra, 2016). Chemical agents such as ethyl methanesulphonate (EMS), methylmethane sulphonate (MMS) and ethidiun bromide, which induce mutations in the genetic constitution of crops have become important in mutation breeding (Figure 1.1) (Porbeni *et al.,* 2014). The chemical mutagens can be broadly classified into three categories i.e. alkylating agents, base analogs or acridine dyes. Alkylating agents, which include EMS, are the most commonly used chemical mutagens (Jain, 2010). The EMS is widely used due to its high effectiveness and potency in inducing random mutations by nucleotide substitution compared to most of the low hazard

chemical mutagens (Anbarasan *et al*., 2013). It poses a low environmental risk and can be easily disposed by hydrolysis (Pathirana, 2011). However, chemical mutagens present an environmental hazard if they are inappropriately disposed or leaked.

Chemical mutagenesis is widely used in developing countries compared to physical mutagenesis because it requires relatively less sophisticated equipment, which are more readily available. Chemical mutagens are also highly useful because they result in high mutation rates, especially point mutations (Jain, 2005). However, chemical mutagens are less potent as they induce milder mutagenic effects on biological materials compared to physical mutagens. Furthermore, it is generally difficult to keep an accurate dosimetry of chemical mutagens (Kodym and Afza, 2003). This has posed challenges during mutagenesis because chemical agents are also affected by changes in environmental conditions. There is always a need to carry out preliminary trials to establish the effective dose of the chemical mutagen before large-scale mutagenesis.

1.4 Progress in wheat improvement using various mutation breeding techniques

Since the early 1900s, mutagenesis has become integral in creating useful genetic variation for crop improvement. Both physical and chemical mutagens have been used successfully to enhance genetic variation for genetic improvement resulting in the release of varieties with improved yield and agro-morphological traits, early flowering, shorter plant height, enhanced pests and disease tolerance, herbicide resistance and improved nutritional quality (Maluszynksi, 2001; Eze and Dambo, 2015). Mutant varieties with improved yield related traits such as dwarfism, early flowering and improved leaf morphology have been developed showing that the opportunities are vast and not limited to single trait selection associated with many breeding programs or sequential stacking of important genes that are time consuming. There are over 3000 mutant varieties that have been released to date in 60 countries, with China, India, Russia, Netherlands, Japan and USA being the top developers (Jain, 2010; IAEA, 2018). Africa has only contributed 2% (66 varieties) of the released mutant varieties globally (FAO, 2015). Rice accounts for the majority of the mutant varieties, with over 700 varieties followed by barley, wheat and maize

(Jain, 2010). A total of 289 mutant wheat varieties have been released, accounting for 8% of the total mutant varieties in the world (Figure 1.1).

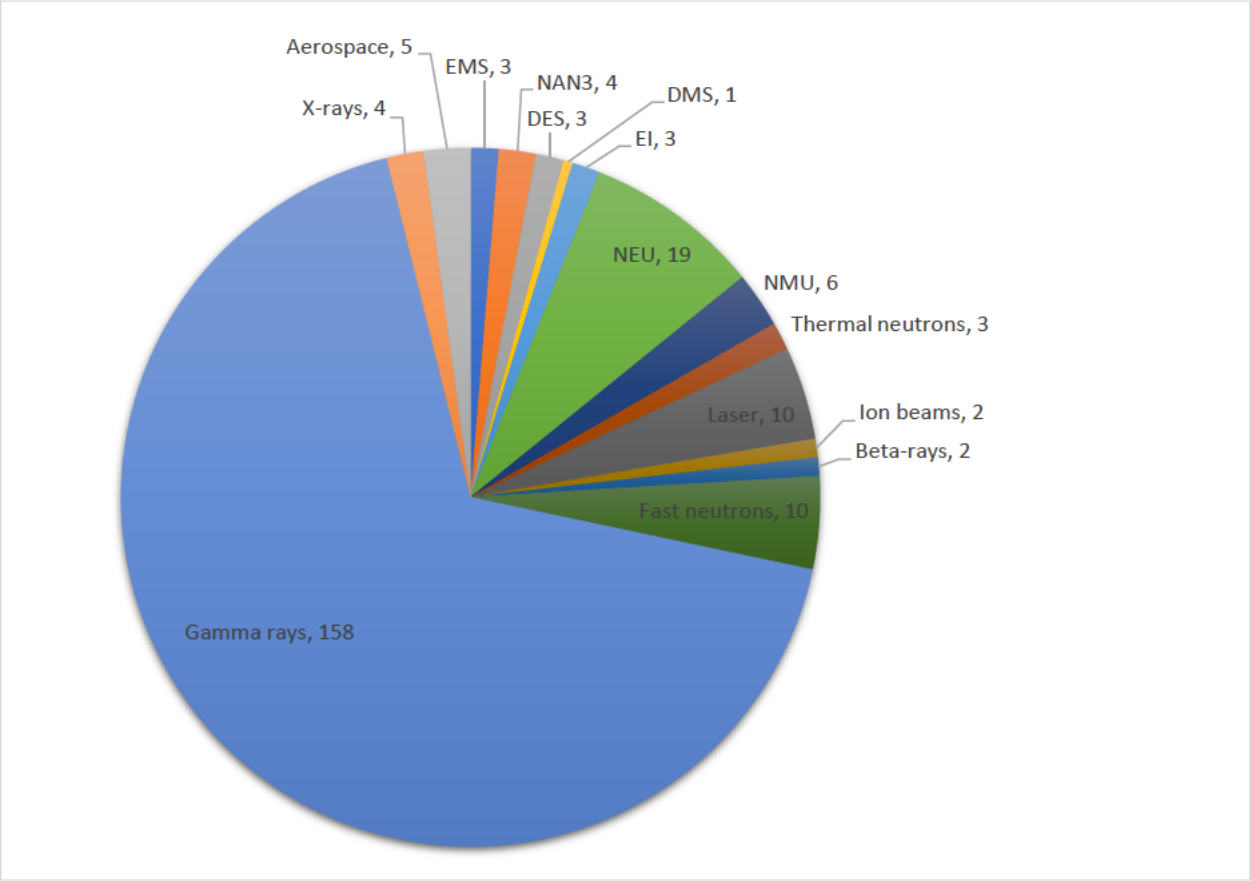

Figure 1.1: Number of wheat mutant varieties developed and released globally using various techniques. DES: Diethyl sulfate, EL: Ethylenimine, NEU: N-Ethyl-N-Nitrosourea, EMS: Ethyl methanesulphonate, NAN_3: Sodium azide, DMS: Dimethyl sulfate, NMU: N-Nitroso-N-methylurea. Adapated from the Joint FAO/IAEA database. http://mvd.iaea.org

While the ultimate goal of most breeding programs is to improve yield, there are some mutant varieties that have been released with improved yield as an indirect result of improvement in biotic and abiotic stress tolerance. Heat, pest, disease and aluminum tolerance have been targeted successfully, especially in the continental South America. In countries such as Brazil, Colombia, Argentina and Peru mutant varieties with aluminium tolerance were developed to improve wheat productivity under acidic soils that are widely distributed in these countries (FAO, 1996). Heat tolerant mutant varieties have been released in India, while the Yunadon No. 3 mutant variety was highly successful, exhibiting complete resistance to rust, powdery

mildew and aphids such that by 1986 it was being cultivated on 200, 000 hectares (Jain, 2010).

With wheat yields stagnating in many parts of the world (Voss-Fels *et al*., 2015), the release of mutant varieties with improved yield potential provides an opportunity to ensure food security for the growing population. While mutation breeding opens vast opportunities, there is still need to optimize the use of mutants as breeding populations. Nazarenko *et al.* (2018) reported that mutant varieties can be used as breeding populations for developing productive varieties. Githinji and Birthia (2015) reported that they obtained high yielding F_1 involving 2 mutant lines showing that mutant lines have breeding value. However, the use of mutant breeding populations is still limited in developing countries and must be integrated into mainstream breeding programs to complement other breeding techniques.

1.4.1 Integrated mutation breeding

Mutation breeding has been used to complement other breeding strategies. Its integration with other breeding techniques such as conventional methods, use of molecular markers and high throughput genomics have played a significant role in crop improvement to alleviate global food security. The International Atomic Energy Agency (IAEA) asserted that a mutant variety can be developed through conventional breeding techniques by continuous self-pollination of a mutant genotype, indirect use of a mutant as parental line in cross breeding or a combination of any of the two methods with double haploid technique (IAEA, 2019). In 2006, a wheat mutant variety “Longfumail 16” with improved fungal resistance and grain yield was developed by gamma irradiation (Table 1.1), while “H6756”, a salt tolerant mutant cultivar was derived from a double cross involving a mutant parental line developed by gamma irradiation (Liu *et al.,* 2007; IAEA, 2019). An example of a successful application of integrated mutation breeding in wheat is the creation of double haploids, which has opened tremendous amount of opportunities in wheat breeding. In 2011, a Beijing wheat mutant variety with high tolerance to drought, developed by the combination of space mutagenesis and doubled haploid technique was approved for varietal release in China (IAEA, 2018). However, phenotypic selection of mutant varieties especially at the segregating generation has been challenging and time consuming. This probably has been due to lack of proper

screening, environmental influence and complexity in the trait of interest. Hence, speed breeding and genotypic selection has been advocated recently (Jain, 2010).

Application of molecular techniques such as using random amplified polymorphic DNA (RAPD), single nucleotide polymorphisms (SNPs), simple sequence repeats (SSR) or microsatellites, sequence target sites (STS) markers have been reported to be more effective and reliable in screening mutant lines compared to phenotyping selection (Bibi *et al*., 2010; Dhillon *et al*., 2014). Marker assisted selection techniques have been adopted in the assessment of genetic diversity and characterization studies in mutant germplasm (Şen and Sarsu, 2018). Regardless of this rapid approach, the use of molecular breeding techniques is still lagging in several developing countries due to resource constraints (Suprasanna *et al*., 2017).

Recently, the use of mutagenesis has expanded into genomic studies (Li *et al.,* 2001) benefitting mutant characterization studies (Penna and Jain, 2017). Integration of mutagenesis with other technologies is termed muta-genomics which is the merging of conventional mutagenesis and functional genomics. Muta-genomics (mutational genomics) has become a faster breeding tool in detecting genetic variation, screening mutations in mutant populations and selecting mutant phenotypes towards genetic stability and improved agronomic performance. The use of high throughput genomics techniques such as microarray, differential display, Targeting Induced Local Lesions In Genomes (TILLING), high resolution melt (HRM) analyses have been used in most plant species for screening in mutant populations (Jain and Suprasanna*,* 2011). The most commonly known high throughput technique that integrates conventional mutagenesis with genomics is TILLING (Uauy *et al.,* 2009; Sestili *et al*., 2010). In this technique, mutagenesis is complemented by the isolation of chromosomal DNA from a mutated line and screening of the population at the DNA level using advanced molecular techniques (Sikora *et al.,* 2011).

The ability to effectively and efficiently detect a mutation is a major advantage of high throughput DNA sequencing methods (King *et al*., 2015) although it can be tedious in species with a complicated genome such as wheat (Sikora *et al.,* 2011). Some logistics involved in TILLING such as handling, harvesting and cleaning procedures

for individual lines without cross-contamination, proper storage of seeds, organization of several thousand bags of seed and their corresponding DNA samples are prerequisites for inducing mutagenesis and future selections (Sikora *et al.,* 2011). Also, tracking a TILLING population and associated data over several generations and maintaining numbers on seed availability requires establishing a database and bar-coding system, which may be a challenge in developing countries.

1.5 Mutation breeding in wheat for drought tolerance, biomass allocation and yield gain

In the last few decades, induced mutations have had positive impact in the creation of crop varieties with improved traits. The major aim in wheat mutation breeding has been to improve varieties of commercial value by altering one or two major traits contributing to increased grain yield. Arain *et al*. (2000) and Ahloowalia *et al*. (2004) opined that the value and economic impact of a new mutant variety are determined by its yield potential, response to agronomic input, breeding value and consumer preference. Mutation breeding would be more useful in improving traits controlled by few genes because mutagenesis results in point mutations and rarely affects a large number of genes simultaneously.

Mutation breeding has been used to improve drought tolerance, increase lodging resistance, reduce plant height, improve tolerance to high density, increase rooting depth and reduce the days to flowering in wheat. However, mutation breeding to optimize biomass allocation has not been attempted except breeding for reduced plant height, which could be indirectly related to above ground biomass (Singh and Balyan, 2009). Increasing biomass allocation to roots could improve drought tolerance by increasing efficiency in water capture and utilization (OlaOlorun *et al.,* 2020b). Phenotyping below ground biomass and roots is generally more difficult relative to above ground. Consequently, root improvement has been neglected in most breeding programs and most modern cultivars have poor root systems that predispose them to drought stress (White *et al.,* 2015). The genetic variation in rooting patterns has almost been completely eroded following years of deliberate focus on improvement of harvest indices, reduced plant height and improved grain yield with negative selection for root or below ground biomass. Attempts to simultaneously improve yield and root traits concurrently with the aid of conventional

methods have not been encouraging due to a negative association between yield and increase in root biomass (Den Herder *et al*., 2010; White *et al.,* 2015). Mutation breeding could provide a means to circumvent these challenges and also assist in creating new genetic variation for high root biomass, grain yield and optimal biomass allocation (OlaOlorun *et al.,* 2020b).

1.6 Outlook and recommendation

Mutagenesis has generated a vast amount of genetic variation that has contributed to crop improvement, genetics and advanced genomic studies. It has also played an important role in improving global food security with 113 wheat mutants varieties having been released in the last two decades (Table 1.1). There is potential to employ mutagenesis to create new genetic variation in root traits to improve drought tolerance and grain yield, and to optimize biomass allocation for ecosystem services such as nutrient recycling and soil restitution. Developing countries are still lagging in mutation breeding due to lack of financial, technical and physical resources, which has led to only a few successful mutants to be released. To enhance cultivar development in these countries, there is a need to complement conventional and molecular breeding techniques with mutagenesis to create genetic variation that would otherwise not be available. The complementarity between mutagenesis and genomic selection has opened opportunities for QTL identification and cultivar development. Mutation breeding will assume an even more important role in crop improvement in the future by creating new genetic variation, breaking unfavourable linkages and identifying genes for important traits.

Table 1.1: Wheat mutant varieties released in the last 20 years with their improved traits and mutagenic methods used

Name of mutant variety developed	Original/mother variety	Improved traits	Method	Mutagenic agent (dose)	Treated material	Reference
Giant	Kalinova	Drought tolerance, high protein content, and grain yield	Induced mutagenesis, continuous self-pollination and selection	Gamma rays (100-250GY)	Seed	Nazarenko *et al.* (2018)
H6765	HHHH	Grain yield, drought and salinity tolerance	Induced mutagenesis, continuous self-pollination and selection	Gamma rays (1.5Gy)	Pollen	Liu *et al*. (2007)
Hangmai901	N/A	Yield, seed weight and drought tolerance	Combination of space mutagenesis and doubled haploid technique	Aerospace	Seed	IAEA (2019)
Leana	Favoritka	Drought tolerance, yield, early maturity and protein content	Induced mutagenesis, continuous self-pollination and selection	N-Nitroso-N-methyl urea (0.0125%,18 hours)	Seed	Nazarenko *et al.* (2018)
Longfu 2	14615	Drought tolerance and resistance to fungal disease	Induced mutagenesis, continuous self-pollination and selection	Ion beams (11～44Gy)	Seed	Zhao *et al*. (2005)
Longfumai 15	83228	Yield and drought tolerance	Induced mutagenesis, continuous self-pollination and selection	Aerospace	Seed	IAEA (2019)
Njoro-BW1	N/A	Drought tolerance, resistance to rust, yield and baking quality	Induced mutagenesis, continuous self-pollination and selection	Gamma rays	Seed	IAEA (2019)
Baichun 5	PH82-2	Yield and nutritional quality	Induced mutagenesis, continuous self-pollination and selection	Gamma rays	Seed	IAEA (2019)
Darkhan-106	RAH-506	Yield	Induced mutagenesis, continuous self-pollination and selection	Gamma rays (180Gy)	Seed	IAEA (2019)
Fermer	Pobeda	Yield, quality, drought and cold tolerance, resistance to leaf rust	Induced mutagenesis, continuous self-pollination and selection	Gamma rays (50Gy)	Seed	Plant Mutation Reports (2010)
NAROWheat 1, NAROWheat 2, NAROWheat 3	Pasa	Yield, short plant height and resistance to stem rust (UG99)	Induced mutagenesis, continuous self-pollination and selection	Gamma rays (250Gy)	Seed	National Crop Variety List for Uganda (2015)

Name of mutant variety developed	Original/mother variety	Improved traits	Method	Mutagenic agent (dose)	Treated material	Reference
Guinness/1322	Katya	Yield, drought tolerance, resistance to lodging and seed shattering	Induced mutagenesis, continuous self-pollination and selection	Gamma rays (50Gy)	Seed	Plant Mutation Reports (2010)
Luyuan 301	121	Seed yield and plant structure	Hybridization with a mutant and continuous self-pollination	Mutant hybrid	Seed	IAEA (2019)
Jingdong 23	Winter 6/92R149	Seed yield, tillering ability, immunity to stripe rust	Hybridization with a mutant and continuous self-pollination	Mutant hybrid	Seed	IAEA (2019)
Hangmai 96	Liaochun	Seed yield	Induced mutagenesis, continuous self-pollination and selection	Aerospace	Seed	National Wheat Varieties (2007)

N/A: Not available

1.7 References

Aguilar R, Quesada M, Ashworth L, Herrerias-Diego Y, Lobo J (2008) Genetic consequences of habitat fragmentation in plant populations: susceptible signals in plant traits and methodological approaches. Mol Ecol 17(24):5177–5188. doi: 10.1111/j.1365-294X.2008.03971.x

Ahloowalia BS, Maluszynski M, Nichterlein K (2004) Global impact of mutation-derived varieties. Euphytica 135:187–204.

Ahmed S, Khan WM, Khan MS, Akhtar N, Umar N, Ali S, Hussain S, Shah SS (2017) Impact of gamma radiations on wheat (*Triticum aestivum* L.) varieties (Batoor and Janbaz). Pure Appl Biol 6(1):218-225.

Alemu H (2016) Review paper on mutation breeding as applied in groundnut (*Arachis hypogea* L.) improvement. Gene Cell Ther 1(5):35-40. doi: 10.11648/j.gct.20160105.11

Alexieva V, Sergiev I, Mapelli S, Karanov E (2001) The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. Plant Cell Environ 24(12):1337-1344.

Anbarasan K, Sivalingam HD, Rajendran R, Anbazhagan M, Chidambaram AA (2013) Studies on the mutagenic effect of EMS on seed germination and seedling characters of sesame (*Sesamum indicum* L.) Var.T MV3. Int J Res Biol Sci 3(1):68-70.

Arain MA, Ahmad A, Sodium KA (2000) Utilization of induced mutations for genetic improvement of wheat. In: mutation techniques and molecular genetics for tropical and subtropical plant improvement in Asia and the Pacific region. IAEA-SR-210/6. IAEA, Vienna, pp 109-111.

Atanassov A, Todorovska E, Trifonova A, Petrova M, Marinova E, Gramatikova M, Valcheva D, Zaprianov S, Mersinkov N (1998) Investigation of the somaclonal and mutagen induced variability in barley by the application of protein and DNA markers. In: Application of DNA based marker mutations for improvement of cereals and other sexually reproduced crop plants. IAEA-TECDOC-1010. IAEA, Vienna, pp 21-31.

Bibi S, Dahot MU, Nizamani GS, Khan IA, Khatri A, Naqvi MH, Oad FC, Burio UA (2010) Molecular marker assisted selection for drought tolerant wheat genotypes. Pak J Bot 42(4):2443-2452.

Den Herder G, Van Isterdael G, Beeckman T, De Smet I (2010) The roots of a new green revolution. Trends Plant Sci 15:600-607. doi: 10.1016/j.tplants.2010.08.009

Dhillon RS, Saharan RP, Jattan M, Rani T, Sheokand RN, Dalal V, Wuehlisch G (2014) Molecular characterization of induced mutagenesis through gamma radiation using RAPD markers in *Jatropha curcas* L. Afr J Biotechnol 13(7):806-813. doi: 10.5897/AJB12.2934

Eze JJ, Dambo A (2015) Mutagenic effects of sodium azide on the quality of maize seeds. J Adv Lab Res Biol 6(3):76-82.

FAO (Food and Agricultural Organization) (1996) Use of mutation techniques for improvement of cereals in Latin America. IAEA-TECDOC--859. Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture.

FAO (Food and Agricultural Organization) (2015) Joint Division of the Food and Agriculture Organization of the United Nations and the International Atomic Energy Agency (Joint FAO/IAEA). IAEA mutant database. http://mvgs.iaea.org/ (Accessed 15 July 2015).

FAO/IAEA (Food and Agricultural Organization/ International Atomic Energy Agency) (2018) Manual on Mutation Breeding - Third edition. Spencer-Lopes MM, Forster BP, Jankuloski L. (eds) Food and Agriculture Organization of the United Nations, Rome, Italy, pp 1-301.

Githinji GG, Birithia RK (2015) Effects of induced mutagenesis and single crossing on agronomic traits of wheat (*Triticum Aestivum* L.). J Agri Life Sci 2(2):1-7.

Govindaraj M, Vetriventhan M, Srinivasan M (2015) Importance of genetic diversity assessment in crop plants and its recent advances: an overview of its analytical perspectives. Genet Res Int 2015:1-14. doi.org/10.1155/2015/431487

Griffiths AJF, Miller JH, Suzuki DT (2000) Sources of variation. In: An introduction to genetic analysis. Seventh edition. Freeman WH and co, New York, pp 1-860. www.ncbi.nlm.nih.gov/books/NBK21766

Hingra D (2016) Mutation Breeding. https://www.slideshare.net/devhingra/mutation-breeding-60180150 (Accessed 14 October 2017)

Hu X, Li Y, Gao Y, Luo A, Zhao D, Hu X (2010) Review and prospect of space mutation application in pepper breeding. China Veg 24:14–18.

IAEA (International Atomic Energy Agency) (2011) Mutation induction for breeding. IAEA Vienna. www.mvgs.iaea.org/LaboratoryProtocals.aspx (Accessed 10 May 2011).

IAEA (International Atomic Energy Agency) (2018) Mutant varieties database. IAEA, Vienna. https://mvd.iaea.org (Accessed 23 August 2018).

IAEA (International Atomic Energy Agency) (2019) Mutant varieties database. IAEA, Vienna. https://mvd.iaea.org (Accessed 19 March 2019).

Jain SM (2005) Major mutation-assisted plant breeding programs supported by FAO/ IAEA. Plant Cell Tiss Org 82:113-123.

Jain SM (2010) Mutagenesis in crop improvement under the climate change. Rom Biotech Lett 15(2):88-106.

Jain SM, Suprasanna P (2011) Induced mutations for enhancing nutrition and food production. Gene Conserve 40:201–215.

Keneni G, Bekele E, Imtiaz M, Dagne K (2012) Genetic vulnerability of modern crop cultivars: causes, mechanism and remedies. Int J Plant Res 2:69–79

Khazaei H, Makela PSA, Stoddard FL (2018) Ion beam irradiation mutagenesis in rye (*Secale cereal* L.), linseed (*Linum usitatissimum* L.) and faba bean (*Vicia faba* L.). Agr Food Sci 27:146-151

King R, Bird N, Ramirez-Gonzalez R, Coghill JA, Patil A, Hassani-Pak K, Cristobal U, Phillips AL (2015) Mutation scanning in wheat by exon capture and next-generation sequencing. PLoS One 10(9):e0137549. doi: 10.1371/journal.pone.0137549

Kodym A, Afza R (2003) Physical and chemical mutagenesis. In: Grotewold E (ed) Methods in molecular biology. Plant functional genomics: methods and protocols, Humana Press, Springer Nature, Switzerland, pp 189-204.

Kristensen PS, Jahoor A, Andersen JR, Cericola F, Orabi J, Janss LL (2018) Genome-wide association studies and comparison of models and cross-validation strategies

for genomic prediction of quality traits in advanced winter wheat breeding lines. Front Plant Sci 9:69. doi.org/10.3389/fpls.2018.00069

Lee YI, Lee IS, Lim YP (2002) Variation in sweet potato regenerates from gamma rays irradiated embriogenic callus. J Plant Biotechnol 4:163-170.

Li X, Song Y, Century K, Straight S, Ronald P, Dong X, Lassner M, Zhang Y (2001) A fast neutron deletion mutagenesis based reverse genetics system for plants. Plant J 27:235–242.

Liu LX, Zhao LS, Guo HJ, Zhao SR, Wang J, Chen WH, Zheng QC (2007) A salt tolerant mutant wheat cultivar 'H6756'. Plant Mut Rep 1(3):50-51

Maluszynksi M (2001) Officially released mutant varieties—The FAO/IAEA database. Plant Cell Tiss Org 65:175–177.

Mba C, Afza R, Bado S (2010) Induced mutagenesis in plants using physical and chemical agents. In: Davey MR, Anthony P (eds) Plant cell culture: essential methods. John Wiley and Sons Ltd, Chichester, pp 111-130.

National Crop Variety List for Uganda (2015) National crop variety list, pp. 16-17. http://tasai.org/wp-content/themes/tasai2016/info_portal/Uganda/National%20Crop%20Variety%20List%20for%20Uganda%20(2015).pdf (Accessed 30 October 2019).

National Wheat Varieties for China (2007) http://www.ampcn.com/info/detail/14910.asp (Accessed 30 October 2019)

Nazarenko M, Lykholat Y, Grygoryuk I, Khromikh N (2018) Optimal doses and concentrations of mutagens for winter wheat breeding purposes. Part I. Grain productivity. J Cent Eur Agric 19(1):194-205.

Novak FJ, Brunner H (1992) Plant breeding: induced mutation technology for crop improvement. IAEA Bulletin. 4:25-33.

Okagaki RJ, Neffer MG, Wessler SR (1991) A deletion common to two independently derived waxy mutations of maize. Genetics 127:425-431.

OlaOlorun BM, Shimelis H, Matthew I, Laing M (2019) Optimizing the dosage of ethyl methanesulphonate mutagenesis in selected wheat genotypes. S Afr J Plant Soil. doi: 10.1080/02571862.2019.1610808

OlaOlorun BM, Shimelis H, Laing M, Mathew I (2020a) Morphological variations of wheat (*Triticum aestivum* L. em. Thell.) under variable ethyl methanesulphonate

mutagenesis. Cereal Research Communications. doi: 10.1007/s42976-020-00092-3

OlaOlorun BM, Shimelis H, Mathew I (2020b) Variability and selection among mutant families of wheat for biomass allocation, yield and yield-related traits under drought-stressed and non-stressed conditions. Journal of Agronomy and Crop Science (Wiley). doi: 10.1111/jac.12459

Oldach KH (2011) Mutagenesis. In: Pratap A, Kumar J (eds) Biology and breeding of food legumes. CABI,Wallingford, pp 208-219.

Paiva E, Lopes MA, Parentoni SN, Martins PR, Torres GA (1998) Searching for RFLP markers to identify genes for aluminum tolerance in maize. In: Application of DNA based marker mutations for improvement of cereals and other sexually reproduced crop plants. IAEA-TECDOC-1010. IAEA, Vienna, pp 33-40.

Pathirana R (2011) Plant mutation breeding in agriculture. CAB Rev: Persp Agri Vet Sci Nutrit Nat Res, 6(032):1-20.

Penna S, Jain SM (2017) Mutant resources and muta-genomics in crop plants. Emir J Food Agr 29(9):651-657.

Pierce BA (2005) Gene illustrations and DNA repair. In: Genetics - A conceptual approach. W. H. Freeman and Company, New-York, pp 473-503.

Plant Mutation Reports (2010) Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture and FAO/IAEA Agriculture and Bio-technology Laboratory, Seibersdorf 2(2):1-60. http://www-naweb.iaea.org/nafa/pbg/public/pmr-02-02.pdf

Porbeni JBO, OlaOlorun BM, Olalekun OJ, Oyetunde OA (2014) Agro-morphological effect of ethidium bromide on *Vigna unguiculata* (L.) Walp. Cv Ife Brown. Res J Agri 1(5):1-10.

Porbeni JBO, OlaOlorun BM, Sansa OO (2016) Effects of season on agro-morphological traits of some mutant cowpea lines. J Agric Sci Envt 16(2):79-87.

Raina A, Laskar RA, Khursheed S, Amin R, Tantray YR, Parveen K, Khan S (2016) Role of mutation breeding in crop improvement- past, present and future. Asian Res J Agric 2(2):1-13.

Rizwan M, Akhtar S, Aslam M, Asghar MJ (2015) Development of herbicide resistant crops through induced mutations. Adv in Life Sci 3(1):1-8.

Şen A, Sarsu F (2018) Genetic diversity in sodium azide induced wheat mutants studied by SSR markers. Trak Univ J Nat Sci 19(2):129-135. doi: 10.23902/trkjnat.424305.

Sestili F, Botticella E, Bedo Z, Phillips A, Lafiandra D (2010) Production of novel allelic variation for genes involved in starch biosynthesis through mutagenesis. Mol Breed 25:145-154.

Shivakumar M, Nataraj V, Kumawat G, Rajesh V, Chandra S, Gupta S, Bhatia VS (2018) Speed breeding for Indian agriculture: a rapid method for development of new crop varieties. Curr Sci 115(7):1241.

Shu QY, Forster BP, Nakagawa H (2012) In: Nakagawa H. (ed) Plant mutation breeding and biotechnology. CABI,Wallingford, pp 1-608.

Sikora P, Chawade A, Larsson M, Olsson J, Olsson O (2011) Mutagenesis as a tool in plant genetics, functional genomics, and breeding**.** Int J Plant Genomics 2011:1-13. Article ID 314829. doi:10.1155/2011/314829.

Singh NK, Balyan HS (2009) Induced mutations in bread wheat (*Triticum aestivum* L.) Cv. 'Kharchia 65' for reduced plant height and improve grain quality traits. Adv Biol Res 3(5-6):215-221.

Singh R, Kole CR (2005) Effect of mutagenic treatments with EMS on germination and some seedling parameters in mungbean. Crop Res 30:236-240.

Smale M, Reynolds MP, Warburton M, Skovmand B, Trethowan R, Singh RP, Ortiz-Monasterio I, Crossa J (2002) Dimensions of diversity in modern spring bread wheat in developing countries from 1965. Crop Sci 42:1766–1779.

Suprasanna P, Ganapathi TR, Ghag SB, Jain SM (2017) Genetic modifications of horticultural plants by induced mutations and transgenic approach. Acta Hortic 1187:219-232. doi: 10.17660/ActaHortic.2017.1187.22.

Tadesse W, Inagaki M, Tawkaz S, Baum M, Van Ginkel M (2012) Recent advances and application of doubled haploids in wheat breeding. Afr J Biotechnol 11(89):15484-15492.

Uauy C, Paraiso F, Colasuonno P, Tran RK, Tsai H, Berardi S, Comai L, Dubcovsky J (2009) A modified TILLING approach to detect induced mutations in tetraploid and hexaploid wheat. BMC Plant Biol 9(115):1-14. doi.org/10.1186/1471-2229-9-115

UPOV (International Union for the Protection of New Varieties of Plants) (2002) General introduction to the examination of distinctness, uniformity, and stability, and the development of harmonized descriptions of new varieties of plants. http://www .upov.int/en/publications/tg-rom/tg001/tg_1_3.pdf. (Accessed 14 October 2017)

van de Wouw M, Kik C, van Hintum T, van Treuren R, Visser B (2010) Genetic erosion in crops: concept, research results and challenges. Plant Genet Resour 8(1):1-15.

Voss-Fels K, Frisch M, Qian L, Kontowski S, Friedt W, Gottwald S, Snowdon RJ (2015) Sub-genomic diversity patterns caused by directional selection in bread wheat gene pools. Plant Genome 8(2):1-13. doi:10.3835/plantgenome2015.03.0013

Wani MR, Kozgar MI, Tomlekova N (2014) Mutation breeding: a novel technique for genetic improvement of pulse crops particularly Chickpea (*Cicer arietinum* L.) In: Parvaiz A, Wani MR, Azooz MM, Lam-son PT (eds) Improvement of crops in the era of climatic changes. New York, Springer, pp 217-248.

White CA, Sylvester-Bradley R, Berry PM (2015) Root length densities of UK wheat and oilseed rape crops with implications for water capture and yield. J Exp Bot 66:2293–2303.

Yasmin GSC, Luciana CV, Ueric JBd, Layara AB (2019) Recent trends in research on the genetic diversity of plants: implications for conservation. Diversity 11(62):1-21. doi:10.3390/d11040062

Zhao L, Wang Y, Zhen D, Li Y, Wang H, Zuo H, Wei Z (2005) The new variety from spring wheat mutation “Long Fu No.2”. Acta Agron Hung 19(1):79-80

Chapter 2

Optimizing the Dose of Ethyl Methanesulphonate Mutagenesis in Selected Wheat Genotypes

Abstract

Narrow genetic variation limits the success of crop improvement programs. Mutagenesis using ethyl Methanesulphonate (EMS) provides an opportunity to increase genetic variation to enhance selection in wheat improvement. This study aimed at establishing the optimum dose and treatment conditions of EMS for effecrive mutagenesisi to induce genetic variation for drought tolerance and enhanced biomass allocation in selected wheat genotypes. Seeds of three genotypes (LM29, LM43 and LM75) were treated with three EMS doses (0.1, 0.4 and 0.7% v/v) at three temperatures (25, 30 and 35 °C) for three exposure periods (1hr, 1.5hrs and 2hrs) using three replicates. The ideal treatment conditions for effective mutagenesis were 0.7% EMS for 2 hours at 35 °C for genotypes LM29 and LM43 and 0.4% EMS for 2 hours at 25 °C for LM75. The estimated EMS doses for LM43, LM29 and LM75 were 0.32, 1.07, and 1.81%v/v EMS, respectively. This information can be used for large-scale mutation induction, exploring new genetic variation, and evaluating genetic improvement and select mutant individuals with drought tolerance, high root-shoot biomass and C sequestration.

Keywords: chemical Mutagenesis, ethyl methanesulphonate, lethal dose, seedling characteristics, wheat

2.1 Introduction

Wheat (*Triticum aestivum* L.; 2n=6x=42, AABBDD) is an important source of food, feed and industrial raw material (Sajjad *et al.,* 2012; Muhmood *et al*., 2014; DAFF, 2016). Despite the global importance of wheat, biotic (e.g. disease and pests) and abiotic (e.g. poor soil fertility and drought) stresses affect wheat production and productivity. Consequently, the main goal in wheat improvement programs is to develop wheat ideotypes with high yield potential, stress resilience and enhanced root-shoot biomass and Carbon (C) sequestration ability (Semenov and Stratonovitch, 2013).

Genetic variation is a precondition to the development of improved wheat cultivars that can tolerate drought stress and contribute to carbon sequestration for improved soil health and climate change mitigation. The narrow genetic variation in wheat is exacerbated by deliberate selection and crosses involving few genetically related and limited number of elite genotypes (Cowling, 2013). Induced mutagenesis offers an opportunity to create the needed genetic variation for successful breeding.

Mutagenesis is induced using physical or chemical agents (Raina *et al.,* 2016). Chemical mutagens such as ethyl methanesulphonate (EMS) has been successfully used on different crops such as in wheat (Bahar and Akkaya, 2009), rice (Ramchander *et al.,* 2014), sesame (Anbarasan *et al.,* 2013), sugar beet (Hohmann *et al.,* 2005), pepper (Devi and Salvakumar, 2013) and ornamental species (Jiang and Dunn, 2016). Ethyl methanesulphonate is the most efficient in inducing higher mutation frequency of crop traits compared to physical mutagens such as gamma radiation (Satpute and Fultambkar, 2012; Mangaiyarkarasi *et al*., 2014). Optimizing mutagenesis is necessary before embarking on large-scale mutagenesis program (Khan and Wani, 2004; Joshi *et al.,* 2011). Exposure of seeds to EMS results in variable response and success rate of selecting ideal mutants due to differences in genotype, dose, temperature and duration of exposure. Therefore, mutation conditions need to be optimized before embarking on large-scale mutagenesis program (Joshi *et al.,* 2011).

Higher doses of EMS reduced shoot or root length in treated seedlings. Anbarasam *et al*. (2013) reported that the shoot length of sesame seedlings treated with 1.8% EMS

was reduce by 46% compared to those treated with 0.4%. Similarly, Dhakshanamoorthy *et al*. (2010) reported a 35% reduction in root length of *Jatropha curcas* treated with 4% EMS compared to 1% EMS treatment. However, Kumar *et al*. (2009) reported that higher concentrations resulted in wider and multiple type variation. The LD_{50}, defined as a dose of the mutagen that results in 50% reduction in seed germination after exposing the seeds to the mutagen for a definite period and specific conditions (Bharathi *et al.,* 2013; Beyaz *et al.,* 2016), is often used to compare the effect of the mutagen in seeds treated under different conditions. Similarly, LD_{50} values vary due to differences in crop species, genotype, mutagen, and ambient conditions during mutagenesis (Aparna *et al.,* 2013; Liamngee *et al.,* 2017). The LD_{50} value for EMS mutagenesis on wheat, *Catharanthus roseus* and pigeon pea were 0.3% (Bahar and Akkaya, 2009), 50mM (Mangaiyarkarasi *et al*., 2014) and 25mM (Ariraman *et al*., 2014), respectively, showing interspecific variation in response to EMS treatment. Intraspecific variations are also known to exist due to genotypic differences. For instance, Karthika and Lakshmi (2006) reported significantly different LD_{50} values of 26.4mM and 25.7mM for two soya bean varieties CO1 and CO2, respectively.

To select unique wheat ideotypes with enhanced C sequestration and drought tolerance. The success of mutation breeding for enhanced C sequestration and drought tolerance will depend on the number of mutants in germination potential, seedling survival, seedling vigour, root biomass and root to shoot ratios. Therefore, it is necessary to determine these parameters in specific populations in order to assess the extent of variation that can be created and evaluated for different traits. Therefore, the aim of this study was to determine the optimum dosage and treatment conditions of EMS for effective mutagenesis of selected wheat genotypes to induce genetic variation for drought tolerance and enhanced biomass allocation.

2.2 Materials and methods

2.2.1 Experimental site and plant materials

The study was carried under laboratory and greenhouse conditions at the Controlled Environmental Facility (CEF) of the University of KwaZulu-Natal. Seeds of three wheat genotypes (LM29, LM43 and LM75) were used for the study. Seeds were sourced from the International Maize and Wheat Improvement Centre (CIMMYT) (Table 2.1). The genotypes were developed in the CIMMYT drought tolerant nursery and were previously evaluated for biomass potential and drought stress tolerance and identified to have high root biomass under drought conditions in subsequent evaluations (Mwadzingeni *et al.*, 2016).

Table 2.1: Names and pedigrees of wheat genotypes used in the study

Name of genotype	Pedigree
LM29	PRL/2*PASTOR*2//SKAUZ/BAV92
LM43	ROLF07*2/6/PVN//CAR422/ANA/5/BOW/CROW//BUC/PVN/3/YR/4/TRAP#1
LM75	BUC/MN72253//PASTOR

2.2.2 Treatment conditions

The experiment consisted of 4 factors (genotype, dose, time and temperature) with three levels each. The wheat genotypes with three levels were selected as described above by Mwadzingeni *et al.* (2016). Three levels of EMS doses (0.1, 0.4 and 0.7%) and three levels of exposure period (1 hour, 1.5 hours and 2 hours) were chosen as previously suggested by Mba *et al*. (2007) for inducing mutation in wheat. Three temperature levels (25, 30 and 35 °C) were used to enable a range of temperatures affecting biological processes following Ndou *et al.* (2013). Each genotype was exposed to all possible combination of the treatment factors.

2.2.2.1 Seed sterilization and pre-soaking

Forty healthy and uniform seeds for each genotype were counted and placed separately in customized 8 cm long and 6 cm wide labelled plastic mesh bag according to each treatment combination. The seeds were surface sterilized to remove contaminants and reduce chances of microbial infection by soaking the mesh bags in 70% ethanol for 1 minute and washing under running water at room temperature for 2 minutes. They were later soaked in 30% JIK (Sodium hypochlorite) for 5 minutes and washed off under running water for 2 minutes and then pre-soaked in distilled water for 24 hours at room temperature before the EMS preparation and treatment (Figure 2.1).

Figure 2.1: Some procedures explained for EMS treatment of wheat seeds. (A) Proper labelling of mesh bags, (B) Soaking of seeds in distilled water for 20-24 hours, (C and D) Mesh bags placed in EMS Treatment in water bath to maintain temperature at 35^{o}C

2.2.2.2 EMS preparation

The procedures to EMS preparation and seed treatment were adapted from Mba *et al.,* (2007). Prior to EMS preparation, a 2% solution of dimethyl sulfoxide (DMSO) was prepared to be used as a carrier agent for EMS treatment. The DMSO was autoclaved at 120 °C and 103.5 kPa for 15 minutes and set to cool down at room temperature for 5-6 hours. The EMS solutions at three concentration levels of 0.1%, 0.4% and 0.7% were prepared accordingly by making up a litre with 2% DMSO solution using a pipette. The solution was mixed thoroughly by vigorously shaking for 5 minutes.

2.2.2.3 EMS mutagenesis

Controls were separated after pre-soaking. The seeds from the three genotypes (LM29, LM43 and LM75), were subjected to three EMS doses (0.1, 0.4 and 0.7% v/v), at three temperatures (25, 30 and 35 °C) for three exposure periods (1, 1.5 and 2 hours) giving 81 treatment combinations. The mesh bags containing the seeds were immersed in EMS at the appropriate concentration in a beaker. The beakers were placed in a water bath maintained at prescribed temperatures for the different time durations. After each treatment condition, excess EMS was washed off under running water for 3 hours to reduce hazard during handling after mutagenesis. The mesh bags were placed on paper towels afterwards for overnight to drain moisture from seeds (Figure 2.1). The seeds were planted in the following morning as described below.

2.2.3 Trial establishment

The EMS treated seeds and controls per genotype were planted at about 1cm depth in seedling trays under greenhouse condition using soil containing pine bark growth media (Figure 2.2). One seed per hole was planted. The seeds were planted using a completely randomized design with three replications. The seedlings were watered four times daily using a mist irrigation system. The relative humidity in the greenhouse was 63% and controlled by a foggier system.

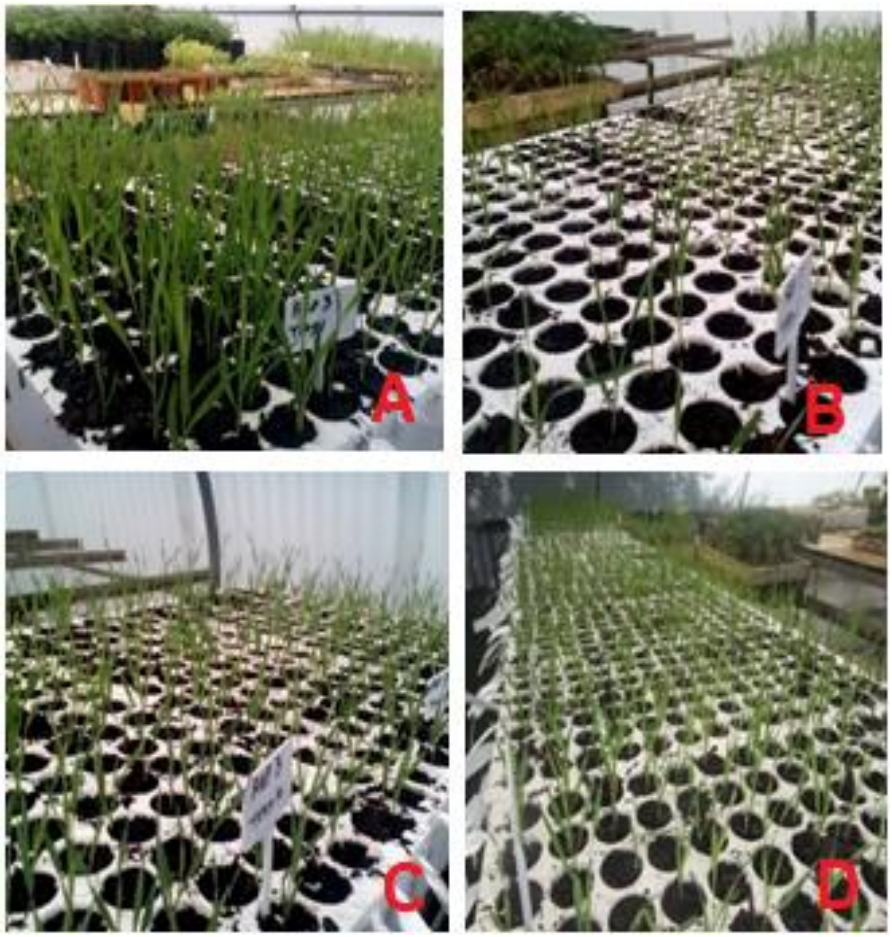

Figure 2.2: Wheat seedling trial in the greenhouse. (A) Seedlings of treated LM29 at 15 days after planting (DAP), (B) Seedlings of treated LM43 at 15 DAP, (C) Seedlings of treated LM75 at 15 DAP, (D) Seedlings of all treatments at 15 DAP

2.2.4 Data collection

The following traits were recorded from germination to 15 days after germination of the seedlings: The days to emergence (DTE) was recorded when 50% of the seeds germinated after sowing, while the percentage germination (%G) was recorded as the proportion of germinated seeds per total number of seeds sown at eight days after sowing. The seedling survival (%SS) was calculated as the proportion of number of survived seedlings per total number of germinated seeds. The shoot length (SHL) was measured as the length from the base of the plant to the tip of the flag leaf, while root length (RL) was measured from the base of the plant to the tip of the longest root. Seedling vigour index (SVI) was estimated as the percentage germination multiplied by seedling height following Abdul-Baki and Anderson (1973), while the root to shoot ratio (RSR) was computed as the proportion of the root length to shoot length. The first three

traits (DTE, %G and %SS) were measured based on 40 seedlings, while the other traits were measured averages of 20 seedlings.

2.2.5 Data analyses

The data collected were analysed using GenStat 18th edition with the analysis of variance (ANOVA) procedure (Payne *et al*., 2017). Treatment means were separated by Fischers' unprotected least significant difference (LSD) at 0.05 significance level. The LD_{50} for each genotype was estimated using the linear regression model by fitting the straight-line equation,

$$y = a + bx$$

where *y* is the dependent variable (germination percentage), *x* is the independent variable (EMS dose) and *a* and *b* are the constant and slope, respectively. LD_{50} was estimated using the germination rates (y) and EMS doses (x), while duration of exposure to EMS and temperature were kept constant at 1.5 hours and 30 °C, respectively, which were the mean ideal conditions in the experiment. The relationships among DTE, %G, SHL, RL, %SS, SVI and RSR were analysed using SPSS version 24 with the Pearson correlations procedure (IBM SPSS, 2016).

2.3 Results

2.3.1 Analysis of variance of trait response

All the factors under consideration (genotype, dose, time and temperature) had significant impact, either individually or in combination, on the response of the traits measured in wheat after mutagenesis (Table 2.2). Seedling survival and DTE exhibited significant differences in response to the four-way interaction of genotype x dose x time x temperature. The three-way interaction (genotype x dose x temperature) resulted in significant ($p<0.01$) differences in seedling vigour. The effects of the interaction involving genotype, time and temperature were significant for percentage germination and shoot length. The genotype x time interaction effect was significant ($p<0.05$) for seedling height.

Table 2.2: Mean square values and significant tests for seed germination and other seedling characters of three EMS-tested wheat genotypes using 81 treatment combinations and 3 replications

Source of Variation	df	DTE	%G	SHL	RL	SH	%SS	RSR	SVI
Genotype (G)	2	557.9***	99531.3***	325.7***	280.6***	1210.6***	75810.3***	0.0015	83510990***
Dose	3	11.9**	1083.3***	43.7***	16.2	102.5***	628.3***	0.0749	2342298***
Time	2	12.6**	1787.0***	148.1***	81.4***	442.6***	425.2*	0.0848	5715330***
Temperature (Temp)	2	8.9*	1084.0**	51.0***	17.9	129.5***	635.3**	0.0419	2868006***
G*Dose	6	1.5	173.7	2.8	7.1	11.4	318.5*	0.0436	280097
G*Time	4	4.7	443.1*	9.5*	17.4	47.9*	172.8	0.0444	1009563***
Dose*Time	4	1.9	556.3*	14.1**	4.9	21.8	279.5	0.0797	405660*
G*Temp	4	8.5*	563.1*	9.6*	4.2	26.0	173.2	0.0046	489518*
Dose*Temp	4	1.8	110.5	6.4	3.1	4.7	420.1*	0.0574	308824
Time*Temp	4	1.4	229.2	2.9	8.3	11.4	170.6	0.0413	64174
G*Dose*Time	8	0.8	381.4*	3.1	2.2	7.7	300.9*	0.0146	183285
G*Dose*Temp	8	4.5	320.7	2.9	10.8	19.0	353.4**	0.0425	507252**
G*Time*Temp	8	1.7	682.5***	8.2*	2.0	16.3	358.6**	0.0155	414802*
Dose*Time*Temp	8	2.3	385.4*	4.7	14.5	23.5	486.5***	0.0663	515626**
G*Dose*Time*Temp	16	5.3*	283.2	3.2	7.9	16.7	263.6**	0.0211	260060
Error	166	2.5	176.9	3.8	7.9	14.6	124.1	0.0363	172898

DTE: Days to 50% emergence, %G: Percentage germination at 8 days, SHL: Shoot length at 15 days, RL: Root length at 15 days, SH: Seedling height at 15 days, %SS: Percentage seedling survival, RSR: Root-shoot ratio, SVI: Seedling vigour index, df: Degree of freedom, * significant at 5% Probability level; ** significant at 1% Probability level, *** significant at ≤ 0.1% Probability level

Significant differences ($p<0.05$) in DTE, %G, SHL, SH, %SS and SVI were recorded in response to temperature. Similarly, the effects of time of exposure to EMS resulted in significant differences ($p<0.05$) in DTE, %G, SHL, RL, SH, %SS and SVI, while the main effect of EMS dose were significant ($p<0.01$) on all traits measured except RL and RSR. DTE, %G, SHL, RL, SH, %SS and SVI exhibited significant differences ($p<0.001$) due to genetic variation.

2.3.2 Genotypic variation for traits performance

The mean performance of genotypes showed significant differences for all the traits assessed in the study except RSR (Table 2.3). The mean days to emergence of LM75 subjected to EMS treatment was 6 days and showed non-significant difference compared to the control. On average, seed of LM29 and LM43 took 4 and10 days to emerge after EMS treatment, respectively. The mean germination percentage for LM29 (94.14%) and LM75 (87.65%) were not statistically significantly different from the 100% germination recorded in their respective controls. In contrast, LM43 recorded significantly lower germination of 32% compared to the other genotypes. In addition, the control treatment for LM43 recorded the lowest germination percentage of 27.78%. The longest mean shoot value of 17.58 cm was recorded in genotype LM29 showing significant differences compared to 15.10 and 13.69 cm recorded in genotypes LM43 and LM75, respectively. There were non-significant differences in the shoot lengths of all genotypes when compared with their respective controls. The mean root lengths of 16.15, 13.62 and 12.44 cm were recorded for genotypes LM29, LM43 and LM75, respectively, due to EMS treatment. The root length among the genotypes were significantly different. The root lengths of LM29 and LM75 seedlings treated with EMS were significantly longer than the comparative controls. EMS treated LM43 had shorter root length compared to its control. Seedling height recorded a similar trend as root length. There were significant differences in seedling survival rate among the genotypes. The highest seedling survival was recorded in genotype LM75 (97.12%) followed by 97.02% and 45.37% for genotypes LM29 and LM43, respectively.

Table 2.3: Mean values for seven traits measured on three wheat genotypes subjected to EMS treatment

Genotypes	DTE		%G		SHL		RL		SH		%SS		SVI	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated
LM29	4[a]	4[a]	100.00[b]	94.14[c]	17.59[c]	17.58[c]	12.59[a]	16.15[c]	30.18[c]	33.73[c]	100[b]	97.02[b]	3018[c]	3285[c]
LM43	11[c]	10[c]	27.78[a]	32.00[a]	15.03[b]	15.10[b]	14.17[b]	13.62[b]	29.19[b]	28.73[b]	38.89[a]	45.37[a]	1129[a]	1310[a]
LM75	6[b]	6[b]	100.00[b]	87.65[b]	13.85[a]	13.69[a]	12.08[a]	12.44[a]	25.93[a]	26.13[a]	100[b]	97.12[b]	2593[b]	2543[b]
LSD (5%)	1.02		4.19		0.61		0.84		1.15		3.50		126.50	
CV (%)	67.9		18.9		12.6		19.3		12.6		14.1		17.1	

DTE: Days to 50% emergence, %G: Percentage germination at 8 days, SHL: Shoot length at 15 days, RL: Root length at 15 days, SH: Seedling height at 15 days, %SS: Percentage seedling survival, SVI: Seedling vigour index. Means in a column followed by the same letter(s) are not significantly different at P=0.05

Genotype LM43 exhibited a higher survival rate (45.37%) compared with the control (38.89%), while EMS treated genotypes LM29 and LM75 recorded a non-significant with <3% drop-in survival rate compared with their respective controls. The test genotypes exhibited significant variation in seedling vigour. The mean seedling vigour of 3285 was recorded in genotype LM29 which was significantly higher than 2543 and 1310 noted for LM75 and LM43, respectively. The seedling vigour of EMS treated genotypes LM29 and LM43 were significantly higher than the comparative controls, whilst LM75 had decreased seedling vigor compared to its control.

2.3.3 Effect of EMS treatment conditions on assessed traits

There was a differential responses of wheat genotypes to varying treatment conditions (Tables 2.4-2.6). Seeds of LM29 treated with the highest EMS dose, under the highest temperature and longest exposure period recorded the lowest %G, %SS and SVI while treatment conditions of 0.1% EMS, 1hour, and 30 °C allowed better response in SHL, RL, SVI (Table 2.4). For genotype LM43, the highest values for %G, %SS, SHL and SVI were recorded in seedlings treated with 0.1% EMS for 1 hour at 25 °C, while seedlings from treatment condition 0.7% EMS, 2 hours, 35 °C recorded the lowest values for %G, %SS, SHL and SVI (Table 2.5). Shoot length, RL and SVI were highest for LM75 seedlings treated with 0.1% EMS for 1 hour at 30 °C and lowest at 0.7% EMS, 1.5 hours and 35 °C (Table 2.6).

Table 2.4: Means for six traits measured on wheat genotype LM29 seedlings treated with three different EMS doses, three temperature regimes and three exposure periods

Dose (%)	Time (hr)	DTE			%G			SHL			RL			%SS			SVI		
		Temperature (°C)																	
		25	30	35	25	30	35	25	30	35	25	30	35	25	30	35	25	30	35
0.1	**1**	3	3	3	100.0	100.0	100.0	19.1	20.5	19.9	18.6	18.7	17.2	100.0	100.0	100	3766	3923	3708
	1.5	3	3	3	100.0	100.0	100.0	20.0	18.4	16.9	17.5	16.8	16.4	100.0	100.0	100	3750	3520	3334
	2	3	3	5	100.0	100.0	97.2	17.8	18.9	17.3	16.0	15.1	15.2	100.0	100.0	100	3380	3398	3247
0.4	**1**	3	3	4	97.2	100.0	97.2	18.3	19.7	17.5	17.5	17.0	15.2	100.0	100.0	100	3570	3669	3267
	1.5	3	3	3	100.0	88.9	91.7	20.7	17.6	15.8	18.5	15.9	15.8	100.0	100.0	100	3914	3347	3152
	2	3	4	5	100.0	97.2	83.3	17.8	15.6	14.0	15.5	16.3	13.7	100.0	100.0	97.2	3334	3193	2677
0.7	**1**	3	3	5	100.0	97.2	91.7	19.1	17.1	15.4	18.1	14.9	16.0	100.0	100.0	94.4	3721	3201	2964
	1.5	3	4	4	100.0	100.0	100.0	20.4	14.6	16.4	16.2	15.4	14.8	100.0	100.0	100	3657	2995	3124
	2	5	4	-	88.9	88.9	22.2	16.2	15.6	14.1	14.7	14.7	14.6	94.4	100.0	33.3	2924	3026	935
Control			4			100.0			17.6			12.6			100.0			3018	

DTE: Days to 50% emergence, %G: Percentage germination at 8 days, SHL: Shoot length at 15 days, RL: Root length at 15 days, %SS: Percentage seedling survival, SVI: Seedling vigour index

Table 2.5: Means for six traits measured on wheat genotype LM43 seedlings treated with three different EMS doses, three temperature regimes and three exposure periods

Dose (%)	Time (hr)	DTE			%G			SHL			RL			%SS			SVI		
		Temperature (°C)																	
		25	**30**	**35**	**25**	**30**	**35**	**25**	**30**	**35**	**25**	**30**	**35**	**25**	**30**	**35**	**25**	**30**	**35**
0.1	**1**	5	10	10	50.0	36.1	22.2	18.9	17.5	16.1	17.8	15.7	13.1	77.8	50.0	38.9	2798	1666	1112
	1.5	8	11	12	25.0	41.7	19.4	13.2	16.3	13.7	14.8	12.5	14.5	38.9	58.3	33.3	1095	1656	847
	2	9	5	12	41.7	38.9	33.3	13.8	17.6	14.7	14.6	12.7	13.0	63.9	38.9	47.2	1821	1180	1310
0.4	**1**	6	12	8	30.6	38.9	44.4	14.7	15.4	14.8	11.6	13.3	12.6	41.7	55.6	61.1	1151	1624	1709
	1.5	10	10	10	27.8	41.7	27.8	16.1	15.5	15.4	13.9	9.13	14.7	36.1	55.6	38.9	1130	1377	1167
	2	8	13	8	38.9	22.2	38.9	14.5	12.6	13.4	12.6	15.6	13.4	52.8	30.6	63.9	1412	860	1819
0.7	**1**	12	7	11	27.8	22.2	27.8	17.5	14.8	17.3	15.9	12.5	14.8	38.9	30.6	33.3	1263	889	1111
	1.5	10	11	10	33.3	30.6	36.1	13.6	17.5	14.8	9.07	19.0	11.5	50.0	36.1	44.4	1131	1174	1163
	2	11	13	-	27.8	25.0	13.9	13.1	14.1	10.8	12.7	15.5	11.3	44.4	44.4	19.4	1144	1333	423
Control			11			27.8			15.0			14.2			38.9			1129.1	

DTE: Days to 50% emergence, %G: Percentage germination at 8 days, SHL: Shoot length at 15 days, RL: Root length at 15 days, %SS: Percentage seedling survival, SVI: Seedling vigour index

Table 2.6: Means for six traits measured on wheat genotype LM75 seedlings treated with three different EMS doses, three temperature regimes and three exposure periods

Dose (%)	Time (hr)	DTE			%G			SHL			RL			%SS			SVI		
		Temperature (°C)																	
		25	**30**	**35**	**25**	**30**	**35**	**25**	**30**	**35**	**25**	**30**	**35**	**25**	**30**	**35**	**25**	**30**	**35**
0.1	**1**	5	4	5	88.9	100.0	100.0	17.3	17.5	15.1	15.2	15.5	13.9	91.7	100.0	100.0	2965	3304	2903
	1.5	6	6	6	86.1	100.0	91.7	13.5	14.3	12.4	10.1	12.1	12.1	100.0	100.0	100.0	2366	2639	2453
	2	6	4	5	80.6	97.2	83.3	13.4	15.2	14.5	11.5	13.4	10.9	94.4	97.2	88.9	2371	2783	2258
0.4	**1**	5	5	6	88.9	100.0	94.4	16.0	16.1	14.9	15.7	13.1	13.2	97.2	100.0	100.0	3075	2918	2814
	1.5	6	5	7	100.0	91.7	63.9	14.6	13.6	10.7	11.1	11.7	11.1	100.0	97.2	86.1	2573	2460	1902
	2	7	5	5	58.3	86.1	88.9	11.8	12.8	11.9	10.1	11.9	11.4	91.7	94.4	97.2	2015	2335	2258
0.7	**1**	4	6	6	100.0	88.9	100.0	15.4	15.0	14.9	15.2	14.7	13.8	100.0	100.0	100.0	3053	2977	2874
	1.5	7	5	7	72.2	100.0	66.7	12.7	13.5	10.5	11.4	12.8	10.4	100.0	100.0	91.7	2413	2622	1934
	2	7	6	7	66.7	86.1	86.1	11.3	11.2	9.38	11.8	11.2	10.7	97.2	97.2	100.0	2230	2173	2004
Control			6			100.0			13.9			12.1			100.0			2593	

DTE: Days to 50% emergence, %G: Percentage germination at 8 days, SHL: Shoot length at 15 days, RL: Root length at 15 days, %SS: Percentage seedling survival, SVI: Seedling vigour index

2.3.4 Effect of exposure time on assessed traits

A general increase in DTE was observed for all genotypes as EMS dose increased (Figure 2.3a). The seedlings of the genotype LM29 emerged earlier (≤5) than the other genotypes while LM43 seedings emerged late (≤12). A similar emergence response was observed for LM29 seedlings treated for 1 and 1.5 hours irrespective of the EMS dose. However, LM29 seeds treated with 0.7% EMS for 2 hours emerged later (5 days) when compared to other treatment conditions. LM43 treated with 0.7% EMS for 2 hours took the longest time (12 days) to emerge, while seeds treated for 1 hour with 0.1% EMS emerged earliest (8 days). There was no significant effect of EMS doses on LM43 seedlings treated for 1.5 hours. LM75 seedlings treated with 0.7% EMS for 1.5 hours emerged later (7 days) than other treatment conditions while seedlings of treated for 1 hour irrespective of the EMS dose emerged earliest (5 days). The seedlings of genotype LM29 maintained a very high germination response (> 93%) except showing a drastic drop (67%) when treated with 0.7% EMS for 2 hours (Figure 2.3b). LM43 recorded a low level of germination (≤ 40%) for all exposure periods and doses used. LM75 recorded a high level of germination (> 80%) irrespective of the EMS doses and exposure periods. Control treatments of LM29 and LM75 maintained 100% germination, while LM43 was low (27.78%) (Table 2.3). High survival rate was maintained for genotypes LM29 and LM75 (Figure 2.3c). LM29 recorded a high seedling survival (≥ 98%) irrespective of the doses and exposure periods, except for the drastic drop which occurred when treated with 0.7% EMS for 2 hours. The survival rate for all treatment of LM43 was below 60%. The different doses did not induce any significant difference for LM43 seeds treated for 1.5 hours. The controls of LM29 and LM75 maintained 100% survival rate, while LM43 recorded 38.89% (Table 2.3).

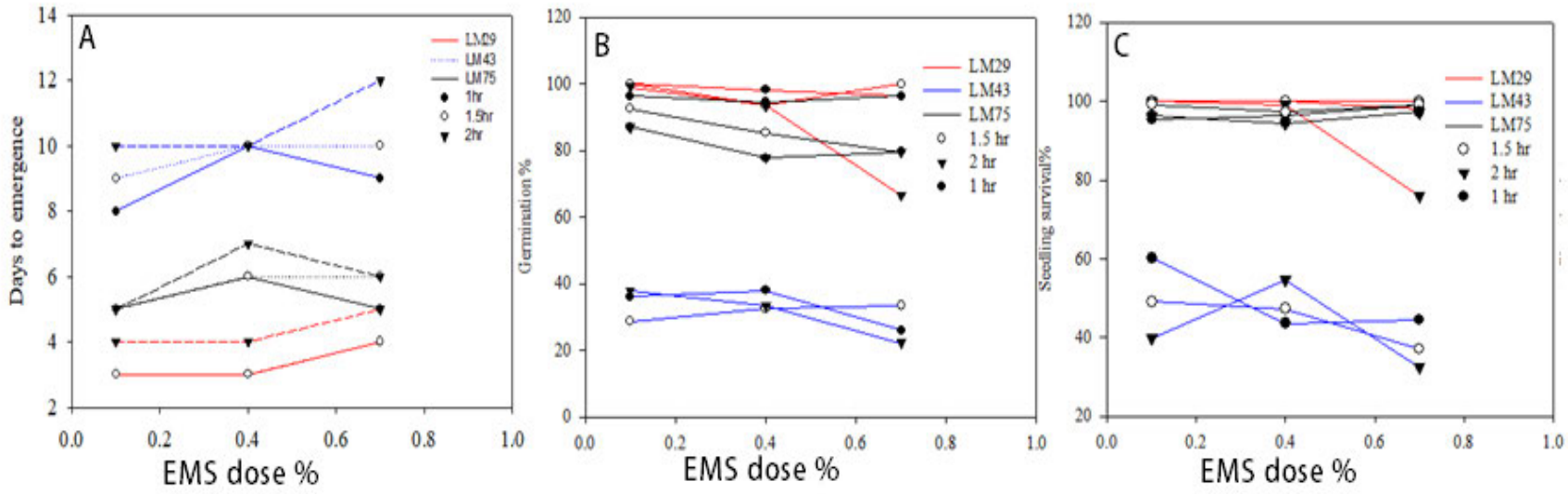

Figure 2.3: Days to emergence, germination percentage and rate of survival in seedlings of three wheat genotypes treated with different doses of EMS for variable durations

2.3.5 Effect of temperature on trait response to genotype and dosage

The trend of DTE for LM43 were irregular and undefined (Figure 2.4a). However, there was a general increase in DTE with increased in EMS dose for LM29 and LM75 irrespective of the varying temperatures. Also, the DTE for genotypes LM29 and LM75 was highest at treatment condition 0.7% EMS and 35 °C. LM29 treated at all temperatures, emerged earlier than other genotypes. However, there was no significant effect of DTE on treated seedlings with 0.1 and 0.4% EMS irrespective of varying temperatures. Treated LM29 seedlings with 0.7% EMS at 35 °C emerged later than other treatment conditions. Treating LM43 seeds with EMS under 30 and 35 °C, resulted in an unclear pattern, as there were sharp rises and falls in DTE with increase in EMS dose. However, for treatments under 25 °C, seedlings emerged earlier when treated with lower doses of EMS. The %SS of LM29 was maintained at 100% irrespective of the doses and temperatures except for seedlings treated with 0.7% EMS for 35 °C which recorded a drastic drop (Figure 2.4b). For LM43 treated under 25 °C, the dose of 0.1% EMS resulted in the highest survival rate, while 0.4 EMS treated seedlings recorded the least. For 30 °C, there was no significant difference in the %SS when treated with 0.1 and 0.4% EMS. However, a sharp drop was observed when treated with 0.7% EMS. Treating LM75 seedlings under 30 °C irrespective of their doses recorded the highest survival. Survival rates of 100% were noted for seedlings of genotypes LM29 and LM75 under control treatment, while LM43 had the lowest value of 38.89% (Tables 2.3). The vigour of LM29 seedlings declined with an increase in EMS doses irrespective of the temperature used (Figure 2.4c). The trend of seedling vigour of LM43 was not well defined. Seedlings obtained from seeds treated with 0.1% EMS were the most vigorous at all the temperatures regimes used compared to seeds treated at the other dosages. For LM75, 0.1% EMS treated seedlings recorded the highest vigour, although there was no significant difference between seedlings treated with 0.4 and 0.7% EMS for all temperatures.

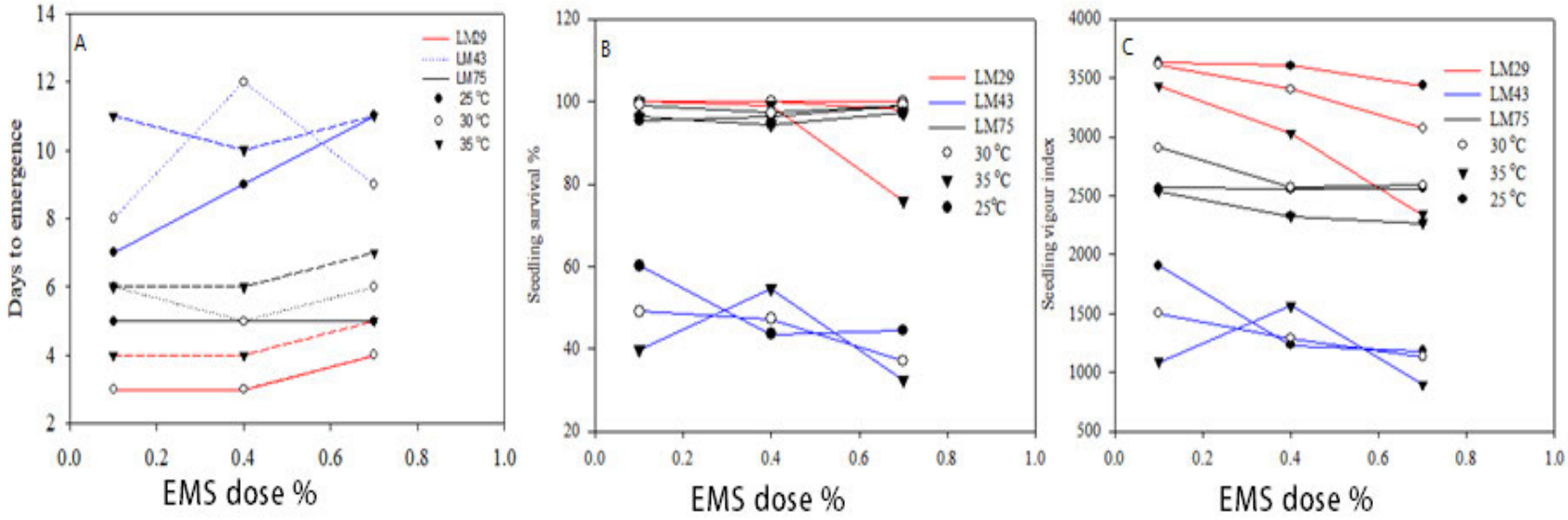

Figure 2.4: Days to emergence, rate of survival and vigor in seedlings of three wheat genotypes treated with different doses of EMS at variable temperatures

2.3.6 LD_{50} values and ideal treatment conditions for test genotypes

The LD_{50} was predicted under a constant EMS exposure time of 1.5 hours at 30 ºC using the linear relationship between percentage germination and dose of EMS. There was a general trend of decreased germination percentage as dose increased (Figure 2.5). However, the response of germination to dose was specific for each genotype resulting in significant differences in LD_{50}. The highest LD_{50} was calculated by linear regression to be 1.81%v/v for LM75, which was significantly higher than 1.07%v/v and 0.32%v/v calculated for LM29 and LM43, respectively.

The ideal mutagenic treatment conditions, defined as the factorial combinations that resulted in the lowest germination % for each genotype, were found to be similar for two of the genotypes. For genotypes LM29 and LM43, the ideal treatment combination was an EMS dose of 0.7% for 2 hours at 35 °C, while an EMS dose of 0.4% for 2 hours at 25 °C for genotype LM75.

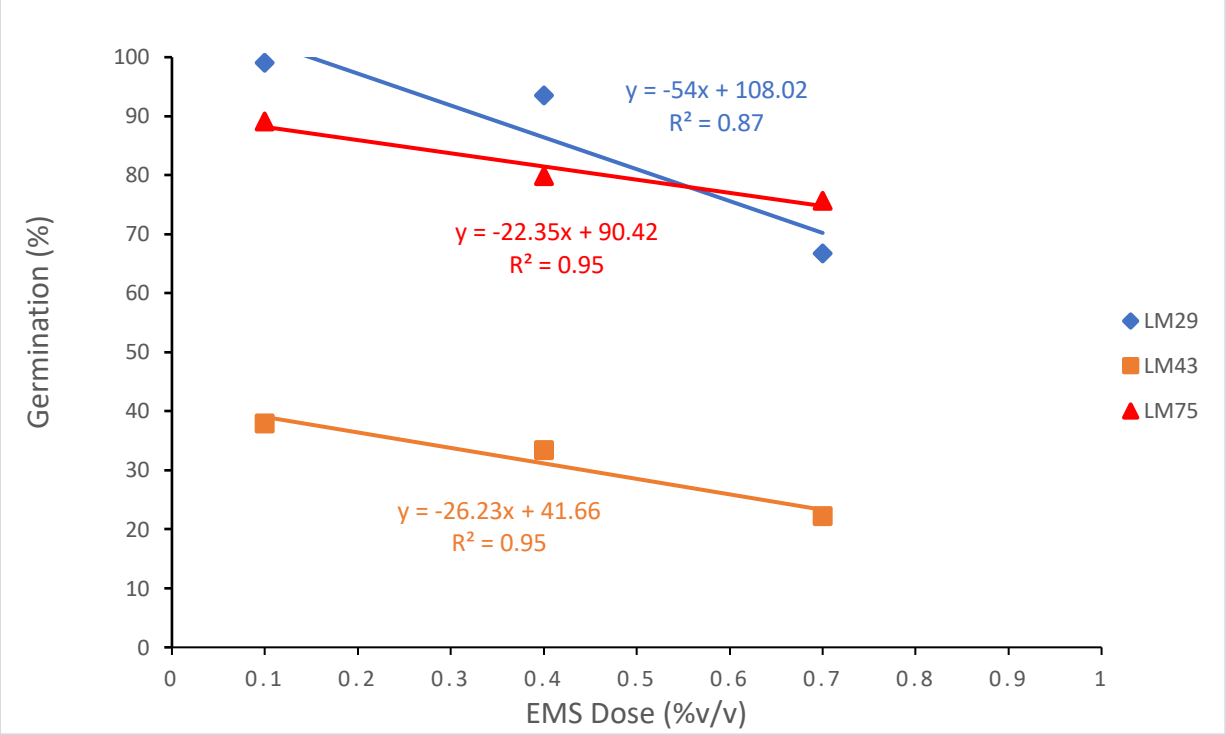

Figure 2.5: Germination percentage fitted against the three EMS doses used to calculate the LD_{50} for three wheat genotypes at constant conditions

2.3.7 Trait associations

The percentage germination and shoot length were positively associated with all characters except days to 50% emergence and root-shoot ratio (Table 2.7). The number of days to 50% emergence exhibited negatively weak correlations with shoot length (*r*= -0.24; p<0.01), root length (*r*= -0.24; p<0.01) and seedling height (*r*= -0.27; p<0.01). Shoot length showed a significant negative association with RSR (*r*= -0.372; p<0.01). Strong positive correlation occurred between shoot length and root length (*r*= 0.53; p<0.01), shoot length and seedling vigour index (*r*= 0.54; p<0.01), root length and root-shoot ratio (*r*= 0.58; p<0.01) and between seedling height and seedling vigour index (*r*= 0.56; p<0.01). There was non-significant association of days to 50% emergence with seedling survival and seedling vigour index.

Table 2.7: Correlation coefficients for pair-wise associations of studied characters in three wheat genotypes

Traits	DTE	%G	SHL	RL	SH	%SS	RSR	SVI
DTE	-							
%G	-0.063	-						
SHL	-0.235**	0.306**	-					
RL	-0.244**	0.192**	0.526**	-				
SH	-0.274**	0.282**	0.863**	0.883**	-			
%SS	0.119	0.929**	0.158*	0.089	0.140*	-		
RSR	-0.043	-0.097	-0.372**	0.575**	0.136*	-0.065	-	
SVI	-0.055	0.894**	0.538**	0.445**	0.560**	0.882**	-0.031	-

DTE: Days to 50% emergence, %G: Percentage germination at 8 days, SHL: Shoot length at 15 days, RL: Root length at 15 days, SH: Seedling height at 15 days, %SS: Percentage seedling survival, RSR: Root-shoot ratio, SVI: Seedling vigour index. * correlation is significant at 5% probability level, ** correlation is significant at 1% probability level

2.4 Discussion

2.4.1 Genotypic variation in trait response

The significant ($p<0.001$) genotypic main effects exhibited for most evaluated traits (Table 2.2) indicate genetic differences among the test genotypes. The presence of high genetic variation allows for possible improvement of seed and seedling qualities through genotype selection. Similar findings were reported in bread wheat (Baloch *et al.,* 2016) and cowpea (Gerrano *et al.,* 2015). Germination percentage and seedling survival rate of genotypes LM29 and LM75 were similar under control conditions owing to the higher germination capacity of these genotypes. However, LM43 had remarkably lower germination percentage, which was unexpected (Table 2.5). The seeds used in this study were the seeds harvested at the same time and stored under similar conditions for a month before replanting. Since the age and storage conditions were similar, we attributed most of the variation in germination to genotypic and treatment effects rather than differences in seed quality.

2.4.2 Impact of treatment factors on trait response

The effects of dose, time and temperature on most traits implies that mutagenesis is also influenced by other factors apart from the genotype. The significant effect of the EMS dose on some traits shows that altering the dosage induces mutation. These findings agree with Horn and Shimelis, (2013) who also found significant effect of mutagen dose on trait response in cowpea. Exposure time was significant for most traits indicating its importance in mutagenesis. Time affects the rate of imbibition and therefore determines how much of the chemical mutagen is taken up by the seed during exposure. Seeds exposed for shorter periods are likely to imbibe lower quantities of the mutagen leading to different responses with those exposed for longer. Subsequently, seeds exposed to EMS for longer periods may imbibe higher amounts of the mutagen leading to longer germination time as the mutagen can interfere with physiological processes that initiates seed germination (Kulkami, 2011). The effect of temperature was significant for some traits since temperature is known to affect biological processes. Higher temperatures accelerate rate of development and maturity in seeds (Edwards,

2010) leading to early emergence. However, excessively high temperatures disrupt biological functioning of enzymes and integrity of genetic material. The different interaction levels among the factors were significant for DTE, %G, %SS and SVI indicating that the combined effects of time, temperature, EMS and genotype were important in determining the optimal mutagen condition. The significant four-way interaction effect on seedling survival implies differential effects to seedling survival, explaining its cumulative contributions of all factors to effectively induce mutation on the treated genotypes.

2.4.3 Mean performance of genotypes under variable EMS treatment conditions

Genotype effects were significant for DTE with LM43 taking longer time to germinate showing differential genotypic response to emergence. Genotypes differ in their response even when exposed to the same stimuli, a phenomenon determined by the underlying genetics. LM29 and LM75 attained an average of 100% germination, implying that they had similar response during mutagenesis. A combination of exposure of seeds to higher doses and higher temperatures for longer periods reduced germination potential indicating effective mutagenesis (Rupinder and Kole, 2005). High EMS dose reduces emergence and germination possibly by disrupting growth promoters, increasing growth inhibitors and inducing chromosomal aberrations (Jayakumar and Selvaraj, 2003). Excessively high temperatures increase rate of respiration and disrupt biological functioning of enzymes leading to restricted hypocotyl elongation and poor emergence (Shah *et al.,* 2008). Long exposure to mutagen can cause the seed to imbibe high amounts of the mutagen leading to interference with the biochemical content and reduces membrane integrity of the seeds. Kiong *et al.* (2008) suggested reduction in germination and survival was due to increasing frequency of chromosomal harm with increasing mutagen concentration. Altered biochemical process cause a delay in emergence or complete failure to emerge. Overall, there was reduction in germination in treated seeds compared to controls in agreement with Khan *et al.* (2004) and Dhakshanamoorthy *et al*. (2010).

Genotypes LM29 and LM75 recorded a mean seedling survival of 100%, implying that both genotypes responded similarly after mutagenesis. Seedling survival rate above 85% for LM29 and LM75 in most treatment combinations indicates that there was no observed mutagenic effect on their survival. Increased level of treatment factors (0.7% EMS for 2 hours at 35 °C) negatively affected the survival rate in LM29 and LM43 seeds in agreement with Khan and Al-Qurainy, (2009) who postulated that high dose, temperature and exposure period disturb meristematic activity and hormonal balance to meristematic tissue. Genotypes performed differently in mean shoot length with LM29 recording the longest shoot length, showing significant genotypic effects (Figure 2.2). Similarly, highest level of treatment combination (0.7% EMS for 2 hours at 35 °C) recorded the shortest shoot length for the test genotypes, through their effect on meristematic activity and hormonal balance. Similar pattern of decreasing shoot length with increased EMS doses was reported by Bahar and Akkaya (2009) in mutant bread wheat.

The significant variation in the average root length explains differential response of the genotypes to various treatment combination. Root length is an important trait used to test for mutagen sensitivity in crops (Joshi *et al.,* 2011). LM43 seedlings exposed to the highest dose and temperature recorded the shortest root length. This finding agrees with Kalia *et al*. (2001) and Shah *et al.* (2008) who observed an inhibitory effect of high EMS doses on the root length of durum wheat and chickpea, respectively. Kumar and Yadav (2010) also reported that the mutagenic effectiveness increased with the increase in the dose and treatment of EMS when treated with sesame seeds. Like other traits, there were significant differences in seedling vigor among the genotypes showing genotypic variation in mutagen tolerance. LM29 recorded the highest average seedling vigour followed by LM75, while LM43 recorded the least in line with their germination potential. In general, highest levels of treatment factors reduced seedling vigor and seedling vigor index due to hormonal imbalance, poor meristem development and poor shoot development, which culminate into weak seedlings. Weak seedlings with low vigor will have problems during establishment under a range of environmental conditions (Sharma *et al.,* 2017).

2.4.4 Genotype response to dosage

The estimated lethal doses of the test genotype showed a general decrease in percentage germination with increase in dosage (Figure 2.5). The differential estimated lethal doses for the 3 test genotypes implies that the wheat genotypes require different dose, time and temperature to achieve optimal mutagenesis in accordance with genotypic variation. LM43 required very low EMS doses to achieve the expected LD_{50} while LM29 was intermediate and LM75 was the most tolerant. For effective mutagenesis in LM29 and LM75, there is need to increase the EMS dose to 1.07 and 1.81%v/v, respectively, while maintaining exposure time at 1.5 hours and temperature at 30 °C. Ramchander *et al.* (2014) reported that lethal dose of EMS for rice treated under *in vitro* condition should be between 0.354% and 0.365%, while Bahar and Akkaya (2009) reported an effective mutagenesis in bread wheat was achieved using 0.3%v/v EMS. Similarly, other studies on mutagenesis have reported LD_{50} outside of the tested range (Horn and Shimelis, 2013; Bind and Dwivedi, 2014; Julia *et al.,* 2018). The knowledge of LD_{50} is of importance and determines sensitivity of different genotypes to the critical mutagen dose. Seedling growth characteristics like percentage germination, seedling survival and height are good indicators in estimating the magnitude of damage cause by the mutagens (Talebi *et al*., 2012; Horn and Shimelis, 2013).

2.4.5 Correlations among traits

The traits exhibited variable correlations across the different treatments. The negative association of days to 50% emergence with shoot and root length suggests greater chances of seedlings that emerge early to develop into taller plants with well-established roots. Early emergence results in taller plant with good field establishment (Alom *et al*., 2016). Seedlings which take longer to emerge may exhaust their food reserves leading to development of stunted shoots and poor root system. However, these associations were weak probably as a result of the fact that the root and shoot lengths were measured on seedlings rather than mature plants. The shoot and root lengths of seedlings may not reflect the full potential of a genotype given that some genotypes may have initial slow growth rate at establishment. In other studies,

Nagashima and Hikosaka (2011) asserted that plants grown under high density regulate their plant height, which may cause week associations due to abiotic stress. The correlations observed between percentage germination and all other characters except days to 50% emergence and root-shoot ratio implies that germination is favourably associated with the other traits and can be selected simultaneously. Similar findings have been reported by Adebisi (2010) in sesame where positive association was observed between germination and other seedling parameters. Good germination and seedling establishment are prerequisites for optimum crop yields (Subedi and Ma, 2005). Ramos and Carvalho (1997) suggested that a successful field establishment indicates a well-developed shoot and root system permitting a better withstand during drought conditions. A good crop establishment increases C sequestration potential plant growth correlates with net carbon gain on a whole plant basis (Kruger and Volin, 2006). Steady germination and a fast seedling establishment leading to high plant growth response as seen in the production of secondary tillers in wheat, will increase the number of leaves per plant thereby, increasing the photosynthetic rates and plant carbon gain. Shoot and root lengths had a positive correlation with, seedling survival and seedling vigor showing that tall plant height, and higher shoot biomass supported by an efficient root system have higher chances to withstand adverse conditions. A positive and strong correlation observed among percentage seedling survival, seedling vigour and percentage germination suggests that selection for one trait could be used to indirectly select the other traits. Harding *et al.* (2012) pointed out a positive association between percentage germination and seedling survival in rice. Simultaneous selection is complicated when two important traits are undesirably correlated. However, for the non-significant correlations, Ramos and Carvalho (1997) suggested independence of association indicating a possibility of selecting two traits independently.

2.5 Conclusion

The study aimed to establish the optimum conditions and the lethal dose (LD_{50}) for effective mutagenesis on seed germination and seedling characteristics of three wheat genotypes. Due to variations in genotypic response to mutagenesis, the lethal dose for the three genotypes LM29, LM43 and LM75 were estimated to be 1.07, 0.32 and

1.81%v/v EMS respectively. The ideal treatment combinations for effective mutagenesis were 0.7% EMS for 2 hours at 35 °C for genotypes LM29 and LM43 and 0.4% EMS for 2 hours at 25 °C for LM75. This may provide the expected genetic variation during the M_2 generation for segregation analysis and selection.

2.6 References

Abdul-Baki A, Anderson JD (1973) Vigor determination in soybean seed by multiple criteria. Crop Sci 13: 630-633.

Adebisi MA, Ajala MO, Adekoya MA, Amira JO, Ajani OO, Adekola TO (2010) Multivariate assessment of variations in seed quality and seed yield components of sesame (*Sesamum indicum* L). Proceedings of the 34th Annual Conference of the Genetics Society of Nigeria (GSN), pp 184-191.

Al-Enezi AN, Al-Bahrany AM, Al-Khayri JM (2012) Effect of x-irradiation on date palm seed germination and seedling growth. Emir J Food Agr 24(5):415-424.

Alom R, Hasan MA, Islam MR, Wang QF (2016) Germination characters and early seedling growth of wheat (*Triticum aestivum* L.) genotypes under salt stress conditions. J Crop Sci Biotechnol 19(5):383-392.

Anbarasan K, Sivalingam HD, Rajendran R, Anbazhagan M, Chidambaram AlA (2013) Studies on the mutagenic effect of EMS on seed germination and seedling characters of sesame (*Sesamum indicum* L.) Var.T MV3. Int J Res Bio Sci 3(1):68-70.

Aparna M, Chaturvedi A, Sreedhar M, Kumar DP, Venu-Babu P, Singhal RK (2013) Impact of gamma rays on the seed germination and seedling parameters of groundnut (*Arachis hypogaea* L.). Asian J Exp Biol Sci 4(1):61-68.

Ariramana M, Gnanamurthy S, Dhanavel D, Bharathi T, Murugan S (2014) Mutagenic effect on seed germination, seedling growth and seedling survival of pigeon pea (*Cajanus cajan* (L.) Millsp). Int Lett Nat Sci 21: 41-49.

Bahar B, Akkaya MS (2009) Effects of EMS treatment on the seed germination in wheat**.** J Appl Biol Sci 3(1):59-64.

Baloch MJ, Channa, GM, Jatoi WA, Baloch AW, Rind IH, Arain MA, Keerio AA (2016) Genetic characterization in 5×5 diallel crosses for yield traits in bread wheat. Sarhad J Agr 32(3):127-133.

Beyaz R, Kahramanogullari CT, Yildiz C, Darcin ES, Yildiz M (2016) The effect of gamma radiation on seed germination and seedling growth of *Lathyrus chrysanthus* Boiss under *in vitro* conditions. J Environ Radioact 15:162-163.

Bharathi T, Gnanamurthy S, Dhanavel D, Murugan S, Ariraman M (2013) Induced physical mutagenesis on seed germination, lethal dosage and morphological mutants of ashwagandha (*Withania somnifera* (L.) Dunal). Int J Adv Res 1(5):136-141.

Bind D, Dwivedi VK (2014) Effect of mutagenesis on germination, plant survival and pollen sterility in M_1 generation of in cowpea (*Vigna unguiculata* (L.) Walp). Indian J Agric Res 48(5):398-401. doi:10.5958/0976-058X.2014.01322.5

Cowling WA (2013) Sustainable plant breeding. Plant Breed 132:1-9.

DAFF (Department of Agriculture, Forestry and Fisheries) (2016) Wheat production guideline. DAFF, Pretoria. https://www.daff.gov.za/Daffweb3/Portals/0/Brochures%20and%20Production%20guidelines/Wheat%20-%20Production%20Guideline.pdf (Accessed June 2019).

Devi SA, Selvakumar G (2013) Chemical mutagens induced alterations in chlorophyll mutants and flower development of chilli (*Capsicum annuum* L.). Int J Mod Agric 2:39-42.

Dhakshanamoorthy D. Selvaraj R, Chidambaram A (2010) Physical and chemical mutagenesis in *Jatropha curcas* L. to induce variability in seed germination, growth and yield traits. Rom J Biol Plant Biol 55(2):113–125.

Edwards MA (2010) Morphological features of wheat grain and genotype affecting flour yield. PhD Thesis, Southern Cross University, Lismore, Australia, pp 1-252.

Forcella F, Benech Arnold RL, Sanchez R, Ghersa CM (2000) Modeling seed emergence. Field Crop Res 67:123-139.

Gerrano AS, Adebola PO, Jansen van Rensburg WS, Laurie SM (2015) Genetic variability in cowpea (*Vigna unguiculata* (L.) Walp.) genotypes. S Afr J Plant Soil 32(3):165–174.

Jayakumar S, Selvaraj R (2003) Mutagenic effectiveness and efficiency of gamma rays and ethyl methanesulphonate in sunflower. Madras Agri J 90(7-9):574-576.

Jiang L, Dunn BL (2016) Ethyl methanesulfonate and caffeine mutagenetic treatment to four ornamental silene species. J Environ Hortic 34(4):95–100.

Joshi N, Ravindran A, Mahajan V (2011) Investigations on chemical mutagen sensitivity in onion (*Allium cepa* L.). Int J Botany 7(3):243-248.

Julia T, Renuka TH, Nanita H, Jambhulkar S (2018) Mutagenic effectiveness and efficiency of gamma rays in Indian mustard (*Brassica juncea* L. Czern and Coss). Int J Curr Microbiol Appl Sci 7(03):1-12. doi: 10.20546/ijcmas.2018.703.390

Harding SS, Johnson SD, Taylor DR, Dixon CA, Turay MY (2012) Effect of gamma rays on seed germination, seedling height, survival percentage and tiller production in some rice varieties cultivated in Sierra Leone. Am J Exp Agric 2(2):247-255.

Hohmann U, Jacobs G, Jung C (2005) An EMS mutagenesis protocol for sugar beet and isolation of non-bolting mutants. Plant Breed 124:317-321.

Horn L, Shimelis HA (2013) Radio-sensitivity of selected cowpea (*Vigna unguiculata*) genotypes to varying gamma irradiation doses. Sci Res Essays. 8:1991-1997.

IBM SPSS I (Statistical Package for the Social Sciences) (2016) IBM corporation released. IBM SPSS Statistics for Windows, Version 24.0. Armonk, New York

Kalia CS, Kharkwal MC, Singh MP, Vari AK (2001) Mutagenic effects of environmental industrial chemical agents in inducing cytogenetical changes in wheat. Indian J Genet Pl Br. 61:203-208.

Kandil AA, Sharief AE, Elokda MA (2012) Germination and seedling characters of different wheat cultivars under salinity stress. Basic Appl 8:585-596.

Karthika R, Lakshmi BS (2006) Effect of gamma rays and EMS on two varieties of soyabean. Asian J Plant Sci 5:721-724.

Khan S, Wani MR (2004) Isolation of high yielding mutants in mungbean (*Vigna radiata* (L.) Wilczek). Tropical Agriculturist 154:51-59.

Khan S, Wani MR, Parveen K (2004) Induced genetic variability for quantitative traits in *Vigna radiata* (l.) wilczek. Pak J Bot 36(4):845-850.

Kiong ALP, Lai AG, Hussein S. Harun AR (2008) Physiological responses of *Orthosiphon stamineus* plants to gamma irradiation. Am-Eurasian J Sustain Agric 2(2):135-149.

Kulkarni GB (2011) Effect of mutagen on pollen-fertitity and other parameter in horse gram (*Imacrotyloma untflorum* lam). Bio Disc 2:146-150.

Kumar G, Yadav RS (2010) EMS induced genomic disorders in sesame. Rom J Biol 55:97-104.

Kumar VA, Kumari RU, Amutha R, Kumar TS, Hepziba SJ, Kumar CRA (2009) Effect of chemical mutagen on expression of characters in arid legume pulse -cowpea (*Vigna unguiculata* (L.) Walp.). Res J Agric Biol 5(6):1115-1120.

Kruger EL, Volin JC (2006) Re-examining the empirical relationship between plant growth and leaf photosynthesis. Funct Plant Biol 33:421–429.

Liamngee SM, Ogah JJ, Amagu KT, Kwon-Ndung EH, Lorkor D, Tervershima JE (2017) Mutagenic action of sodium azide on germination and emergence in landraces of *Phaseolus vulgaris* L. on the Jos Plateau agro-ecological zone. J Agri Vet Sci 10(2):64-70.

Mangaiyarkarasi R, Girija M, Gnanamurthy S (2014) Mutagenic effectiveness and efficiency of gamma rays and ethyl methanesulphonate in *Catharanthus roseus*. Int J Curr Microbiol Appl Sci 3(5):881-889.

Mba C, Afza R, Jain SM (2007) Crop improvement. In: Jenks MA, Hasegawa PM, Jain SM (eds) Advances in molecular breeding towards drought and salt tolerant crops. Springer-Verlag, Berlin, Heidelberg, pp 413-454.

Muhmood A, Javid S, Niaz A, Majeed A, Majeed T, Anwar M (2014) Effect of boron on seed germination, seedling vigor and wheat yield. Soil Envt 33(1):17-22.

Mwadzingeni L, Shimelis H, Tesfay S, Tsilo TJ (2016) Screening of bread wheat genotypes for drought tolerance using phenotypic and proline analyses. Front. Plant Sci 7(1276):1-12.

Nair R, Mehta AK (2014) Induced mutagenesis in cowpea (*Vigna unguiculata* (l.) Walp) var. Arka Garima. Indian J Agric Sci 48(4):247-257.

Nagashima H, Hikosaka K (2011) Plants in a crowded stand regulate their height growth so as to maintain similar heights to neighbours even when they have potential advantages in height growth. Ann Bot 108(1):207–214. doi: 10.1093/aob/mcr109

Ndou VN, Shimelis H, Odindo A, Modi AT (2013) Response of selected wheat genotypes to ethyl methanesulphonate concentration, treatment temperature and duration. S Afr J Plant Soil 8(4):189 -196.

Olasupo FO, Ilori CO, Forster BP, Bado S (2016) Mutagenic effects of gamma radiation on eight accessions of cowpea (*Vigna unguiculata* L. Walp.). Am J Plant Sci 7:339-351.

Payne R, Murray D, Harding S (2017) An introduction to the Genstat command language (18th Edition). VSN International Ltd, Hemel, Hempstead, UK, pp 1-137

Petrenko V (2014) Influence of storage conditions on germination of winter wheat seeds (*Triticum aestivum* L.) in relation to agriculture systems. Agric Sci 21(3):173–180.

Raina A, Laskar RA, Khursheed S, Amin R, Tantray YR, Parveen K, Khan S (2016) Role of mutation breeding in crop improvement- Past, present and future. Asian Res J Agri 2(2):1-13.

Ramchander S, Pillai MA, Ushakumari R (2014) Determination of lethal dose and effect of ethyl methanesulphonate in rice varieties. Trends in Biosci 7(11):1151-1156.

Ramos LC, Carvalho A (1997) Shoot and root evaluations on seedlings from coffea genotypes. Bragantia 56(1):59-68.

Rupinder S, Kole CR (2005) Effect of mutagenic treatment with EMS on germination and some seedling parameters in mungbean. Crop Res 30(2):236-240.

Sajjad M, Khan SH, Ashfaq M, Nasim W (2012) Association of seed morphology with seedling vigor in wheat (*Triticum aestivum* L). Res Plant Bio 2(5):07-12.

Satpute RA, Fultambkar RV (2012) Mutagenic effectiveness and efficiency of gamma rays and EMS in soybean (*Glycine max* (L.) Merrill). Curr Bot 3(2):18-20.

Semenov MA, Stratonovitch P (2013) Designing high-yielding wheat ideotypes for a changing climate. Food Energy Secur 2(3):185–196.

Shah TM, Mirza JI, Haq MA, Atta BM (2008) Radio sensitivity of various chickpea genotypes in M_1 generation 1 - Laboratory studies. Pak J Bot 40:649-665.

Sharma S, Punia RC, Singh V, Mor VS, Hemender (2017) Prediction of seedling establishment in field by using various seed vigour parameters in wheat (*Triticum spp*.). Int J Curr Microbiol Appl Sci 6(11):410-414.

Song J, Fan H, Zhao YY, Jia YH, Du XH, Wang BS (2008) Effect of salinity on germination, seedling growth and ion accumulation of a euhalophyte suaeda salsa in an intertidal zone and on saline inland. Aquat Bot 88:331-337.

Songsri P, Suriham B, Sanitchon J, Srisawangwong S, Kesmala T (2011) Effects of gamma radiation on germination and growth characteristics of physic nut (*Jatropha curcas* L.). J Biol Sci**.** doi:10.3923/jbs.2011

Chapter 3

Agro-Mophological Variations of Wheat (*Triticum aestivum* L.) under Variable Ethyl Methanesulphonate Mutagenesis

Abstract

Genetic gains in wheat yield have stagnated over the years due to both genetic and non-genetic causes, prompting efforts to create new genetic variation for yield improvement to meet current and future demands for wheat. Chemical mutagenesis using ethyl methanesulphonate (EMS) has the potential to generate genetically stable mutants with improved agro-morphological traits to increase genetic variation for grain yield and yield components. However, there is a need to optimize EMS mutagenesis due to variations in lethality, efficiency and effectiveness affecting response to selection under different treatment conditions. The objective of this study was to determine the agro-morphological variations induced through mutagenesis using three pre-determined EMS treatments for a specific wheat genotype to develop breeding populations. The wheat genotype LM43 was subjected to EMS mutagenesis under the following treatment conditions: 0.1% v/v for 1 hour at 25 °C, 0.1% v/v for 1 hour at 30 °C and 0.7% v/v for 1.5 hours at 25 °C. After EMS treatments, some mutant plants in M_1 had significantly ($p< 0.05$) increased number of spikelets per spike, number of kernels per spike and grain yield while tiller number, number of kernels per spike and grain yield increased significantly at M_2. EMS treatment with 0.1% v/v for 1 hour at 30 °C was the most effective and efficient in inducing mutation with the minimum of biological damage in this population. Macro-mutations were also exhibited as abnormalities in spike, peduncle, awn and flag leaf morphology. The study identified early generation mutant populations with a variety of desirable characteristics that could be exploited for increased drought tolerance and grain yield improvement, or for genetic analysis to identify quantitative trait loci in wheat.

Keywords: agronomic traits, EMS mutagenesis, morphological variations, mutation efficiency, wheat breeding

3.1 Introduction

Bread wheat (*Triticum aestivum* L.; 2n=6x=42, AABBDD) is an important source of food for about seven billion people around the world (UN, 2017). However, recurrent droughts and climate change threaten global production and productivity of wheat. For instance, drought stress has significantly reduced wheat production in South Africa, creating a national deficit in wheat supply (Esterhuizen, 2018). Improved cultivars with high yield potential under the prevailing adverse conditions are required in order to reduce the gap between supply and demand. The success of developing improved cultivars hinges on the availability of adequate genetic variation. However, genetic variation in cultivated crops such as wheat has decreased over the years due to intensive selective breeding using limited breeding populations (Cowling, 2013). Genetic gains in wheat yield and agronomic traits have stagnated in many parts of the world as a result of loss of genetic diversity, among other factors (Voss-Fels *et al.,* 2015). Thus, there is need to create new genetic variation in order to improve yield and yield related traits. Variations can be introduced via conventional breeding by crossing divergent genotypes, or through induced mutation. Artificial mutagenesis offers the possibility of inducing desired attributes that cannot be found in nature or to reconstitute genetic variation that have been lost during evolution and selection of finite populations (Srivastava *et al.,* 2011).

Conventional breeding techniques such as hybridization require relatively long period of time to create adequate genetic variation, which creates a critical bottleneck for cultivar development under a rapidly changing environment (Shivakumar *et al.,* 2018). Thus, a rapid method, such as mutation breeding, can be used to complement conventional breeding methods. Mutation is a sudden alteration of the genetic constitution of individuals at one or more loci that can be passed on to the offspring (Porbeni *et al.,* 2014). Mutations can be either natural or induced. Naturally, mutations can occur during DNA replication and can be passed on to their offspring during reproduction (Novak and Brunner, 1992; Srivastava *et al.,* 2011). Natural mutations are usually minor and may not be useful or desirable if the resultant offspring possess inferior traits. Alternatively, mutations can be induced physically or chemically to increase the frequency of useful

mutations for breeding compared to natural mutations (Jain, 2010). Selecting a mutagen type should be based on its efficiency and specificity to cause mutations. Chromosome rearrangements and deletions mainly occur due to irradiation mutagenesis while chemical mutagens create point mutations resulting in change of function mutations (Talebi *et al.,* 2012). Physical mutagenesis occurs when radiation suddenly alters the genetic structure of biological materials (Kodym and Afza, 2003; Nurmansyah *et al.,* 2018). However, the use of physical mutagenesis in developing countries is limited because the equipment required to produce the effective dose of radiation is not available or is too expensive (Kodym and Afza, 2003). On the other hand, chemical mutagenesis occurs when biological material is exposed to chemical agents that alter the genetic composition of individuals (Adekola and Oluleye, 2007). Chemical mutagenesis does not require the use of highly expensive equipment and is widely used in developing countries (Jain, 2005). The use of chemical mutagenesis has gained considerable importance in mutation breeding of wheat due to its ability to induce high mutation rates, especially point mutations (Singh *et al*., 2014; Dhaliwal *et al.,* 2015).

Several types of chemical mutagens including sodium azide, ethidium bromide and ethyl methanesulphonate (EMS) have been used successfully to induce mutations (Siddiqui *et al.,* 2007; Girija *et al*., 2013). The efficiency and effectiveness of the mutagen in inducing the desirable mutations is influenced by its chemical properties, and by biological and environmental factors (Kodym and Afza, 2003). An effective mutagen induces a high frequency of mutations with a high probability of creating new variation. In addition, the mutagen must be efficient enough to induce a higher proportion of mutations with minimal biological damage (Kharkwal, 1998). Ethyl methanesulphonate (EMS) is the most widely used mutagen due to its efficiency and effectiveness in inducing frequent mutations (Espina *et al.,* 2018), it produces random mutations in genetic materials by nucleotide substitution (Ambarasan *et al.,* 2013). Furthermore, it can easily be disposed of by hydrolysis, posing a limited hazard during handling, and its potential negative effect on the environment (Pathirana, 2011).

EMS mutagenesis has been used widely to improve grain quality (FAO, 2010), herbicide resistance (Rizwan *et al.*, 2015), disease resistance (IAEA, 2015) and to induce male sterility (Maan and Williams, 1984) and morphological variations (Dhaliwal *et al.,* 2015) in wheat. These have led to increased genetic variation and variable yield responses in mutants compared to their normal parents. Several crop varieties generated from EMS induced mutations have been released that have enhanced crop production under marginal growing conditions (Kharkwal and Shu, 2009). Ahloowalia *et al.* (2004), Nazarenko (2018) and IAEA (2019) reported that some released mutant wheat varieties such as Darkhan-106, Deada, Baichun 5, Emai 23 and Fumail 2008 have higher grain yield potential and improved agro-morphological traits than some conventional varieties. The increases in yield and variations in morphological traits documented in various studies have shown that mutagenesis can create important genetic variation to complement conventional breeding methods. Genetic variation for root traits and biomass allocation in wheat has narrowed down over the years due to systematic breeding (Cowling, 2013) for high harvest indices and yield. The reduction in genetic variation, especially for root biomass, has reduced gains for the drought tolerance and carbon sequestration capacity of novel wheat varieties (Mathew *et al.,* 2019). Mutagenesis has the potential to rapidly create new genetic variation for traits such as root traits that are usually neglected or otherwise take multiple breeding cycles to improve using conventional breeding methods. Prior to embarking on a large-scale mutagenesis programme, there is a need to evaluate the different treatment combinations and to select the optimal conditions that efficiently and effectively generate wide genetic variation. The effectiveness and efficiency of mutagenesis must be pre-tested for each specific genotype before embarking on a large-scale mutation breeding programme in order to recover high frequency of desirable mutations (Solanki and Sharma, 1994). Previous studies on mutagenesis of grain crops such as rice, chickpea, sunflower and finger millet have reported that the effectiveness and efficiency of a mutagen is usually genotype specific (Bansal *et al.,* 1990; Wani, 2009; Kumar and Ratnam, 2010; Ambavane *et al.,* 2015). OlaOlorun *et al.* (2019) evaluated three genotypes using a combination of treatment conditions but only evaluated the agronomic performance of the genotypes under greenhouse conditions and at seedling

level. The current study extends to large scale evaluation of a single genotype under field conditions (*in situ*) up to maturity and across generations. Thus, the new information generated in this study is complementary to the previous study as it uses the pretested EMS dosages and treatment conditions. The objective of this study was to evaluate agro-morphological variations induced in wheat through mutagenesis, using three pre-determined EMS treatments for a specific genotype to develop breeding populations. This information will be useful in selection of early generation mutants for yield and drought tolerance improvement or provide opportunities for genetic analysis to identify quantitative trait loci in wheat.

3.2 Materials and methods

3.2.1 Treatment conditions and mutagenesis

The study used the bread wheat genotype LM43, initially obtained from the International Maize and Wheat Improvement Centre (CIMMYT). A description of the variety is presented in Table 3.1. The genotype was selected from three genotypes based on its desirable phenotypic variation and performance after EMS mutagenesis in a preliminary study (OlaOlorun *et al.,* 2019). Three treatment combinations were established from preliminary experiments, based on LD_{50} tests and survival rate, which caused little or minimal biological damage at the seedling growth stage. Mutagenesis was carried out in a biocontrol laboratory in the Plant Pathology Department of the University of KwaZulu-Natal. Labelled mesh bags containing seeds were subjected to 0.1% v/v for 1 hour at 25°C (Treatment 1), 0.1% v/v for 1 hour at 30°C (Treatment 2) and 0.7% v/v for 1.5 hours at 25°C (Treatment 3). The procedure followed was detailed in OlaOlorun *et al.* (2019). For each treatment, 1200 healthy and equal-sized seeds were selected and placed separately in a specially designed and labelled mesh bag. Codes were assigned to treatment combinations for ease of labelling of mesh bags and identification purpose (Table 3.1). After mutagenesis, seeds were immediately planted out in the field to avoid seed damage and limit undesirable mutagenesis post-treatment.

Table 3.3: Ethyl methanesulphonate treatment combinations, their assigned codes and pedigree for the wheat genotype LM43 used in this study

Treatment Code	EMS Dose (% v/v)	Duration (Hour)	Temperature (°C)
Treatment 1	0.1	1	25
Treatment 2	0.1	1	30
Treatment 3	0.7	1.5	25
Control	0	24	25
LM43 Pedigree	ROLF07*2/6/PVN//CAR422/ANA/5/BOW/CROW//BUC/PVN/3/YR/4/TRAP#1		

3.2.2 Study location, field arrangement and trial set-up

Two experiments, one with the first mutation generation (M_1), and the other with the second mutation generation (M_2), were conducted from April to August 2018 and from October 2018 to January 2019, respectively. The experiments were conducted under field conditions at the Ukulinga Research Farm of the University of KwaZulu-Natal (latitude 29.67, longitude 30.41, 811 m above sea level). The total rainfall and mean temperature during the M_1 experiment were 193 mm and 16°C, respectively. For the second experiment (M_2 experiment), the total rainfall and mean temperature were 179 mm and 20°C, respectively.

For the M_1 generation experiment, seeds from all treatments were planted in the field using a randomized complete block design with two replications. The plot size was 31m by 8.6m and each replicate comprised of 12 rows. Each row represented a treatment maintaining an intra- and inter-row spacing of 10cm and 60cm, respectively. Three seeds were planted per station. Other cultural and plant protection practices were carried out as recommended in the South Africa standard guidelines for wheat production (DAFF, 2010). The M_1 plants were grown to maturity and M_2 seeds were harvested and bulked for each treatment. 2500 M_2 seeds of each treatment were then planted following the same design and field arrangement that was used for the M_1 experiment.

3.2.3 Data collection

Data on agronomic traits were collected during the growing period and at maturity. The percentage germination (%G) was determined two weeks after planting as a proportion of germinated seeds to the total number of seeds planted. Days to heading (DTH) were recorded as the number of days between sowing and when 50% of the spikes in each row were fully emerged from the flag leaf. Days to maturity (DTM) were calculated from the planting date to physiological maturity when 90% of the plants in a row showed senescence. The number of tillers (TN) and productive tillers (PTN) in each row were counted at physiological maturity, while plant height (PH) was measured in centimeters from the base of the primary tiller to the tip of the spike. The length of the spike (SL) was measured in centimetres from base to the tip while spikelets per spike (SPS) and kernels per spike (KPS) were counted from spikes harvested from 10 randomly selected primary tillers in each row. The thousand seed weight (TSW), expressed in grams, was determined by weighing 1000 randomly selected seeds on a digital laboratory precision balance (Kern & Sohn, PLJ 3000-2FM, Germany). Grain yield (GY) was estimated as the mean weight (grams) of grains harvested. Above ground biomass (AGB) was estimated as the mean weight of plant biomass cut at the soil surface and dried in an oven with forced air circulation at 65°C for 72 hours. Tiller number, PTN, PH and AGB were recorded on single plant basis by randomly tagging 25 plants from each row. Viable and non-viable mutants were identified and counted as mature plants with or without spikes, respectively. Complete sterility was observed as spikes bearing barren spikelets, while partial sterility was observed when spikes contained a mixture of barren and fertile spikelets.

3.2.4 Data analysis

The data was subjected to the analysis of variance (ANOVA) procedure, and descriptive statistics were computed for each generation and treatment using GenStat 18th edition (Payne *et al*., 2017). Lethality, mutation frequency (M Freq), effectiveness (ME) and efficiency (Me) were estimated using the following formulae (Konzak *et al*. 1965): $Lethality = 100 - \%G$

$$M\ Freq = \frac{NOM}{NPO} \times 100$$

$$ME = \frac{M\ Freq}{EMS\ Conc * Temp * Time}$$

$$Me = \frac{M\ Freq}{Lethality} \times 100$$

where, %G: germination percentage, NOM: number of observed mutants, NPO= number of plants observed, Conc= mutagen concentration, Temp= temperature, Time= exposure period, M Freq= mutation frequency, ME= mutation effectiveness and Me= mutation efficiency.

3.3 Results

3.3.1 Analysis of variance of agro-morphological traits observed in the M_1 and M_2 generations

The ANOVA revealed that the different EMS treatments had significantly different ($p<0.01$) effects on grain yield (Table 3.2). The treatment effects showed a cumulative impact on DTH, SL and TSW in the second generation as exhibited by the significant treatment × generation interaction (Table 3.2). The M_1 and M_2 generations exhibited significant ($p<0.05$) differences in all traits except AGB.

3.3.2 Effects of EMS on agronomic traits of wheat at M_1 and M_2 generations

The exposure of wheat to EMS treatments induced significant ($P< 0.05$) variation in DTM, TN, PTN, SPS, KPS and GY of individuals at M_1 generation (Table 3.3). The M_1 generation of plants under Treatment 1 had significantly ($p< 0.05$) higher PTN and KPS compared to those under Treatments 2 and 3. In comparison, M_1 generation plants subjected to Treatment 2 had the highest number of SPS (24.73) and AGB (330.28 g/25 plants), while Treatment 3 induced the mutants to flower and mature earlier (82 and 121 days, respectively) than plants after the other treatments. However, the M_1 plants of the control treatment exhibited higher means for TN, PH and TSW. EMS treatments had non-significant effects on %G, DTH, PH, SL, TSW and GY (Table 3.3).

Table 3.2: Mean square values and significant tests for agronomic traits of wheat subjected to different EMS treatments in the M_1 and M_2 generations

Source of Variation	df	Traits											
		%G	DTH	DTM	TN	PTN	PH	SL	SPS	KPS	TSW	AGB	GY
Replication	1	6.33	6.25	58.14	6.67	4.84	2.51	0.36	0.06	10.90	4.64	712.00	39.49
Treatment (T)	3	366.49	4.19	9.39	2.06	1.41	18.70	0.06	1.97	69.77	11.48	31156.00**	17.19
Generation (G)	1	641.86**	2475.06***	5058.77***	17.37*	48.86***	617.71***	34.65***	93.34***	599.09**	1123.42***	1349.00	176.13*
T X G	3	512.92	12.35*	3.02	2.36	0.43	21.05	0.37*	1.95	100.66	47.02*	42.00	22.82
Error	7	379.74	2.79	28.78	2.32	0.95	16.81	0.09	1.03	36.51	7.91	2961.00	26.10
LSD (0.05)		12.32	2.79	8.97	2.55	1.63	6.86	0.49	1.69	10.10	4.70	90.98	8.54

df: degree of freedom, %G: percentage germination, DTH: days to 50% heading, DTM: days to 90% maturity, TN: tiller number, PTN: productive tiller number, PH: plant height, SL: spike length, SPS: number of spikelets per spike, KPS: number of kernels per spike, TSW: 1000-seed weight, AGB: above ground biomass, GY: grain yield, LSD: least significant difference ($p< 0.05$), * significant at P≤0.05 probability level; ** significant at P≤0.01 probability level, *** significant at P≤ 0.001 probability level

Table 3.3: Means of agronomic trait of wheat subjected to different EMS treatments and their control in the M_1 generation

Treatments	Traits											
	%G	DTH	DTM	TN	PTN	PH	SL	SPS	KPS	TSW	AGB	GY
Treatment 1	94.42	86.25	123.00 [b]	10.50 [ab]	7.25 [b]	99.84	13.96	22.65 [ab]	67.77 [b]	54.15	311.86 [b]	41.95
Treatment 2	94.95	87.25	123.00 [b]	9.75 [ab]	6.00 [a]	96.23	13.85	24.73 [b]	57.99 [ab]	63.14	330.28 [b]	43.49
Treatment 3	93.95 [a]	82.50	121.00 [a]	9.50 [a]	6.75 [ab]	101.55	13.55	21.50 [a]	56.76 [ab]	63.56	318.51 [b]	42.60
Control	91.68	86.50	121.80 [ab]	11.00 [b]	7.25 [b]	102.43	13.20	22.39 [ab]	51.96 [a]	65.59	246.68 [a]	43.37
GM	93.75	85.62	122.20	10.19	6.81	100.01	13.64	22.82	58.62	61.61	301.83	42.85
CV (%)	4.86	3.62	0.93	9.00	9.86	8.61	3.54	7.97	15.58	13.62	28.82	17.91
LSD (0.05)	7.29	5.00	1.81	1.47	1.08	13.77	0.77	2.91	14.61	13.42	127.6	12.28

%G: percentage germination, DTH: days to 50% heading, DTM: days to 90% maturity, TN: tiller number, PTN: productive tiller number, PH: plant height, SL: spike length, SPS: number of spikelets per spike, KPS: number of kernels per spike, TSW: 1000-seed weight, AGB: above ground biomass, GY: grain yield, GM: grand mean, CV: coefficient of variation, LSD: least significant difference ($p< 0.05$)

Likewise, in the M_2 generation, EMS treatments induced significantly ($p< 0.05$) higher mean values in DTM, SL, KPS and AGB than the control treatment (Table 3.4). Means for TN, PTN and TSW were significantly higher in M_2 plants exposed to Treatment 1 than plants exposed to the other treatments. In addition, days to heading were significantly less after Treatment 1 than after Treatments 2 and 3 for M_2 plants. Mutants of the M_2 generation had significantly higher mean values for GY (38.96 g/25 plants) after Treatment 2, while M_2 plants subjected to Treatment 3 had the lowest number of days to maturity. M_2 plants exposed to Treatment 3 recorded significantly higher mean values for SPS (18.34), KPS (54.83) and AGB (309.12 g), while means for %G, PH and SL were significantly higher in the control plants.

A comparison of the EMS effects on wheat plants for both generations showed a higher means for all other agronomic traits studied except for TN and PTN in the M_1 generation (Table 3.5).The lower mean values for DTH, DTM and PH, and higher mean values for TN and PTN in the M_2 generation, are desirable for drought escape and reduced plant height.

3.3.3 Mutagenic frequency, efficiency, and effectiveness of EMS in wheat in the M_2 generation

The EMS treatments resulted in variable responses in mutation frequency, lethality, mutation effectiveness and mutation efficiency (Tables 3.4 and 3.6). The maximum mutation frequency of 3.22% was obtained from Treatment 2 in the M_2 population, while the minimum mutation frequency was observed under Treatment 3 (1.48%). The same trend was observed for mutation effectiveness. Treatments 1 and 3 had higher lethality (31.8% and 24.48% respectively), being less efficient in the M_2 generation, with the same efficiency rate of 6%, while Treatment 2 was the most efficient (21%) in inducing mutagenesis with minimal lethality (15.48%) (Table 3.6).

Table 3.4: Means of agronomic trait of wheat subjected to different EMS treatments and their control in the M_2 generation

Treatments	Traits											
	%G	DTH	DTM	TN	PTN	PH	SL	SPS	KPS	TSW	AGB	GY
Treatment 1	68.20	58.00	86.00 [ab]	14.06	11.22	83.71	10.75 [ab]	17.77	41.77 [a]	47.29	293.97 [b]	38.62
Treatment 2	84.52	61.00	89.50 [c]	11.56	9.59	89.76	10.30 [a]	18.15	47.91 [b]	44.28	306.24 [b]	38.96
Treatment 3	75.52	62.50	84.00 [a]	12.42	10.64	85.62	10.69 [ab]	18.34	54.83 [c]	44.91	309.12 [b]	37.63
Control	96.08	61.50	87.00 [b]	11.05	9.79	91.25	11.06 [b]	17.68	41.02 [a]	42.92	224.57 [a]	29.65
GM	81.08	60.75	86.62	12.27	10.31	87.59	10.70	17.99	46.38	44.85	283.48	36.22
CV (%)	12.99	2.51	0.78	10.30	11.17	5.16	1.76	3.49	3.24	4.05	11.76	9.14
LSD (0.05)	33.51	4.86	2.16	4.02	3.66	14.37	0.60	2.00	4.79	5.78	96.76	10.54

%G: percentage germination, DTH: days to 50% heading, DTM: days to 90% maturity, TN: tiller number, PTN: productive tiller number, PH: plant height, SL: spike length, SPS: number of spikelets per spike, KPS: number of kernels per spike, TSW: 1000-seed weight, AGB: above ground biomass, GY: grain yield, GM: grand mean, CV: coefficient of variation, LSD: least significant difference ($p< 0.05$)

Table 3.5: Comparison of trait means of wheat treated with EMS in the M_1 and M_2 generations

Generations	Traits											
	%G	DTH	DTM	TN	PTN	PH	SL	SPS	KPS	TSW	AGB	GY
M_1	94.44	85.33	122.33	9.92	6.67	99.21	13.79	22.96	60.84	60.28	320.22	42.68
M_2	76.08	60.50	86.50	12.68	10.48	86.36	10.58	18.09	48.17	45.49	303.11	38.40

%G: percentage germination, DTH: days to 50% heading, DTM: days to 90% maturity, TN: tiller number, PTN: productive tiller number, PH: plant height, SL: spike length, SPS: number of spikelets per spike, KPS: number of kernels per spike, TSW: 1000-seed weight, AGB: above ground biomass, GY: grain yield, M_1: first mutation generation, M_2: second mutation generation

Table 3.6: Mutagenic frequency, effectiveness, and efficiency of EMS treatment on wheat in the M_2 generation

Treatments	NPO	Observed Mutants			M Freq (%)	ME	Lethality (%)	Me (%)
		NSS	NSG	NPS				
Treatment 1	1705	15	14	3	1.88	0.75	31.80	6.00
Treatment 2	2113	40	28	0	3.22	1.07	15.48	21.00
Treatment 3	1888	13	14	1	1.48	0.06	24.48	6.00

NPO= number of plants observed, NSS= number of plants with seedless spike (sterility), NSG= number of plants with stunted growth, NPS= number of plants with shattering spikes, M Freq= mutation frequency, ME= mutation effectiveness, Me= mutation efficiency

3.3.4 Identification of morphological variations in the M_2 generation

Several morphological mutations were observed in the plants in the M_2 generation (Figure 3.1). Plants in the control treatment plots (Figure 3.1A) developed normal spikes and spikelets, compared to closely packed spikelets (Figure 3.1B-C), sparsely arranged spikelets (Figure 3.1E, H-J) and deformed plants (Figure 3.1D, L) obtained from plants subjected to EMS treatment. There were 68 mutants that were identified to be either partial or completely sterile, exhibiting deformed spikelets (Figure 3.1F-M). Mutation also resulted in variations in spike and peduncle morphology, such as wrinkling or leafy spikes, and the absence or shortening of peduncles (Figure 3.1D, H-O). Shattering was also observed in some of the mutants (Figure 3.1H-K). Figure 3.1P and 3.1Q showed variation in awn morphology, while flag leaf variations resulting from mutagenesis are illustrated in Figure 3.1E-I and Figure 3.1L and 3.1N.

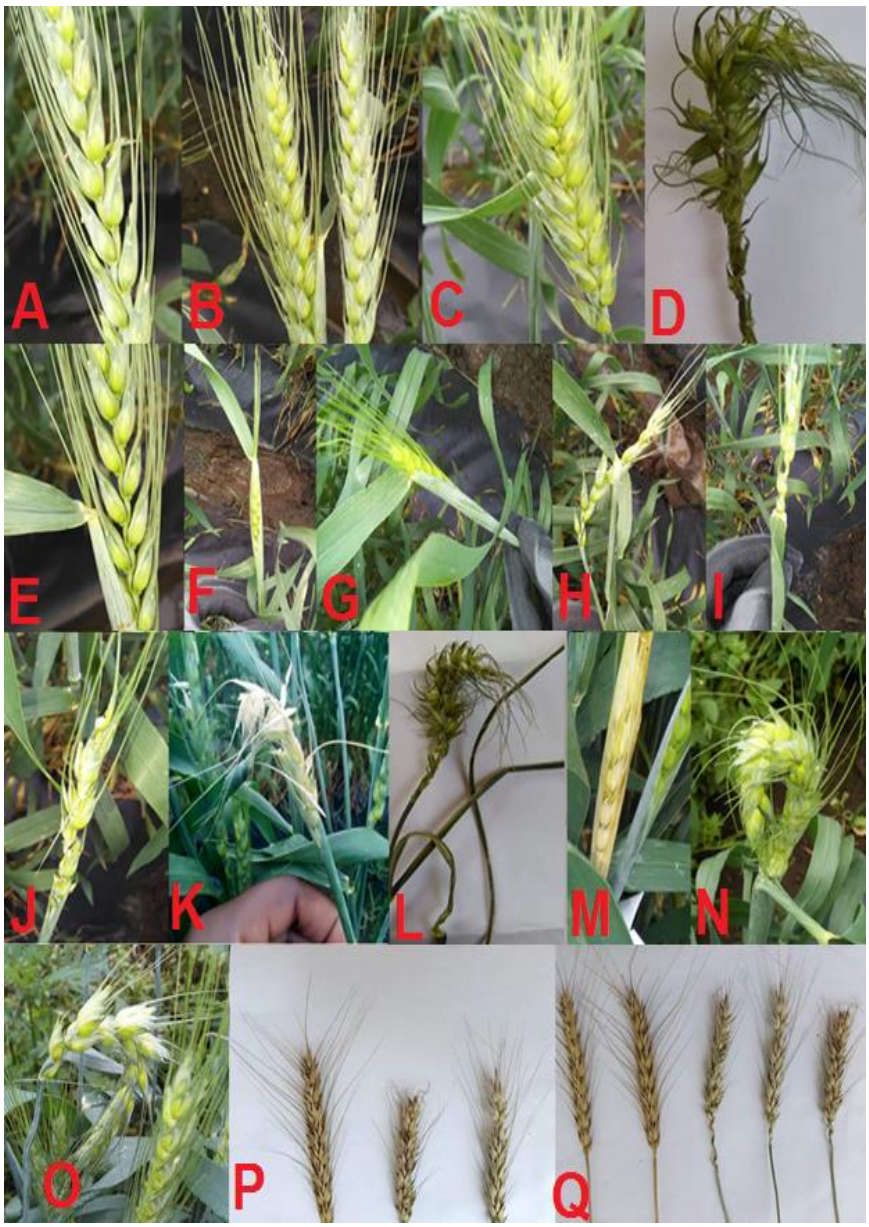

Figure 3.1: Morphological variations of bread wheat genotype LM43 in the M_2 generation: Control (A), spikelet arrangement on the spike (B-E), stunted growth (D, L, M), absence of peduncle (M-N), complete spikelet sterility (D, G, L, M), partial spikelet sterility (N, O), seed shattering (J, K), wavy peduncle (O, Q), awn appearance on spikelet (D, J, K, N, P, Q), bending spike (N, O), appearance of spike from flag leaf (E-I, M-N) and variable spike length (P-Q)

3.4 Discussion

3.4.1 Variations in agro-morphological traits in the M_1 and M_2 generations

The ANOVA revealed that the treatment by generation interaction effects were significant for DTH, SL and TSW, showing that variations in such traits may be noticeable in some generations but not others. The lack of significant variation due to EMS effects for some traits could have been due to the low dosage, or because the changes were not noticeable in the first generation. Roychowdhury and Tah (2013) noted that non-significant variations in some traits occur in the early mutant generations, especially the M_1 generation because gene mutations are generally in their heterozygote state and recessive allele are not expressed. In addition, identifying plants with maximum genetic damage are likely to occur with high frequency of micro mutations in M_2 and M_3 generations (Wani, 2009). It is therefore recommended that further studies on evaluation and mutant screening should be carried out in subsequent segregating generations. The significant differences in trait performance after mutagenesis between generations allows for selection of high performing mutants from each generation.

3.4.2 Mean agronomic performance of individuals exposed to EMS

The agronomic performance and morphology of plants generated from wheat seeds subjected to EMS treatment showed the potential of mutagenesis to create variation in quantitative traits (Tables 3.3 and 3.4). The significant differences in DTM, SL, PTN, TN, SPS, KPS and AGB between treated and untreated seeds showed that EMS contributed significantly to variable agronomic performance. Sakin and Sencar (2002) also observed significant variation in the agronomic traits of wheat exposed to EMS and concluded that mutagenesis creates variation. Similarly, Sakin and Yildirim (2004) found that EMS increased variation in grain yield of durum wheat. These variations, attributing to random mutations, could be useful in wheat breeding programmes, and would assist in circumventing the challenges encountered during hand emasculation of crop species such as wheat that are inherently adapted to self-pollination, which limits the production of novel gene combinations.

In the M_2 generation, the reduction in %G under EMS treatments may have been due to the disruption of physiological and biological processes necessary for germination (Srivastava *et al.,* 2011). These processes include enzyme activities,

hormonal balance and mitotic processes. Sakin and Sencar (2002) also observed that an increase in the EMS treatment temperature resulted in improved germination rates for wheat seeds treated with EMS. Temperature is an important factor of biological processes and enzymes responsible for catalyzing most biological processes in plants have optimal range around 20-25°C (Somero, 1978). The germination would be expected to be higher under Treatment 1, but the interactive effect of temperature and EMS dosage may have caused a reduction in germination potential.

The significant treatment by generation interaction effects for DTH, SL and TSW implies that EMS treatments had variable effects the two generations studied (Table 3.2). The high levels of phenotypic variation observed in M_2 plants compared to M_1 plants corroborated with the findings by Srivastava *et al.* (2011), who found that mutants for several quantitative traits could only be identified in the M_2 generation. Differences in agronomic performances observed between the generations would be due to the increasing variations found in the M_2 generation caused by gene segregation and the cumulative effects of the mutagen. Gregory (1956) explained that the variations observed in subsequent generations were cumulative and that they were a combination of genetic and mutagenic effects. The better mean response of yield-related traits observed among the treated population was an indication of the potential of mutagenesis to create genetic variation for agronomic traits, yield and yield components. However, yield itself did not change in either the M_1 or M_2 generations. In mutation breeding, mutant plants with desirable characteristics can be selected for breeding in yield improvement programmes. They can also be used for genetic analyses to identify important quantitative trait loci.

3.4.3 Mutation frequency, effectiveness, and efficiency

The EMS treatments caused variable responses in frequency, effectiveness, efficiency and lethality of mutations, showing that there was no definite relationship between these variables and the dose of EMS, possibly because mutagenesis is affected by several factors such as temperature, duration of exposure and their interactions. The lack of a definite dose-dependent relationship of lethality, mutation frequency, effectiveness and efficiency has been attributed to variable genetic changes after a mutation (Aliyu *et al.,* 2017). The biological impact of any mutagen depends on the nature of the resultant mutation, and the efficiency and accuracy

with which they are repaired (Britt, 1996). The genetic changes in the DNA may be repaired, depending on the magnitude and location of the change, with smaller mutations being more easily repaired than larger ones (Manova and Gruszka, 2015). Thus, the DNA damage caused by mutagenesis can be repaired limiting the mutations to non-observable levels. In such cases, it could either be that the combination of the dose of the mutagen, temperature and exposure time was not appropriate, and did not induce irreparable mutations.

The mutagenic effect of each treatment on wheat seeds resulted in varying mutation frequencies, with the mutant population from Treatment 2 recording the highest mutation effectiveness and mutation efficiency. Similarly, Treatment 2 produced the highest number of segregants during the second generation, creating the widest phenotypic variation, with the least biological damage. Chemical mutagenesis induces a spectrum of genetic variations in plants (Lasker and Khan, 2017), which can be used for crop improvement, provided that the mutagen does not inflict irreparable and undesirable biological damage. Biological damage or lethality can result from deleterious mutations, or a failure to repair critical segments of the damaged DNA (Golubov *et al.,* 2010).

3.4.4 Morphological abnormalities induced by EMS mutagenesis

Several abnormalities in spike, peduncle, awn and flag leaf morphology were identified, indicating that a number of macro mutations occurred during mutagenesis. Macro-mutations are known to cause significant changes in the morphology of plants (Waghmare *et al.,* 2001; Ramadoss *et al.,* 2014). The process of DNA transcription is prone to error, which means that every individual gene responsible for a quantitative trait can potentially mutate, giving rise to a wide spectrum of viable morphological mutants, as expected in mutation experiments (Manova and Gruszka, 2015; Raina *et al*., 2017). However, in this study few plants were observed with useful variations in spike length and spikelet morphology, indicating the low efficiency of the EMS treatments used. Viable mutants possessing longer spikes, bigger seeds and closely packed spikelets were selected because they would be expected to possess higher KPS and TSW, which are critical components to improve grain yield. Similarly, Ramadoss *et al.* (2014), Eze and Dambo (2015) and Nazarenko (2018) obtained viable sesame, maize and wheat mutants, respectively, with a higher number of seeds after exposure to mutagens.

3.5 Conclusion

EMS mutagenesis induced genetic variation in agronomic traits of wheat such as TN, SPS, PTN, KPS, TSW, GY and AGB compared to the untreated plants. These variations could be exploited to improve a wide range of traits in wheat. EMS treatment with 0.1% v/v for 1 hour at 30°C was the most efficient and effective treatment combination for inducing desirable changes in %G, PH, AGB and GY. Phenotypic expression of genetic variations due to mutagenesis increased in the M_2 generation and would be expected to increase in subsequent generations due to the cumulative mutagenic effect and further genetic recombination. Therefore, the selection of the identified mutants with desirable characteristics could be useful in wheat improvement and genetic studies for quantitative trait loci identification. The results obtained in this study are specific to genotype LM43 but could be useful as a guide for other genotypes. It would be expected that EMS mutagenesis will cause genetic variation in other genotypes with the only differences being in the magnitude and direction of the change dependent on the test genotype.

3.6 References

Adekola OF, Oluleye F (2007) Influence of mutation induction on the chemical composition *Vigna unguiculata* (L.) Walp. Afr J Biotechnol 6:2-4.

Ahloowalia BS, Maluszynski M, Nichterlein K (2004) Global impact of mutation derived varieties. Euphytica 135:187-204.

Aliyu RE, Aliyu A, Adamu AK (2017) Inducing phenotypic variants in sesame (*Sesamum indicum* L.) with fast neutron irradiation. J Res Forest Wild Envt 9(1):1-9.

Ambavane AR, Sawardekar SV, Sawantdesai SA, Gokhale NB (2015) Studies on mutagenic effectiveness and efficiency of gamma rays and its effect on quantitative traits in finger millet (*Eleusine coracana* L. Gaertn). J Radiat Res Appl Sci 8(1):120-125.

Anbarasan K, Sivalingam HD, Rajendran R, Anbazhagan M, Chidambaram AlA (2013) Studies on the mutagenic effect of EMS on seed germination and seedling characters of sesame (*Sesamum indicum* L.) Var.T MV3. Int J Res Bio Sci 3(1):68-70.

Bansal V, Katoch PC, Plaha P (1990) Mutagenic effectiveness of gamma rays, ethyl methane sulphonate and their combined treatments in rice. Crop improv 17(1):73-75

Britt AB (1996) DNA Damage and Repair in Plants. Annu Rev Plant Physiol Plant Mol Biol 47:75–100. doi: 10.1146/annurev.arplant.47.1.75

Cowling WA (2013) Sustainable plant breeding. Plant Breed 132:1-9.

DAFF (Department of Agriculture, Forestry and Fisheries) (2010) Wheat production guideline. DAFF, Pretoria.

Dhaliwal AK, Mohan A, Sidhu G, Maqbool R, Gill KS (2015) An ethylmethane sulfonate mutant resource in Pre-Green revolution hexaploid wheat. PLoS ONE 10(12):1-12. e0145227. doi: 10.1371/journal.pone.0145227

Espina MJ, Ahmed CMS, Bernardini A, Adeleke E, Yadegari Z, Arelli P, Pantalone V, Taheri A (2018) Development and phenotypic screening of an ethyl methane sulfonate mutant population in soybean. Front Plant Sci 9:394. doi: 10.3389/fpls.2018.00394

Esterhuizen D (2018) Grain and Feed Annual: focus on the supply and demand for grain and feed in South Africa. United States Department of Agriculture Grain Report, pp 1-17

Eze JJ, Dambo A (2015) Mutagenic effects of sodium azide on the quality of maize seeds. J Adv Lab Res Biol 5(3):76-82

FAO (Food and Agricultural Organization) (2010) IAEA Plant Mutation Reports. Joint Division of the Food and Agriculture Organization of the United Nations and the International Atomic Energy Agency (Joint FAO/IAEA). 2(2):1-60. ISSN 1011-260X.

Girija M, Dhanavel D, Gnanamurthy S (2013) Gamma rays and EMS induced flower colour and seed mutants in cowpea (*Vigna unguiculata* L. Walp). Adv Appl Sci Res 4(2):134-139.

Gregory WC (1956) The comparative effects of radiation and hybridization in plant breeding. International Conference for Peaceful Uses of Atomic Energy. Proceedings. 12:48-51

Golubov A, Yao Y, Maheshwari P, Bilichak A, Boyko A, Belzile F (2010) Microsatellite instability in Arabidopsis increases with plant development. Plant Physiol 154:1415-1427. doi: 10.1104/pp.110.162933

IAEA (International Atomic Energy Agency) (2015) IAEA mutant database. IAEA, Vienna. http://mvd.iaea.org/ (Accessed July 2015).

IAEA (International Atomic Energy Agency) (2019) IAEA mutant database. IAEA, Vienna. http://mvd.iaea.org/ (Accessed May 2019).

Jain SM (2005) Major mutation-assisted plant breeding programs supported by FAO/ IAEA. Plant Cell Tiss Org 82:113-123.

Jain SM (2010) Mutagenesis in crop improvement under the climate change. Rom Biotech Lett 15(2):88-106.

Kharkwal MC (1998) Induced mutations in chickpea (*Cicer arietinum* L.). I. Comparative mutagenic effectiveness and efficiency of physical and chemical mutagens. Indian J Genet Pl Br 58:159-167

Kharkwal MC, Shu QY (2009) The role of induced mutations in world food security. In: Shu QY (ed) Induced plant mutations in the genomics era. Food and Agriculture Organization of the United Nations. Rome, Italy, pp 33-38.

Kodym A, Afza R (2003) Physical and chemical mutagenesis. In: Grotewold E (ed) Methods in molecular biology. Plant functional genomics: methods and protocols. 236: 189-204. doi: 10.1385/1-59259-413-1:189

Konzak CF, Nilan RA, Wagner J, Faster RJ (1965) Efficient chemical mutagenesis. In: Use of induced mutations in plant breeding. Radiat Bot 5:49-70

Kumar PRR, Ratnam SV (2010) Mutagenic effectiveness and efficiency in varieties of sunflower (*Helianthus annuus* L.) by separate and combined treatment with gamma-rays and sodium azide. Afr J Biotechnol 9(39):6517-6521.

Lasker RA, Khan S (2017) Mutagenic effectiveness and efficiency of gamma rays and HZ with phenotyping of induced mutations in lentil cultivars. Int Lett Nat Sci 64:17-31. doi: 10.18052/www.scipress.com/ILNS.64.17

Maan SS, Williams ND (1984) An EMS induced dominant allele for male sterility transferred to euplasmic wheat. Crop Sci 24:851-852.

Manova V, Gruszka D (2015) DNA damage and repair in plants– From models to crops. Front Plant Sci 6:885.

Mathew I, Shimelis H, Mutema M, Clulow A, Zengeni R, Mbava N, Chaplot V (2019) Selection of wheat genotypes for biomass allocation to improve drought tolerance and carbon sequestration into soils. J Agron Crop Sci 00:1-16. doi: 10.1111/jac.12332

Nazarenko M (2018) Optimal doses and concentrations of mutagens for winter wheat breeding purposes. In: Nazarenko M, Lykholat Y, Grigoryuk I, Khromykh N (eds) Part I. Grain productivity. J Cent Eur Agric 19(1):194–205. doi: /10.5513/JCEA01/19.1.2037

Novak FJ, Brunner H (1992) Plant breeding: induced mutation technology for crop improvement. IAEA Bulletin. 4:25-32.

Nurmansyah, Alghamdi SS, Migdadi HM, Farooq M (2018) Morphological and chromosomal abnormalities in gamma radiation-induced mutagenized faba bean genotypes. Int J Radiat Biol 94:(2):174-185. doi: 10.1080/09553002.2018.1409913

OlaOlorun BM, Shimelis H, Matthew I, Laing M (2019) Optimizing the dosage of ethyl methanesulphonate mutagenesis in selected wheat genotypes. S Afr J Plant Soil. doi: 10.1080/02571862.2019.1610808

Pathirana R (2011) Plant mutation breeding in agriculture. CAB Rev: Persp Agri Vet Sci Nutrit Nat Res 6:032.

Payne R, Murray D, Harding S (2017) An introduction to the Genstat command language (18th Edition). VSN International Ltd, Hemel, Hempstead, UK, pp 1-137.

Porbeni JBO, OlaOlorun BM, Olalekun OJ, Oyetunde OA (2014) Agro-morphological effect of ethidium bromide on *Vigna unguiculata* (L.) Walp. Cv Ife Brown. Res J Agri 1(5):1-10.

Raina A, Laskar RA, Khursheed S, Khan S, Parveen K, Amin R, Khan S (2017) Induced physical and chemical mutagenesis for improvement of yield attributing traits and their correlation analysis in chickpea. Int Lett Nat Sci 61:14-22. doi: 10.18052/www.scipress.com/ILNS.61.14

Ramadoss BR, Ganesamurthy K, Angappan K, Gunasekaran M (2014) Evaluation of effect of gamma rays on sesame genotype TTVS 51 and TTVS 19 in M_1 generation. Int J Dev Res 4(2):273-277.

Rizwan M, Akhtar S, Aslam M, Asghar MJ (2015) Development of herbicide resistant crops through induced mutations. Adv Life Sci 3(1):01-08.

Roychowdhury R, Tah J (2013) Mutagenesis: a potential approach for crop improvement. In: Hakeem KR, Ahmad P, Ozturk M (eds) Crop improvement: new approaches and modern techniques. Springer, New York, pp 149-187.

Sakin MA, Sencar O (2002) The effects of different doses of gamma ray and EMS on formation of chlorophyll mutations in durum wheat (*Triticum durum* Desf.). J Agric Sci 8(1):15-21.

Sakin MA, Yildirim A (2004) Induced mutations for yield and its components in durum wheat (*Triticum durum* Desf.). J Food Agric Environ 2(1):285-290.

Shivakumar M, Nataraj V, Kumawat G, Rajesh V, Chandra S, Gupta S, Bhatia VS (2018) Speed breeding for Indian Agriculture: a rapid method for development of new crop varieties. Curr Sci 115(7):1241.

Siddiqui S, Meghvansi MK, Zia-ul H (2007) Cytogenetic changes induced by sodium azide (NaN_3) on *Trigonella foenum*-graecum seeds. S Afr J Bot 73:632-635.

Singh R, Tiwari R, Sharma D, Tiwari V, Sharma I (2014) Mutagenesis for wheat improvement in the genomics era. J Wheat Res 6(2):120-125.

Solanki IS, Sharma B (1994) Mutagenic effectiveness and efficiency of gamma rays, ethyl imine and N-nitroso-N-ethyl urea in macrosperma lentil (*Lens culinaris* Medik.). Indian J Genet Pl Br 54:72-76.

Somero GN (1978) Temperature adaptation of enzymes: biological optimization through structure-function compromises. Ann Rev Ecol Syst 9(1):1-29.

Srivastava P, Marker S, Pandey P, Tiwari DK (2011) Mutagenic effects of sodium azide on the growth and yield characteristics in wheat (*Triticum aestivum* L. em. Thell.). Asian J Plant Sci 10(3):190-201. doi: 10.3923/ajps.2011.190.201

Talebi AB, Talebi AB, Shahrokhifa B (2012) Ethyl methanesulphonate (EMS) induced mutagenesis in Malaysian rice (cv. MR219) for lethal dose determination. Am J Plant Sci 3:1661-1665.

United Nations, Department of Economic and Social Affairs, Population Division (2017) World Population Prospects: The 2017 Revision, Key Findings and Advance Tables. Working Paper No. ESA/P/WP/248. (Accessed May 2019). https://esa.un.org/unpd/wpp/Publications/Files/WPP2017_KeyFindings.pdf

Voss-Fels K, Frisch M, Qian L, Kontowski S, Friedt W, Gottwald S, Snowdon RJ (2015) Subgenomic diversity patterns caused by directional selection in bread wheat gene pools. Plant Genome 8(2). doi:10.3835/plantgenome2015.03.0013

Waghmare VN, Mehra RB (2001) Induced chlorophyll mutants, mutagenic effectiveness and efficiency in *Lathyrus sativus* L. Indian J Genet Pl Br 61(1):53-56.

Wani AA (2009) Mutagenic effectiveness and efficiency of gamma rays, ethyl methane sulphonate and their combination treatments in chickpea (*Cicer arietinum* L.). Asian J Plant Sci 8:318-321. doi: 10.3923/ajps.2009.318.321

Chapter 4

Variability and selection among mutant families of wheat for biomass allocation, yield and yield-related traits under drought-stressed and non-stressed conditions

Abstract

Genetic variation is fundamental for plant breeding programs. Exploiting the genetic variation of wheat for biomass allocation, yield and yield-related traits enhances breeding for drought tolerance. The aim of this study was to evaluate genetic variation and to select best individuals among 180 M_3 mutant families of wheat developed through EMS mutagenesis with superior biomass allocation, grain yield and agronomic performance evaluated in the controlled and field environments under non-stressed and drought-stressed conditions. Experiments were conducted using a randomized complete block design with two replications. Days to 50% heading (DTH), days to 90% maturity (DTM), plant height (PH), number of productive tillers (PTN), shoot biomass (SB), root biomass (RB), total biomass (TB), root-shoot ratio (RSR), spike length (SL), spikelet per spike (SPS), one thousand seed weight (TSW) and grain yield (GY) were collected. Mutant families showed significant genotypic ($p<0.05$) variation for yield and biomass traits while genotype × site × water regime interaction effects were significant ($p<0.05$) for DTM, SB, TB, TSW and GY. Superior families designated as 52, 159, 103, 126, 145 were selected for improved drought tolerance and high biomass allocation to roots. The selected families of wheat are recommended for genetic advancement and genetic analysis to identify genomic regions controlling biomass allocation and yield gains under drought stress.

Keywords: biomass allocation, drought stress, genetic variation, mutagenesis, root-to-shoot ratio, yield-related traits

4.1 Introduction

Bread wheat (*Triticum aestivum* L. 2n=6x=42, AABBDD) is among the most widely grown cereal crops serving various value chains in the world (Nhemachana and Kirsten, 2017). In 2017, wheat was produced on an estimated area of 218 million hectares with grain output of 772 million tons globally (FAO, 2018). About 30% of the world's population depends on wheat as a primary source of calories. Wheat provides up to 60% of proteins derived from cereals (Shewry and Hey, 2015; Khalil *et al*., 2019).

Despite its dietary and economic importance, wheat yields have stagnated or decreased significantly in southern Africa over the last 20 years (van der Merwe and Cloete, 2018). As a result, the region depends on wheat imports to fulfil domestic consumption requirements. Various constraints including poor soils, pests and diseases and climatic change-induced heat and drought stresses are among the major causes of low yields in sub-Sahara Africa (Rehman *et al*., 2009; Dube *et al*., 2016). Drought stress is the leading most important constraint of wheat production and productivity globally (Tambussi *et al.,* 2007). Wheat is sensitive to drought stress at all stages of growth although drought occurrence at booting, anthesis or grain-filling stages has significantly higher adverse impact on grain yield and quality (Shamuyarira *et al.,* 2019). It is imperative to develop drought tolerant cultivars for use as a part of an integrated suite of tools to reduce the impact of drought stress and other constraints on wheat yield and quality.

Genetic variation is fundamental for developing cultivars with enhanced tolerance to abiotic and biotic stresses. Genetic variation in agronomic traits such as flowering and maturity period, tillering capacity, kernel weight, spike morphology, plant height and grain yield has been targeted in drought tolerance breeding programs (Sallam *et al*., 2019). For instance, early flowering and maturity in wheat are widely targeted because they are strongly associated with higher terminal drought stress tolerance and drought escape. In some studies, genotypes possessing the height reducing genes (*Rht* genes), were selected to improve the ability of wheat to withstand prolonged moisture deficit (Grover *et al*., 2018). Consequently, strategies that allow simultaneous selection of multiple traits were developed and used to improve drought tolerance and increase grain yield in wheat. However, modern wheat germplasm has lost substantial genetic diversity in economic traits due to continuous

selection within a narrow range of elite lines (van de Wouw *et al*., 2010; Govindaraj *et al*., 2015). In addition, emphasis on selection of yield related traits such as high harvest indices have eroded genetic diversity in root traits, which has contributed to the poor rooting capacity and high susceptibility to moisture stress in most modern wheat cultivars (White *et al.,* 2015). There is a need to create genetic variation for economic traits including root traits to increase the prospects of developing drought tolerant cultivars.

Genetic variation in crop plants is created through sexual recombination during cross-pollination or mutation induction (Tadesse *et al*., 2012). Sexual recombination is important in creating new genetic variation and potentially improving selection response for yield and related traits. For instance, a 10-41% increase in yield potential has been reported in wheat due to heterosis that occurs after genetic recombination when divergent parental lines were crossed (Fu *et al*., 2014). However, the exploitation of heterosis is limited in inherently self-pollinating crops such as wheat. The highly cleistogamous nature of wheat requires emasculation to facilitate outcrossing with a suitable pollen donor to create recombinant genetic variation. The process of emasculation is tedious and limits the number of potential recombinants that can be generated, which curtails creation of new genetic variation for heterosis breeding. Several methods such as the application of gametocides have been used successfully to replace hand emasculation and pollination in wheat. The genetic variation created by sexual recombination is limited by the initial genetic divergence of the parental lines.

Genetic variation can be harnessed through mutation induction. Mutation is a change in the genetic constitution of an individual either naturally or by exposure to mutagens (Porbeni *et al*., 2014). Natural mutations occur randomly at relatively low frequencies and have limited use for breeding purposes. Induced mutation by exposure of plant parts e.g. seeds to mutagens such as ethyl methanesulphonate (EMS) leads higher frequencies of mutation events. This may be exploited to create useful genetic variation for breeding. Mutation breeding circumvents the need for emasculation in cleistogamous species such as wheat and can create genetic variation irrespective of the initial diversity in the parental population. The amount of genetic variation created by mutation breeding is not limited by the initial diversity in the base population but depends on the potency of the mutagen. However, mutation

breeding creates a large number of mutants that would require tedious and costly evaluation under different conditions to identify superior and stable mutants. The early generation selection approach often used in crop hybridization programs is recommended to reduce the cost and improve selection efficiency in mutation breeding (Luz *et al*., 2016; Abraha *et al*., 2017). Mutation breeding can be complemented with conventional breeding where superior mutants identified in early generation selection can serve as parental lines in crosses or for selfing to fix desirable traits (Singh *et al*., 2017). Mutation breeding provides an opportunity to widen genetic diversity in agronomic traits such as earliness to flowering and maturity, plant height and tillering capacity, which are traditionally targeted for breeding for drought tolerance, and biomass allocation to roots.

Biomass allocation pattern influences drought tolerance in wheat (Fang *et al*., 2017). Plants that invest significantly in root biomass increase their potential for water and nutrient absorption, which directly influence their growth potential (Wasaya *et al.,* 2018). The capacity to absorb moisture and nutrients is more important in drought prone environments, such as in sub-Sahara Africa where wheat is grown under residual moisture and nutrients from a preceding crop (Negassa *et al.,* 2013). Large root biomass is important in dryland farming conditions where crops have to explore large volumes of soil to extract enough moisture for growth (Tsuji *et al*., 2005; Palta *et al*., 2011; Ehdaie *et al*., 2012). However, the source-sink competition that exists between above and below ground parts might compromise yield production in genotypes with excessively large root systems (Zhu and Zhang, 2013; Fang *et al*., 2017). Mutation breeding could assist in creating new genetic variation for both above and below ground traits and also provide an opportunity to break unfavorable linkage drag between root traits and yield. Historically, root-related traits have largely been neglected during breeding programs because root phenotyping is difficult and the available methods for root assessment are inefficient (Den Herder *et al*., 2010; White *et al.,* 2015).

Assessing genetic diversity in above and below ground traits among mutant genotypes and evaluating trait associations will assist in devising appropriate strategies to develop improved wheat cultivars. Understanding trait associations enables indirect selection for optimal biomass allocation between above and below ground parts and superior agronomic performance for drought tolerance and high

grain yield production. Prior to this study, seeds of a wheat genotype selected for drought tolerance were subjected to mutagenesis and mutant individuals at the third generation were selected for this study. The mutants were grown with the objective to evaluate genetic variation in the third mutant generation, and to select families with superior biomass allocation, grain yield and agronomic performance evaluated in the controlled and field environments under non-stressed and drought-stressed conditions.

4.2 Materials and methods

4.2.1 Source of mutant families

Third mutation generation (M_3) seeds of a wheat genotype, LM43, were used in this study. Genotype LM43 was selected from three genotypes based on its desirable phenotypic variation and performance after EMS mutagenesis in a preliminary study (OlaOlorun *et al.,* 2019). Mutant genotypes were obtained by treating LM43 seeds with EMS under three different conditions. Previously, three conditions involving exposure of LM43 seeds to different dosages of EMS for different durations at different temperature regimes were evaluated for efficiency in inducing mutation with minimal biological damage. Three treatment conditions: i) exposure of seeds to 0.1% EMS for 1 hour at 25°C, ii) exposure of seeds to 0.1% EMS for 1 hour at 30°C and iii) exposure of seeds to 0.7% EMS for 1.5 hours at 25°C were found to be efficient and effective in inducing mutagenesis with minimal biological damage to LM43 seeds (OlaOlorun *et al.,* 2020). After exposure to each of the three conditions, seeds were planted in a field and subsequently harvested to raise the M_1 generation. Each generation was sequentially planted and harvested until the M_3 generation, which was used in this study. Under each set of the three treatment conditions, 60 mutant families were selected to give a total of 180 families used in this study. Each family was number coded in respect of the treatment conditions from which it was obtained. The first 60 families coded from 1 to 60 were obtained from seeds exposed to the first treatment conditions of 0.1% EMS for 1 hour at 25°C. The second set of families with number codes from 61 to 120 were obtained from seeds exposed to the second treatment conditions of 0.1% EMS for 1 hour at 30°C. Finally, the third set of families with number codes 121 to 180 were generated from seeds exposed to the third treatment conditions of 0.7% EMS for 1.5 hours at 25°C.

4.2.2 Study sites and trial management

The experiments were carried out under greenhouse and field conditions at the University of KwaZulu Natal (UKZN). Plants that were obtained from seeds treated under the different set of conditions were evaluated under two contrasting water regimes (well-watered and drought-stressed treatments). The greenhouse experiment was set up at the Controlled Environment Facility between February and July in 2019. The average day and night temperatures in the greenhouse were 26°C and 20°C, respectively, with a mean relative humidity of 75%. Ten seeds per family were sown in 10 litre plastic pots filled with composted pine bark growing media and thereafter, thinned to seven plants per family. The experiment was set up as a randomized complete block design with two replications. Drip irrigation was applied from emergence to the heading stage for all treatments. At the 50% heading, the drought treatment was imposed by reducing water supply from the dripper lines to maintain soil moisture at 30% field capacity while adequate water supply was maintained until maturity for plants subjected to the well-watered control treatment.

The field experiment was conducted at the Ukulinga Research Farm of the UKZN between March and August in 2019. The average temperature, relative humidity and total rainfall during the growing period were 18°C, 64% and 203 mm, respectively. The experiment was set up as a randomized complete block design with two replications. Ten seeds per family were planted on a 1.5 m long row with 10 cm between plants and 60 cm between the rows. Mechanical weeding was carried out when necessary and, pests and diseases were chemically controlled. The other agronomic practices were carried out following the South Africa guidelines for wheat production (DAFF, 2010). The plants were established under adequate moisture until the heading stage. The drought stress treatment was imposed by withholding irrigation when 50% of the plants reached anthesis. The moisture content in the drought treatment was maintained at 35% field capacity from the heading stage. The moisture content in the well-watered treatment was maintained at above 80% throughout the growing period. The moisture content was monitored by soil moisture meters inserted at strategic points in the field at 0.30 and 0.60m soil depths. A custom-made plastic mulch was placed to cover the soil surface and prevent entry of rainwater or surface runoff.

4.2.3 Data collection

The days to 50% heading (DTH) was recorded as the number of days from sowing date to the date when 50% of the plants in a row had fully emerged spikes while days to 90% maturity (DTM) was measured as the number of days from sowing to the date when 90% of the plants had reached senescence. Plant height (PH) was measured in centimetres from the base of the plant to the tip of the spike while the number of productive tillers per plant (PTN) was counted at physiological maturity. The shoot biomass (SB) was estimated as the weight of above ground biomass (including spikes) cut at the soil surface and while root biomass (RB) was the mean weight of below ground biomass. The roots were harvested following a method modified from Hirte *et al.* (2018). Root and shoots were separated at the soil surface and the roots were washed under running tap water to remove excess soil. The root and shoot biomass were oven-dried with forced air circulation at 60°C for 72 hours prior to weighing. The roots and shoots of five plants were used to estimate the biomass and were measured in grams. The total biomass (TB) and root to shoot ratio (RSR) were computed after weighing root and shoot biomass. The length of the spike (SL) was measured in centimetres from base to the tip of the spike while spikelets per spike (SPS) were counted from spikes harvested from five selected primary tillers in each row. One thousand seed weight (TSW) was expressed in grams and determined by weighing 1000 randomly selected seeds on a digital laboratory precision balance (Kern & Sohn, PLJ 3000-2FM, Germany). Grain yield (GY) was estimated as the mean weight (grams) of grains harvested from 5 plants selected from each row.

4.2.4 Data analyses

Data on phenotypic traits measured under the two testing sites and contrasting water regimes were subjected to a combined analysis of variance after testing for homogeneity of variance in GenStat 18th edition (Payne *et al*., 2017). Means were separated by the Fisher's Unprotected least significant difference (LSD) at 5%. Pearson's correlation coefficients were computed among traits under each treatment using the SPSS version 24 statistical software (IBM SPSS, 2016). The strength of the correlations were categorized into weak, moderate and strong following Zou *et al.* (2003). Principal component analysis (PCA) based on the correlation matrix was conducted to deduce multivariate associations among traits and families. The multivariate associations were depicted in PCA biplots using the first two principal

components axis for non-stressed and drought-stressed conditions separately using the R software version 3.6.3 (R Core Team 2020).

4.3 Results

4.3.1 Analysis of variance for phenotypic traits across sites and water regimes

A combined analysis of variance showed that the effects of genotype × site × water regime interaction were significant ($p<0.05$) for DTM, SB, TB, TSW and GY. The interaction effects of genotype × site was significant ($p<0.05$) for RB while the genotype × water regime effects were significant ($p<0.05$) for PTN, SPS and TSW (Table 4.1). Significant ($p< 0.05$) differences among genotypes were recorded for DTH, PH, PTN, SB, RB, TB, TSW and GY. The site main effects had highly significant ($p<0.001$) impact on all the measured traits except RB and GY. Significant differences ($p<0.05$) were observed between the water regimes for all the traits except DTH and TSW.

4.3.2 Mean performance of mutant families across water regimes

The mean performance for the top 10 and bottom 5 of the 180 M_3 wheat families and the untreated control are presented in Table 4.2. Water stress reduced the average number of days to maturity by 7.63% to 121 days. The mean response for biomass traits SB, RB and TB decreased by 5.48, 6.62 and 5.55%, respectively under water stressed conditions. The family designated as 52 produced the highest shoot biomass of 79g while family 79 recorded the lowest (27.5 g) under water stressed conditions. Families 101, 131 and 161 recorded the highest SB (above 100 g) under non-stress conditions. Among the top 10 families with high RB under non-stress conditions were families 101, 52 and 126 while families 32, 52 and 101 had the highest RB under water stressed conditions. The TB was highest for family 101 (146.9 g) under non-stress while families 52 and 103 recorded the highest (94 and 89.9 g, respectively) under water stressed conditions. The RSR increased by 13.04% from 0.23 under non-stressed conditions to 0.26 for water stressed conditions. Family 52 had the highest root to shoot ratio of 0.28 under non-stressed conditions while the highest RSR (0.43) under water stressed conditions was recorded for family 161. A 15.56% decline in grain yield was recorded under water stressed compared to non-stressed conditions. Families 161, 131 and 32 with grain yield means of 33.6, 28.6 and 27.8 g, respectively, were the top performing families under non-stress while families 52 and 159 were the highest yielding families with respective mean grain yield of 19.3 and 17.2 g under water-stressed conditions.

Table 4.4: Mean squares and significant tests for twelve phenotypic traits of 180 M_3 wheat families and a control across two testing sites and two water regimes

Source of Variation	df	Traits											
		DTH	DTM	PH	PTN	SB	RB	TB	RSR	SL	SPS	TSW	GY
Genotype (G)	180	108.8*	197.1	211.6*	65.1**	2681.0*	70.8*	3098.0*	0.03	4.8	44.7	105.2*	186.3***
Site (S)	1	4084.7***	9271.3***	20869.2***	3352.2***	110017.0***	103.9	103359.0***	4.00***	843.9***	299.1**	2215.3***	0.1
Water Regime (WR)	1	119.5	1852.9***	1569.4***	354.9**	32895.0***	3158.1***	15668.0**	2.35***	28.4*	393.1***	10.1	2115.8***
G × S	180	38.7	89.7*	43.0	23.3	1095.0	40.8*	1303.0	0.03	0.9	34.6	36.0	44.2
G × WR	180	39.6	84.5	34.4	16.7*	1061.0	34.5	1177.0	0.03	0.9	34.4*	33.8*	32.1
WR × S	1	4254.4***	9347.7***	1425.5***	476.0*	260.0	746.3***	125.0	0.02	23.5*	60.5	25876.3***	2152.7***
G × WR × S	180	94.7	252.1*	168.9	55.6	2963.0**	59.7	3415.0**	0.03	3.9	40.0	115.5***	148.6***
Replication	1	362.0*	1650.6***	157.5*	794.7***	74413.0***	3776.5***	44662.0***	4.61***	10.8	294.6**	1029.6***	1512.6***
Residual	723	88.9	207.4	176.2	51.4	2252.0	55.0	2469.0	0.04	4.7	40.2	86.9	103.2
CV (%)		12.0	11.1	13.3	64.1	57.0	50.2	50.7	89.7	17.8	29.7	21.2	75.4

* P < 0.05; ** P< 0.01; *** P < 0.001; df: degrees of freedom, DTH: days to 50% heading, DTM: days to 90% maturity, PH: plant height, PTN: productive tiller number, SB: shoot biomass, RB: root biomass, TB: total biomass, RSR: root-shoot ratio, SL: spike length, SPS: spikelet per spike, TSW: thousand seed weight, GY: grain yield

Table 4.2: Mean values for biomass, yield and yield related traits of 180 M_3 wheat families and the control showing the top 10 and bottom 5 ranked families across two testing sites and two water regimes, ranked according to total biomass and grain yield performance

Families	Traits																							
	DTH		DTM		PH		PTN		SB		RB		TB		RSR		SL		SPS		TSW		GY	
	NS	WS	NS	WS	NS	WS	NS	WS	NS	WS	NS	WS	NS	WS	NS	WS	NS	WS	NS	WS	NS	WS	NS	WS
Top 10 families																								
161	79	72	136	112	93.0	83.1	23	8	108.1	42.8	12.1	8.8	120.1	51.5	0.17	0.43	11.5	11.0	21	21	42.5	31.3	33.6	6.0
131	80	81	133	113	104.4	96.7	20	12	114.9	62.9	14.5	11.0	129.4	73.9	0.14	0.27	11.0	11.8	20	22	47.5	26.3	28.6	10.7
32	75	77	126	119	100.3	98.3	17	11	96.6	71.7	13.0	16.9	109.5	88.6	0.12	0.31	12.3	11.8	22	22	50.0	31.3	27.8	7.7
145	77	76	136	134	96.1	89.4	18	12	99.4	51.9	11.9	6.6	111.3	58.6	0.12	0.13	11.6	10.1	21	20	47.5	41.3	26.6	11.3
101	81	83	144	126	96.1	89.6	17	12	126.8	63.2	20.1	14.5	146.9	77.7	0.17	0.30	11.5	11.8	21	21	45.0	35.0	26.1	8.9
96	75	71	127	112	96.9	85.8	17	9	93.9	50.6	16.5	10.7	110.4	61.3	0.17	0.33	11.2	11.0	21	20	47.5	38.8	26.0	9.1
159	81	76	126	124	95.2	96.6	20	17	95.3	74.4	16.6	13.9	111.9	88.3	0.17	0.23	11.5	11.6	20	20	43.8	38.8	24.3	17.2
52	76	71	131	110	90.2	98.8	15	16	96.7	79.0	18.0	15.0	114.7	94.0	0.28	0.38	11.3	11.6	19	20	48.8	35.0	23.9	19.3
103	71	76	123	118	92.0	92.6	17	11	87.5	76.6	13.2	13.3	100.6	89.9	0.16	0.31	11.4	12.2	18	21	45.0	40.0	23.0	12.0
126	74	77	131	129	95.5	91.9	14	11	87.0	56.9	17.6	13.3	104.7	70.2	0.19	0.29	10.8	12.1	20	22	51.3	38.8	22.6	12.0
Control	82	83	137	136	92.5	85.9	12	9	60.4	58.4	11.9	7.0	72.2	65.5	0.20	0.16	10.2	11.0	19	20	50.0	38.8	14.1	10.3
Bottom five families																								
125	78	76	129	115	92.8	89.8	8	5	48.0	39.6	15.3	9.0	63.3	48.6	0.60	0.25	9.8	10.7	16	20	43.8	33.8	5.9	3.9
20	75	81	125	127	91.4	97.8	6	15	74.9	39.0	16.2	12.7	91.0	51.7	0.27	0.45	11.2	12.1	20	22	40.0	40.0	5.8	5.1
2	81	75	124	117	89.2	97.1	7	11	65.2	43.9	15.7	8.6	80.9	52.6	0.35	0.18	10.1	12.5	18	23	36.3	35.0	5.3	5.0
66	83	77	130	122	89.6	103.8	6	10	79.7	45.5	19.5	19.3	99.1	64.9	0.40	0.46	10.6	11.8	20	23	41.3	31.3	5.1	3.6
79	71	72	115	119	82.6	90.7	6	11	64.8	27.5	11.1	10.5	75.9	38.0	0.22	0.67	10.6	11.8	19	21	38.8	30.0	4.7	4.4
Mean	78	77	131	121	94.9	94.5	12	12	73	69	13.6	12.7	86.5	81.7	0.23	0.26	11.5	11.1	21	21	46.8	36.4	13.5	11.4
SE	0.4	0.4	0.6	0.6	0.5	0.5	0.3	0.2	1.9	1.5	0.3	0.3	2.1	1.6	0.008	0.01	0.08	0.08	0.32	0.11	0.3	0.4	0.4	0.3
CV (%)	12.4	12.3	13.2	12.8	14.4	13.8	60.5	49	70.4	59.6	67.1	57	63.8	52.7	88.1	113.4	19.3	19	41.4	14.3	17.5	30	78.7	68.6
LSD (5%)	12.23		22.05		13.59		8.74		57.11		10.63		60.25		0.33		1.83		8.96		11.99		12.84	

NS: non-stressed conditions, WS: water stressed conditions, CV (%): coefficient of variation, SE: standard error, LSD: least significant difference, DTH: days to 50% heading, DTM: days to 90% maturity, PH: plant height, PTN: productive tiller number, SB: shoot biomass, RB: root biomass, TB: total biomass, RSR: root-shoot ratio, SL: spike length, SPS: spikelet per spike, TSW: thousand seed weight, GY: grain yield

4.3.3 Correlations among quantitative traits

Under non-stressed conditions, GY exhibited positive and significant associations ($p<0.01$) with all traits except DTH, RSR and SPS (Table 4.3, upper diagonal). The RSR exhibited a negative association with GY (r=-0.36, $p<0.01$). Shoot biomass exhibited moderate correlations with RB (r =0.34, $p<0.01$), and RSR (r=-0.31, $p<0.01$) while it had strong association with TB (r=0.992, $p<0.01$). Root biomass exhibited significant and moderately positive correlations with TB (r=0.453, $p<0.01$) and RSR (r=0.335, $p<0.01$) while TB exhibited significant but weak correlations with RSR (r=-0.249, $p<0.01$). Under water stressed conditions, GY showed significant association ($p<0.01$) with all traits except DTH, RB and SPS (Table 4.3, lower diagonal). The correlations of GY with TSW (r=0.36, $p<0.01$) and PH (r=0.30, $p<0.01$) were moderate under water stressed compared to non-stressed conditions. The RSR also exhibited a negative association with GY (r=-0.28, $p<0.01$). Among the biomass traits, positive and significant correlations ($p<0.01$) were recorded between SB and RB (r=0.35), and SB and TB (r=0.98).). Likewise, RB was correlated to TB (r=0.54, $p<0.01$) and RSR (r=0.53, $p<0.01$). There was a negative and significant association between SB and RSR (r=-0.20, $p<0.01$).

4.3.4 Cluster analysis

The hierarchical clustering grouped all mutant families obtained from seeds treated with 0.1% EMS for 1 hour at 25 ^{o}C into either cluster 1 or cluster 2 (Table 4.4). All mutant families found in cluster 3 were progenies derived from a mutagenized seed with 0.1% EMS for 1 hour at 30 ^{o}C except family 59, which consisted of progenies of seeds treated with 0.1% EMS for 1 hour at 25 ^{o}C. Clusters 4 and 5 were admixtures of families obtained from seeds that were mutagenized under different EMS dosage and conditions. Twenty mutant families with high grain yield and total biomass under water stressed conditions were selected from each EMS treatment condition for breeding purpose.

Table 4.3: Correlation coefficients of twelve phenotypic traits of 180 M_3 wheat families and control LM43 evaluated in two testing sites under water stressed (lower diagonal) and non-stressed (upper diagonal) conditions

Traits	DTH	DTM	PH	PTN	SB	RB	TB	RSR	SL	SPS	TSW	GY
DTH	-	0.56**	0.11	0.04	0.18*	0.28**	0.20**	0.01	0.16*	0.04	0.17*	0.07
DTM	0.51**	-	0.21**	0.11	0.36**	0.28**	0.38**	-0.19*	0.10	0.22**	0.39**	0.17*
PH	0.05	0.02	-	0.20**	0.24**	0.16*	0.27**	-0.27**	0.35**	0.15*	0.36**	0.24**
PTN	-0.03	0.11	0.25**	-	0.44**	0.24**	0.45**	-0.41**	0.20**	0.04	0.06	0.83**
SB	0.01	0.24**	0.41**	0.53**	-	0.34**	0.99**	-0.31**	0.08	0.10	0.09	0.46**
RB	0.21**	0.01	0.23**	0.16*	0.35**	-	0.45**	0.34**	0.11	0.03	-0.01	0.26**
TB	0.05	0.22**	0.42**	0.51**	0.98**	0.54**	-	-0.25**	0.09	0.10	0.09	0.47**
RSR	0.11	-0.17*	-0.08	-0.25**	-0.20**	0.53**	-0.06	-	-0.15*	-0.12	-0.33**	-0.36**
SL	0.08	0.06	0.29**	0.15*	0.34**	0.28**	0.37**	-0.01	-	0.25**	0.22**	0.33**
SPS	0.20**	0.14	0.38**	0.14	0.39**	0.41**	0.44**	0.03	0.59**	-	0.04	0.09
TSW	-0.11	0.33**	0.02	0.07	0.18*	-0.14	0.13	-0.20**	0.12	-0.10	-	0.25**
GY	-0.12	0.19*	0.30**	0.81**	0.58**	0.02	0.53**	-0.28**	0.22**	0.13	0.36**	-

** Correlation is significant at the 0.01 level (2-tailed), * Correlation is significant at the 0.05 level (2-tailed). NS: non-stressed conditions, WS: water stressed conditions, DTH: days to 50% heading, DTM: days to 90% maturity, PH: plant height, PTN: productive tiller number, SB: shoot biomass, RB: root biomass, TB: total biomass, RSR: root-shoot ratio, SL: spike length, SPS: spikelet per spike, TSW: thousand seed weight, GY: grain yield

Table 4.4: Clustering of the 180 M_3 wheat families and control LM43 based on phenotypic similarity across two testing sites and two water regimes

Cluster	Families		Selected families	
	Designations	**Total**	**Designations**	**Total**
1	1 to 28, 31, 33	30	3, 4, 5, 7, 8, 12, 15, 16, 18, 19, 21, 23, 27, 31	14
2	29, 30, 32, 34 to 58, 60, 61, 64	31	32, 35, 45, 48, 49, 52, 56, 60, 61	9
3	59, 62, 63, 65 to 106, 111, 115	47	63, 71, 73, 78, 80, 85, 88, 93, 94, 96, 99, 101, 103, 113	14
4	107, 108, 109, 110, 112, 113, 114, 116 to 131, 133, 135, 136, 138, 139, 140	29	108, 116, 126, 128, 129, 131, 140	7
5	132, 134, 137, 141 to 180, LM43 (control)	44	143, 145, 148, 152, 158, 159, 161, 162, 163, 164, 165, 169, 170, 172, 175, 179	16

4.3.5 Principal component analysis

Under the non-stressed treatment, the first five principal components (PC) with Eigen values ≥1.00 accounted for 77.04% of the total variation (Table 4.5). SB, TB and GY had the highest loadings of 0.80, 0.81 and 0.75, respectively on PC-1. The dominant traits on PC-2 were RB and RSR. Other traits such as DTH, DTM, PH, PTN, and TSW had moderate loadings on either one of the first three PCs while SL and SPS contributed highly to PC-4 and PC-5, respectively. In the water stressed treatment, the first four PCs with Eigen values ≥1.00 accounted for a cumulative 71.87% of the variation in the mutant population. The 32.65% variation explained by PC-1 was largely contributed by PTN, SB, TB and GY. Similar to the non-stressed treatment, RB and RSR were the largest contributors to the variation explained by the PC-2. DTH and DTM had high contributions (>0.75) on PC-3. The PC-4 accounted for 9.18% of the variation, which was largely attributed to the negative loadings by SL (-0.56) and SPS (-0.45) and the positive loading of RSR (0.41).

The multi-variate family-trait relationships among the top 15 and bottom 5 of the 180 M_3 wheat families and the untreated control were illustrated by the PC biplot in Figures 4.1 and 4.2 for the non-stressed and water stressed conditions, respectively. The proximity of a family to a trait vector indicates the correlation of the family and the particular trait while a family vector predicts the performance of that family for a particular trait. Under non-stress, most of the families and traits were more concentrated in the positive quadrants of the PC-1 with families 57, 61, 93, 145 and 160 excelling in PH, PTN, SL, SPS and GY (Figure 4.1). Families 98, 100, 106 and 134 were associated with DTH, DTM and TSW while families 20, 25 and 91 showed strong correlations with RSR. Families 2, 79 and 161 exhibited low performance for most traits. Unlike under non-stress condition, families and traits in water stressed conditions were dispersed in all the four quadrants of the PCA biplot with families 16, 35 and 142 being inclined towards SL, SPS, RB and TB vectors (Figure 4.2). Families 31, 52, 55, 73, 80 and 103 were strongly associated with PH, PTN, SB, TSW and GY. Families 122, 145 and 181 had high mean values for DTM while families 125 and 156 were late flowering with high values for DTH.

Table 4.5: Principal component matrix for phenotypic traits of 180 M_3 wheat families and a control evaluated across two testing sites under non-stressed and stressed conditions.

Traits	DTH	DTM	PH	PTN	SB	RB	TB	RSR	SL	SPS	TSW	GY	Eigen value	% of variance	Cumulative % of variance
Non-Stressed															
PC-1	0.36	0.56	0.49	0.69	0.80	0.44	0.81	-0.47	0.38	0.23	0.38	0.75	3.78	31.52	31.52
PC-2	0.46	0.32	-0.24	-0.26	0.22	0.65	0.30	0.65	-0.29	-0.10	-0.30	-0.27	1.68	14.02	45.54
PC-3	0.50	0.52	0.34	-0.44	-0.31	-0.07	-0.30	-0.03	0.33	0.29	0.56	-0.30	1.62	13.51	59.05
PC-4	-0.10	-0.30	0.20	0.15	-0.25	0.40	-0.18	0.42	0.61	0.31	-0.22	0.20	1.16	9.64	68.69
PC-5	0.18	-0.06	0.07	0.20	-0.23	0.18	-0.20	0.12	0.02	-0.79	0.32	0.23	1.00	8.35	77.04
Stressed															
PC-1	0.12	0.31	0.56	0.67	0.89	0.46	0.90	-0.16	0.54	0.58	0.23	0.71	3.92	32.65	32.65
PC-2	0.37	-0.12	0.12	-0.36	-0.08	0.70	0.08	0.71	0.28	0.47	-0.49	-0.51	2.09	17.45	50.10
PC-3	0.76	0.85	-0.14	-0.19	-0.07	-0.11	-0.09	-0.13	-0.003	0.06	0.30	-0.12	1.51	12.59	62.69
PC-4	0.13	0.12	-0.29	0.30	0.12	0.35	0.19	0.41	-0.56	-0.45	-0.07	0.17	1.10	9.18	71.87

%: percentage, PC: principal component axis, DTH: days to 50% heading, DTM: days to 90% maturity, PH: plant height, PTN: productive tiller number, SB: shoot biomass, RB: root biomass, TB: total biomass, RSR: root-shoot ratio, SL: spike length, SPS: spikelet per spike, TSW: thousand seed weight, GY: grain yield

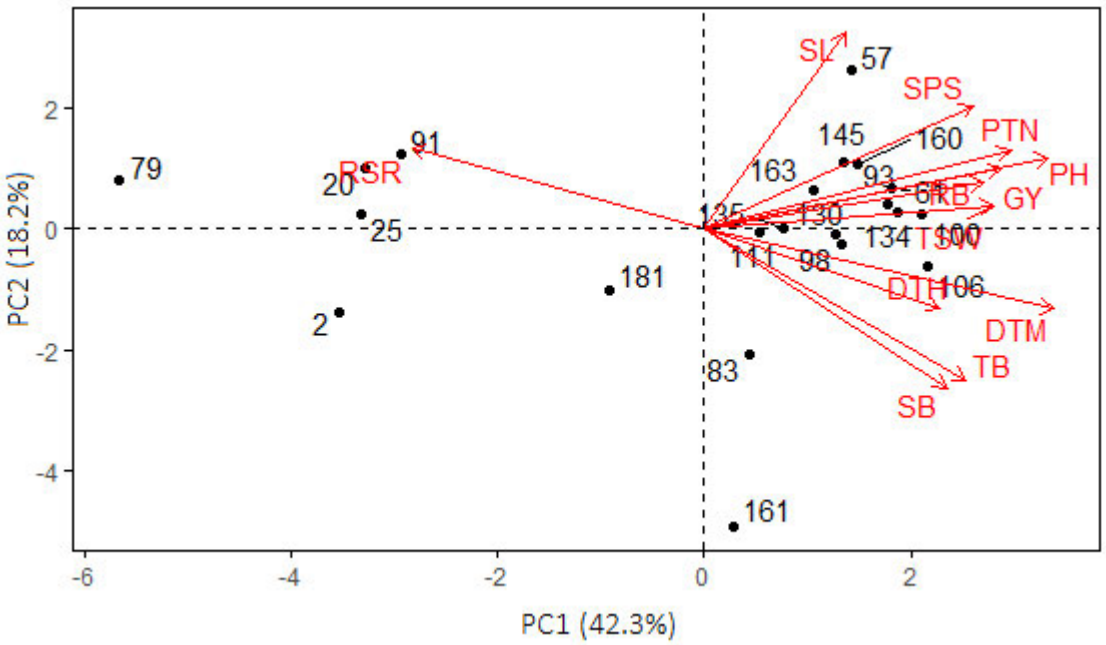

Figure 4.1: Principal component biplot showing families-trait relationship among the top 15 and bottom 5 of the 180 M3 wheat families and a control genotype LM43 under non-stressed conditions. DTH: days to 50% heading, DTM: days to 90% maturity, PH: plant height, PTN: productive tiller number, SB: shoot biomass, RB: root biomass, TB: total biomass, RSR: root-shoot ratio, SL: spike length, SPS: spikelet per spike, TSW: thousand seed weight, GY: grain yield

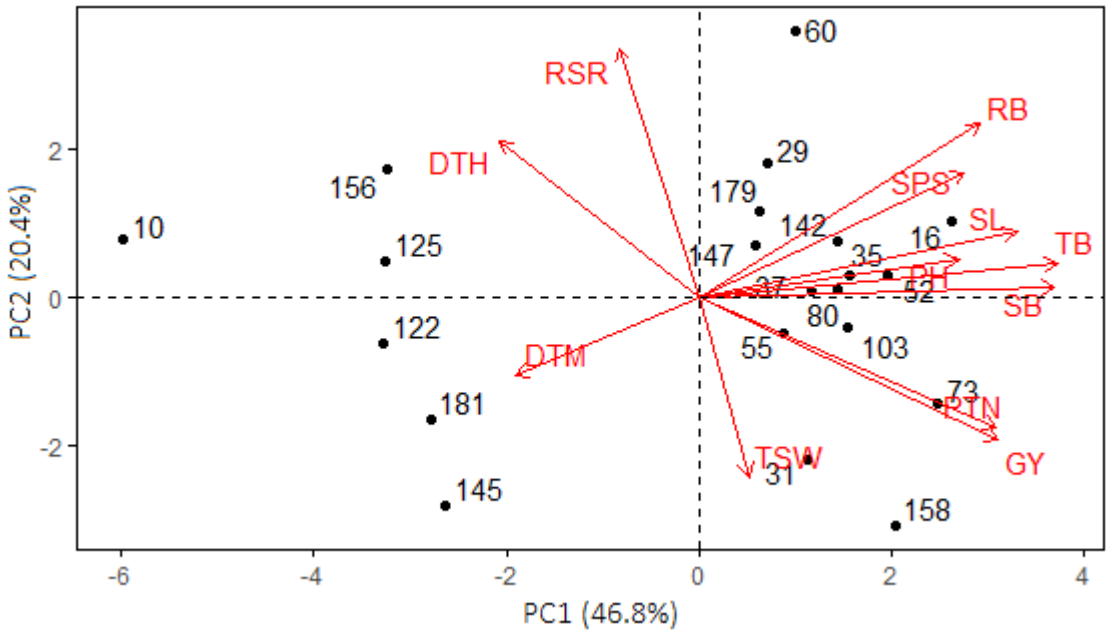

Figure 4.2: Principal component biplot showing families-trait relationship among the top 15 and bottom 5 of the 180 M3 wheat families and a control genotype LM43 under water stressed conditions. DTH: days to 50% heading, DTM: days to 90% maturity, PH: plant height, PTN: productive tiller number, SB: shoot biomass, RB: root biomass, TB: total biomass, RSR: root-shoot ratio, SL: spike length, SPS: spikelet per spike, TSW: thousand seed weight, GY: grain yield

4.4 Discussion

4.4.1 Genotypic variation in agronomic traits

The significant ($p<0.05$) effects of the interactions involving genotype, site and water regimes on the traits such as DTM, SB, RB, TB, PTN, SPS, TSW and GY (Table 4.1) suggest that genotypic and environmental factors are crucial for biomass allocation and yield improvement. The confounding effects of genotype × site × water regime effects have been recognized as an impediment to efficient selection of superior genotypes evaluated in different environments. Thus, differences in sites and water availability that constitute environmental conditions in this study can either accelerate or delay maturity and alter biomass accumulation in roots and spikes. Dube *et al.* (2016) and Matlala *et al*. (2019) also found that environmental conditions played a vital role in influencing yield and yield related traits and potential cultivar development in wheat through significant genotype × environment interaction. The significant effects of genotypic main effect exhibited for most traits indicate the presence of genetic variation among the mutant families. Since the families were derived from seeds of one selected drought tolerant genotype, the observed differences emanate from the genetic changes induced during mutation. Mutagenesis using EMS creates opportunities to increase genetic variation and enhances selection of superior mutant genotypes for wheat improvement. This offers an opportunity to identify superior families for mass selection or individual genotypes for pure line development. Previously, Luz *et al*. (2016) also found that mutagenesis in rice increased genetic variation for selecting individuals with superior agronomic performance. The impact of water stress on agronomic performance among the mutant families shows the important role of water in plant growth and development. Plant response to water availability has been widely reported previously (e.g. Osakabe *et al*., 2014; Tátrai *et al*., 2016; Robbins and Dinneny, 2018; Marchin *et al*., 2020). Mwadzingeni *et al*. (2017) reported significant interaction between genotype and water regime influencing yield and yield component traits of wheat genotypes under contrasting water levels showing that water availability affects plant growth in general although the actual extent of impact is dependent on genetic constitution of the plant and the intensity of the stress. Selection of genotypes with superior agronomic performance and biomass allocation under drought stress facilitates the

development of cultivars adapted for water constrained environments but there is a need to assess the dynamic stability of such genotypes when moisture conditions improve to avoid yield penalties. For instance, it has been reported that some cultivars with high yield potential under drought conditions were not as superior under irrigated conditions (Abdolshahi *et al.,* 2013; Mehraban *et al.,* 2018; Hooshmandi, 2019). Ideally, a desirable cultivar should have high and stable yield potential under diverse conditions. Thus, it would be necessary to conduct additional studies to evaluate the yield stability of identified mutant families across multiple environments.

4.4.2 Mean performance for biomass and agronomic traits under variable drought stress

The higher trait means for most mutant families under water stress condition in comparison to the untreated control imply that the EMS mutagen had positive impact on the genetic performance (Table 4.2). Mutagenesis resulted in changes in the genetic constitution on progeny that often induces higher performance in agronomic performance compared to the non-mutagenized controls. This study confirms that genetic modification through mutation can improve agronomic and biomass performance (Figures 4.3-4.6). These findings agreed with Luz *et al*. (2016) who found that EMS enhanced agronomic performance of mutant rice families compared to the non-mutagenized control families. EMS mutagenesis induces desirable changes in the gene structure, which produces mutants with altered agronomic traits such as increased spike length, tiller number, heavier kernel weight and biomass (Mohapatra *et al*., 2014; Feldman *et al*., 2017). This confirmed the potential use of EMS mutation to increase agronomic performance for the development of high yielding genotypes. Kontz *et al.* (2009) selected mutant lines of wheat resistant to drought stress while Singh and Balyan (2009) identified wheat mutant lines with improved grain quality and reduced height compared to the untreated controls. Other studies reported an improvement in grain yield and yield components in millet (Addai and Salifu, 2016), wheat (Nazarenko *et al*., 2018) and rice (Oladosu *et al*., 2014).

The significant differences in trait means between non-stressed and water-stressed conditions for TSW, GY and biomass traits, confirmed that drought stress has a

negative impact on genotype performance. Drought stress causes stomatal closure and leaf rolling, leads to osmotic adjustment and increases cell wall elasticity, which lead to reduced gaseous exchange and translocation of water and nutrients for photosynthesis (Reddy *et al.,* 2004; Yi *et al.,* 2016; Yu *et al.,* 2017; Abid *et al.,* 2018). Consequently, a reduction in photosynthesis results in low biomass production under drought stress. Farooq *et al*. (2014) and Mwadzingeni *et al*. (2017) reported significant reduction in yield, seed size, plant height and tiller numbers due to drought stress, which were corroborated by the findings of this study. In contrast, drought stress resulted in an increase in RSR, which implied that drought stress promoted root biomass accumulation or had higher negative impact on shoot compared to root growth in the mutant families. Increased allocation of assimilates to below ground biomass in plants under soil moisture stress has been reported previously as a mechanism to counter the negative effects of edaphic factors to maintain productivity (Zhu and Zhang, 2013). This environmental plasticity can be exploited to improve crop response to drought stress by identifying genotypes that maintain high RSR coupled with high GY in water limited conditions. Several studies have reported the importance of deep root systems for water uptake from deeper soil layers under water-stressed environments in cereal crops such as sorghum (Steele *et al*., 2013), rice (Manschadi *et al*., 2006; Wasson *et al*., 2012), maize (Prudhomme *et al.,* 2014), and wheat (Kashiwagi *et al*., 2006). However, undertaking to increase root biomass in a cultivar must be pursued after thorough understanding of the causes of drought stress in a particular environment and the maintenance costs associated with a large root system (Tuberosa, 2012). Bigger root systems would not be cost-effective in cases where moisture is available at shallow depths and the large root biomass may reduce grain yield potential due to high metabolic costs. Conversely, large root biomass would be more beneficial in soils where the moisture is available in deeper horizons (Manschadi *et al*., 2010; Wasson *et al*., 2012)

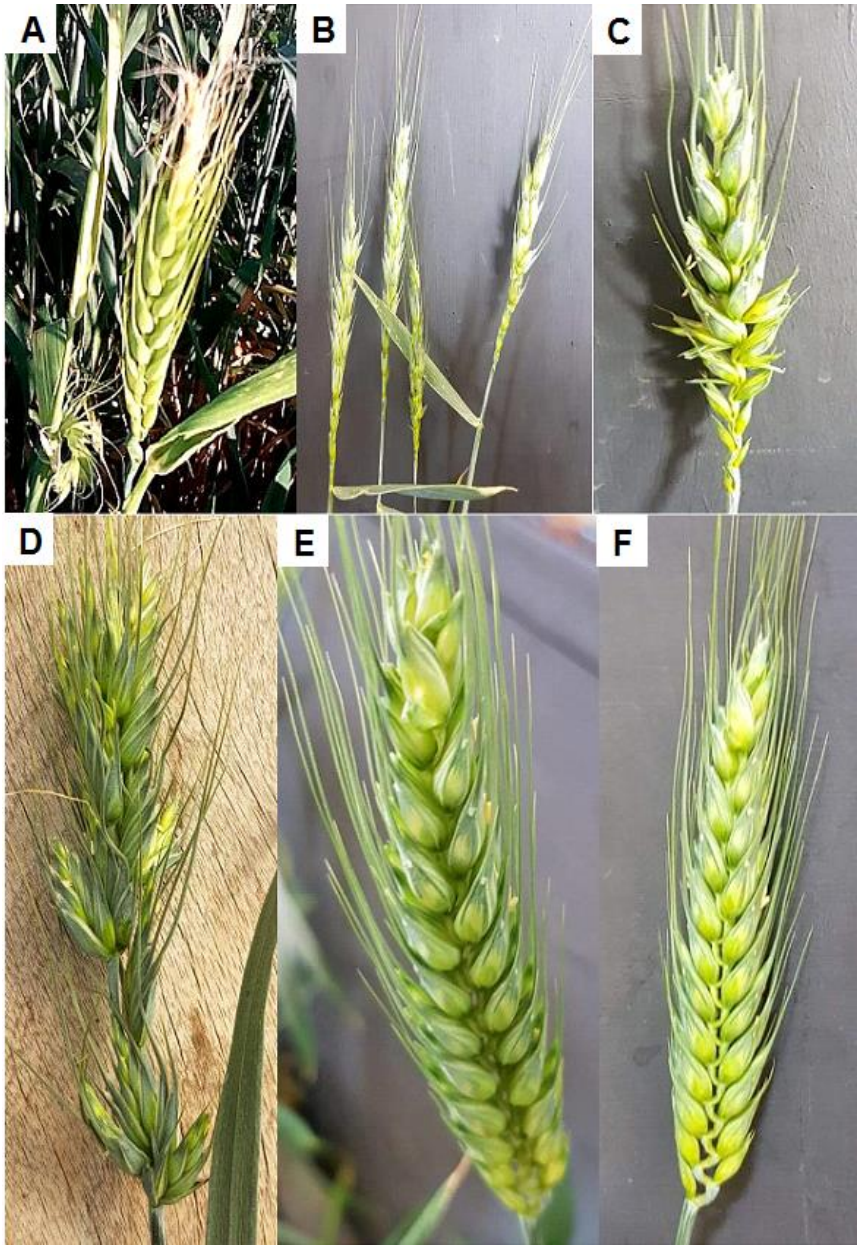

Figure 4.3: Differences in spike morphology among mutant wheat families (A-F) at Ukulinga Research Farm of the University of KwaZulu-Natal. Note: A (Familiy 2), B (Familiy 125), C (Familiy 85), D (Familiy 66), E (Familiy 161) and F (Control)

Figure 4.4: Differences in shoot biomass produced among mutant wheat families (A-L) at the controlled environment facility of the University of KwaZulu-Natal. Note: A (Control), B (Familiy 12), C (Familiy 101), D (Familiy 2), E (Familiy 140), F (Familiy 66), G (Familiy 1), H (Familiy 96), I (Familiy 79), J (Familiy 32), K (Family 103) and L (Family 91)

Figure 4.5: Variation in root biomass production among mutant wheat families. Note: A (Control), B (Familiy 161), C (Familiy 52), D (Familiy 103), E (Familiy 159), F (Familiy 52), G (Familiy 96) and H (Familiy 145)

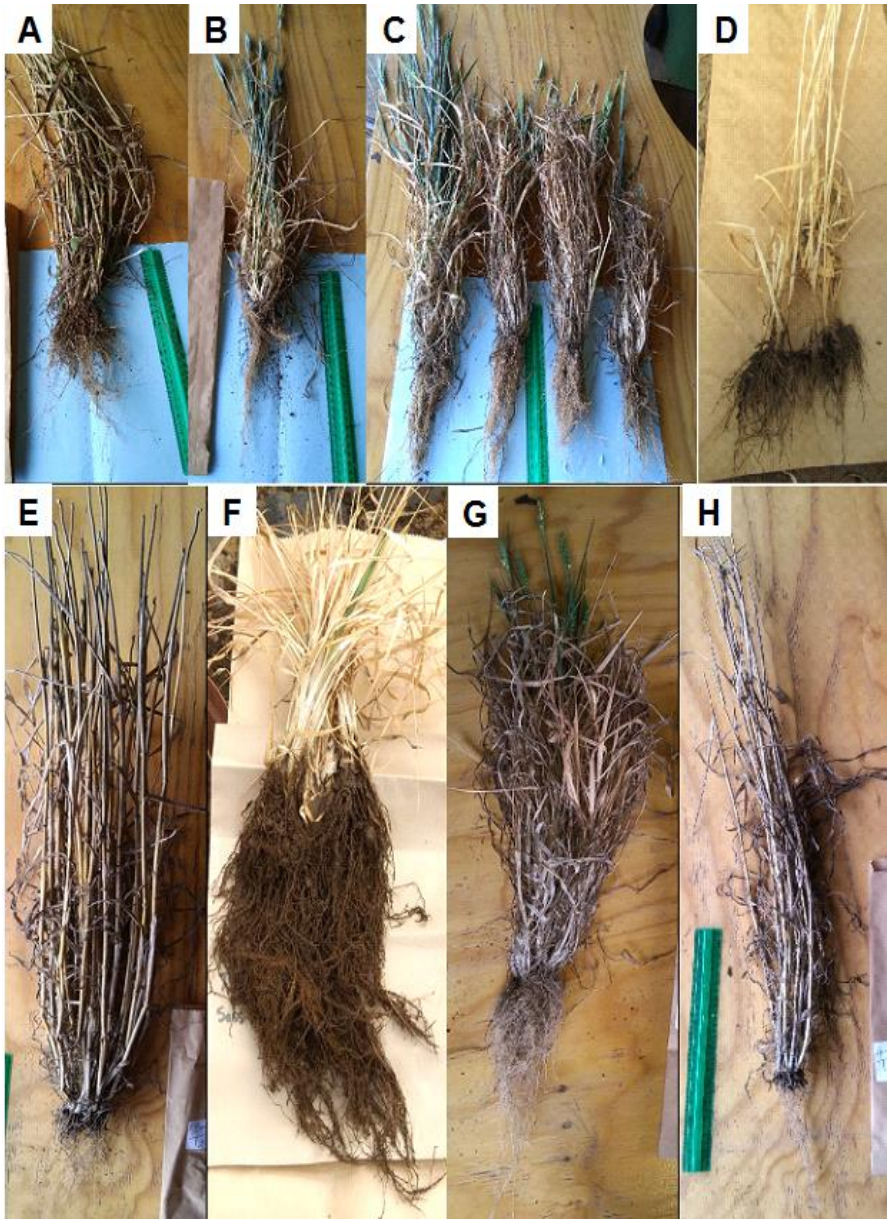

Figure 4.6: Differences in biomass partitioning between roots and shoots among mutant wheat families. Note: A (Familiy 103), B (Familiy 40), C (Familiy 79), D (Familiy 2), E (Familiy 159), F (Familiy 124), G (Familiy 126) and H (Control)

4.4.3 Trait associations

The significant and positive correlations between GY and yield related trait such as DTM, PH, PTN, SB, TB, SL and TSW under both water regimes imply that these traits are directly related to GY accumulation irrespective of water availability conditions. Above ground traits such as SB, PH and PTN that are directly related to biomass accumulation are known to have direct impact on GY due to their influence on solar radiation interception, provision of photosynthetic area and supporting yield vessels. For instance, taller plants have higher ability to compete for light interception, which increases yield potential of taller plants (Nagashima and Hikosaka, 2011; Onoda *et al.,* 2014; Tang *et al.,* 2019; Zhang *et al.,* 2019). Higher number of productive tillers provides vegetative growth to support spikes that are directly linked to the amount of grain harvested per plant (Xie *et al*., 2016; Chen *et al.,* 2019; Bastos *et al*., 2020). Similarly, higher shoot biomass provides vegetative growth to support photosynthesis and resource mobilization for grain yield. It is reported that the ear and flag leaves of cereal plants are major contributors of assimilates (contributing between 10 and 76%) during grain filling (Tambussi *et al*., 2007; Aranjuelo *et al*., 2011). Tambussi *et al*. (2005) and Sanchez-Bragado *et al*. (2014) reported that wheat ears have a higher contribution of assimilates under drought stress conditions. This highlights the direct and positive impact of above ground traits on grain yield. The slight reduction in the strength of correlations between GY and most traits under water -stressed compared to non-stressed treatment is expected because trait associations are dynamic and environmental stress tends to weaken the correlations between genotype and phenotypic expression (Bustos-Korts *et al.,* 2018). The reduction in the correlations is subject to the extent and duration of drought stress. The study found that RB was positively correlated to GY under non-stressed conditions only. This relationship can be explained by improved water and nutrient acquisition by large rooted genotypes, which has been reported previously (Liao *et al*., 2006; Palta *et al*., 2011). The lack of association between RB and GY under drought-stressed conditions could be due to increased inter-root competition (King *et al*., 2003) that aggravates the effects of water stress and reduces photosynthesis (Du *et al*., 2013). Alternatively, plants with smaller root mass could be unable to capture sufficient soil moisture necessary for grain filling (Ehdaie *et al*., 2012). The negative association between RSR and GY under both conditions suggest that there must be a limit to partitioning biomass to

roots at the expense of shoots in order to maintain high GY. While a large root system is important for nutrient and water acquisition, an excessively large root system with increased sink capacity for assimilates and maintenance requirements can potentially compete with above ground components resulting in reduced grain yield production. Source-sink competition has been reported widely and becomes more critical when resources are limiting especially in water and nutrient limited conditions (Liao *et al*., 2006; Fang *et al*., 2017).

The significant associations between above ground traits with SB and GY show that the above ground traits could be simultaneously selected to improve GY and SB. The number of tillers and leaf characteristics such as chlorophyll content and leaf area directly influence photosynthetic capacity (Zhang *et al*., 2009; Aditya and Bhartiya, 2013). The accumulation of large SB could potentially lead to a large canopy to prevent direct moisture loss from the soil and thus promote water utilization for high GY production in wheat (Botwright *et al*., 2002). The large canopy would provide an advantage where transpirational losses are minimized during drought stress. Selection for improved yield in non-stressed environments has indirectly increased grain yield in many drought stress environments (Cattivelli *et al*., 2008). However, Abdolshahi *et al.* (2013) suggested that indirect selection of mean yield and yield potential genotypes under non-stressed environments may not be appropriate for water-stressed environments.

4.4.4 Clustering of mutant families

The clustering of M_3 families based on their phenotypic similarities revealed the relatedness of the mutant progenies. The groupings were mainly based on families generated from seeds subjected to similar mutagenic conditions. Similarly, Luz *et al.* (2016) clustered mutant rice families in the same clusters derived from the same EMS treatments. However, families of the same EMS treatment condition that clustered differently could be as a result of environmental factors or effect of continuous gene segregation of the individual mutants. Mutations are random and unpredictable resulting in variation even among progeny derived from seeds treated under similar mutagenic conditions (Gregory, 1956). Most families from clusters 1 and 5 showed high mean performances in biomass and grain yield production

especially under water stressed conditions, reflecting their ability to withstand unfavourable environmental conditions. This could be a useful strategy to select parental lines for hybridization in subsequent breeding programs (Luz *et al.,* 2016).

4.4.5 Trait contribution to total variation within the mutant population under different water regimes

The principal component analysis showed that SB, RB and GY contributed much to the total variation followed by DTH, DTM, PH, PTN and TSW (Table 4.5) suggesting that the traits exhibited variable importance in distinguishing the mutant families. These traits could be simultaneously selected based on their importance in discriminating the genotypes and their interrelationships. Indirect selection for GY through related traits is a well-known and widely used strategy for GY improvement (Bankole *et al*., 2017; Guo *et al*., 2018; Baye *et al*., 2020). Similarly, the strategy could be extended to select genotypes with favorable biomass allocation using RSR and SB, which are more easily measurable compared to RB. Mathew *et al*. (2019) used selection for root to shoot ratios and SB to indirectly improve biomass allocation for drought tolerance and carbon sequestration in wheat. Under water stressed treatment, the high positive loadings of PTN, SB, TB, RB, RSR and GY on the first two PC axes, indicate the importance of selecting families based on these traits for drought tolerance and increased biomass (Table 4.5). Traits with high loading on the first and second PCs are important for selection as they are able to discriminate the genotypes more effectively compared to traits with less contributions (Shlens, 2014; Zhang and Castelló, 2017; Zuśka *et al*., 2019).

The differences in trait contributions to the total variation observed among the genotypes under different water regimes was in line with findings from Mwadzingeni *et al*. (2016) and Mathew *et al*. (2019). Similarly, families plotted in the positive quadrants of the first principal component axis (Figures 4.1 and 4.2) can be selected as genetic resources for improving above ground traits. For both water regimes, biomass traits except RSR contributed positively to the variation on PC1 showing that there was wide variation in these traits among the genotypes. The higher contribution by SB compared to RB showed that there was wider genetic variation for SB among the genotypes, which corroborated previous assertions that there is

narrow genetic variation in root biomass (White *et al.,* 2015). It also shows that there may be limited variation created in the RB after mutagenesis.

4.5 Conclusion

The EMS treatments generated wide genetic variation and created several families with superior traits compared to the untreated control. The high yielding families designated as 52, 159, 103, 126, 145 under drought stress are recommended for developing breeding populations with high grain yield potential, improved drought tolerance and increased biomass allocation to roots while families selected in each cluster can be considered for genetic advancement due to their genetic dissimilarities and high mean performance in grain yield and total biomass production. Improved grain yield production by large rooted genotypes under non-stressed conditions shows that rooting systems confer advantages in moisture extraction but the lack of correlations under drought stress could be a result of high cost of metabolic maintenance for roots. This shows that there is an urgent need for inclusion of root-related traits in breeding programs to limit loss of genetic diversity for rooting systems. In addition, improved root phenotyping techniques coupled genetic tools are required to improve selection efficiency and identification of genomic loci controlling roots for marker-assisted selection.

4.6 References

Abdolshahi R, Safarian A, Nazari M, Pourseyedi S, Mohamadi-Nejad G (2013) Screening drought-tolerant genotypes in bread wheat (*Triticum aestivum* L.) using different multivariate methods. Arch Agron Soil Sci 59:685-704. doi: 10.1080/03650340.2012.667080

Abid M, Ali S, Qi LK, Zahoor R, Tian Z, Jiang D, Sinder JL, Dai T (2018) Physiological and biochemical changes during drought and recovery periods at tillering and jointing stages in wheat (*Triticum aestivum* L.). Sci Rep 8:4615. doi:10.1038/s41598-018-21441-7

Abraha MT, Hussein S, Laing M, Assefa K (2017) Early generation genetic variation and heritability of yield and related traits among tef populations. J Crop Sci Biotechnol 20:379-386.

Addai IK, Salifu B (2016) Selection of mutants with improved growth and total grain yield in the M_2 generation of pearl millet (*Pennicetum glaceum* L.) in the Northern region of Ghana. J Agron 15:88-93. doi: 10.3923/ja.2016.88.93

Aditya JP, Bhartiya A (2013) Genetic variability, correlation and path analysis for quantitative characters in rainfed upland rice of Uttarakhand Hills. J Rice Res 6:24-34.

Aranjuelo I, Cabrera-Bosquet L, Morcuende R, Avice JC, Nogués S, Araus JL, Martínez-Carrasco R, Pérez P (2011) Does ear C sink strength contribute to overcoming photosynthetic acclimation of wheat plants exposed to elevated CO_2? J Exp Bot 62:3957-3969

Bankole F, Menkir A, Olaoye G, Crossa J, Hearne S, Unachukwu N, Gedil M (2017) Genetic gains in yield and yield related traits under drought stress and favorable environments in a maize population improved using marker assisted recurrent selection. Front Plant Sci 8:808. doi: 10.3389/fpls.2017.00808

Baye A, Berihun B, Bantayehu M, Derebe B (2020) Genotypic and phenotypic correlation and path coefficient analysis for yield and yield-related traits in advanced bread wheat (*Triticum aestivum* L.) lines. Cogent Food Agric 6(1):1752603. doi: org/10.1080/23311932.2020.1752603

Botwright TL, Condon AG, Rebetzke GJ, Richards RA (2002) Field evaluation of early vigour for genetic improvement of grain yield in wheat. Australian J Agric Res 53:1137-1145. doi: 10.1071/AR02007

Bastos LM, Carciochi W, Lollato RP, Jaenisch BR, Rezende CR, Schwalbert R, Vara Prasad PV, Zhang G, Fritz AK, Foster C, Wright Y, Young S, Bradley P, Ciampitti IA (2020) Winter wheat yield response to plant density as a function of yield environment and tillering potential: a review and field studies. Front Plant Sci 11:54. doi: 10.3389/fpls.2020.00054

Bustos-Korts D, Romagosa I, Borràs-Gelonch G, Casas AM, Slafer GA, van Eeuwijk F (2018) Genotype by environment interaction and adaptation. In: Meyers R (eds) Encyclopedia of sustainability science and technology. Springer, New York, NY

Cattivelli L, Rizza F, Badeck FW, Mazzucotelli E, Mastrangelo AM, Francia E (2008) Drought tolerance improvement in crop plants: an integrated view from breeding to genomics. Field Crops Res 105:1-14. doi: 10.1016/j.fcr.2007.07.004

Chen X, Zhang W, Liang X, Liu Y-M, Xu S-J, Zhao Q-Y, Du Y-F, Zhang L, Chen X-P, Zou C-Q (2019) Physiological and developmental traits associated with the grain yield of winter wheat as affected by phosphorus fertilizer management. Sci Rep 9:16580. doi: 10.1038/s41598-019-53000-z

DAFF (Department of Agriculture, Forestry and Fisheries) (2010) Wheat production guideline. DAFF, Pretoria.

Den Herder G, Van Isterdael G, Beeckman T, De Smet I (2010) The roots of a new green revolution. Trends Plant Sci 15:600-607. doi: 10.1016/j.tplants.2010.08.009

Du YL, Wang ZY, Fan JW, Turner NC, He J, Wang T (2013) Exogenous abscisic acid reduces water loss and improves antioxidant defence, desiccation tolerance and transpiration efficiency in two spring wheat cultivars subjected to a soil water deficit. Funct Plant Biol 40:494-506. doi: 10.1071/fp12250

Dube E, Mare-Patose R, Kilian W, Barnard A, Tsilo TJ (2016) Identifying high-yielding dryland wheat cultivars for the summer rainfall area of South Africa. S Afr J Plant Soil 33:77-81

Ehdaie B, Layne AP, Waines JG (2012) Root system plasticity to drought influences grain yield in bread wheat. Euphytica 186:219-232. doi: 10.1007/s10681-011-0585-9

FAO (Food and Agricultural Organization) (2018) World food and agriculture-statistical pocketbook. Rome, pp 1-254.

Fang Y, Du Y, Wang J, Wu A, Qiao S, Xu B, Zhang S, Siddique KHM, Chen Y (2017) Moderate drought stress affected root growth and grain yield in old, modern and newly released cultivars of winter wheat. Front Plant Sci doi: 10.3389/fpls.2017.00672

Farooq M, Hussain M, Siddique KH (2014) Drought stress in wheat during flowering and grain-filling periods. Crit Rev Plant Sci 33:331-349

Feldman AB, Leung H, Baraoidan M, Elmido-Mabilangan A, Canicosa I, Quick WP, Sheehy J, Murchie EH (2017) Increasing leaf vein density via mutagenesis in rice results in an enhanced rate of photosynthesis, smaller cell sizes and can reduce interveinal mesophyll cell number. Front Plant Sci 8:1-10. doi: 10.3389/fpls.2017.01883

Fu D, Xiao M, Hayward A, Fu Y, Liu G, Jiang G, Zhang H (2014) Utilization of crop heterosis: a review. Euphytica 197(2):161-173

Govindaraj M, Vetriventhan M, Srinivasan M (2015) Importance of genetic diversity assessment in crop plants and its recent advances: an overview of its analytical perspectives. Genet Res Int 2015:1-14. doi: 10.1155/2015/431487

Gregory WC (1956) The comparative effects of radiation and hybridization in plant breeding. International Conference for Peaceful Uses of Atomic Energy. Proceedings. 12:48-51

Grover G, Sharma A, Gill HS, Srivastava P, Bains NS (2018) Rht8 gene as an alternate dwarfing gene in elite Indian spring wheat cultivars. PLoS One doi: 10.1371/journal.pone.0199330

Guo J, Shi W, Zhang Z, Cheng J, Sun D, Yu J, Li X, Guo P, Hao C (2018) Association of yield-related traits in founder genotypes and derivatives of common wheat (*Triticum aestivum* L.). BMC Plant Biol 18(1):38. doi: 10.1186/s12870-018-1234-4

Hirte J, Leifeld J, Abiven S, Oberholzer HR, Mayer J (2018) Below ground carbon inputs to soil via root biomass and rhizo-deposition of field-grown maize and wheat at harvest are independent of net primary productivity. Agric Ecosyst Environ 265:556-566

Hooshmandi B (2019) Evaluation of tolerance to drought stress in wheat genotypes. Res Paper 37(2):37-43

IBM SPSS I (Statistical Package for the Social Sciences) (2016) IBM corporation released. IBM SPSS Statistics for Windows, Version 24.0. Armonk, New York

Kashiwagi J, Krishnamurthy L, Crouch JH, Serraj R (2006) Variability of root length density and its contributions to seed yield in chickpea (*Cicer arietinum* L.) under terminal drought stress. Field Crops Res 95:171-181

Khalil, MU, Akram Z, Rana M, Shah ZH Ahmed Z (2019) Molecular marker assisted selection of promising wheat genotypes for high grain protein content. Adv Plant Agri Res 9(2):348-353. doi: 10.15406/apar.2019.09.00447

King J, Gay A, Sylvester-Bradley R, Bingham I, Foulkes J, Gregory P (2003) Modelling cereal root systems for water and nitrogen capture: towards an economic optimum. Ann Bot 91:383-390. doi: 10.1093/aob/mcg033

Kontz B, Franklin S, Brunel C (2009) Selection of winter wheat mutant lines resistant to drought stress. J Undergrad Res 7(9). http://openprairie.sdstate.edu/jur/vol7/iss1/9

Liao MT, Palta JA, Fillery IRP (2006) Root characteristics of vigorous wheat improve early nitrogen uptake. Aust J Agric Res 57:1097-1107. doi: 10.1071/ar05439

Luz VK, Silveira SF, Magalhães da Fonseca G, Groli EL, Figueiredo RG, Baretta D, Kopp MM, Junior AM, Carlos da Maia L, Costa de Oliveira A (2016) Identification of variability for agronomically important traits in rice mutant families. Plant Breed 75(1):41-50. doi: 10.1590/1678-4499.283

Manschadi AM, Christopher JT, DeVoil P, Hammer GL (2006) The role of root architectural traits in adaptation of wheat to water-limited environments. Funct Plant Biol 33:823-837.

Manschadi AM, Christopher JT, Hammer GL, DeVoil P (2010) Experimental and modelling studies of drought-adaptive root architectural traits in wheat (*Triticum aestivum* L.). Plant Biosystem 144:458-462

Mathew I, Shimelis H, Mutema M, Clulow A, Zengeni R, Mbava N, Chaplot V (2019) Selection of wheat genotypes for biomass allocation to improve drought tolerance and carbon sequestration into soils. J Agron Crop Sci 205:385-400. doi:10.1111/jac.12332

Matlala M, Shimelis H, Mashilo J (2019) Genotype-by-environment interaction of grain yield among candidate dryland wheat genotypes. S Afr J Plant Soil. 36(4): 299-306. doi: 10.1080/02571862.2019.1566502

Marchin RM, Ossola A, Leishman MR, Ellsworth DS (2020) A simple method for simulating drought effects on plants. Front Plant Sci 10:1715. doi: 10.3389/fpls.2019.01715

Mehraban SA, Tobe A, Gholipouri A, Amiri E, Ghafari A, Rostaii M (2018) Evaluation of drought tolerance indices and yield stability of wheat cultivars to drought stress in different growth. World J Environ Biosci 7(1): 8-14

Mohapatra T, Robin S, Sarla N, Sheshasayee M, Singh AK, Singh K, Singh NK, Mitha SVA, Sharma RP (2014) EMS induced mutants of upland rice variety Nagina22: generation and characterization. P Natl A Sci India A. 80(1):163-172. doi: 10.16943/ptinsa/2014/v80i1/55094

Mwadzingeni L, Shimelis H, Tesfay S, Tsilo TJ (2016) Screening of bread wheat genotypes for drought tolerance using phenotypic and proline analyses. Front Plant Sci 7(1276):1-12.

Mwadzingeni L, Shimelis H, Tsilo, TJ (2017) Variance components and heritability of yield and yield components of wheat under drought-stressed and non-stressed conditions. Aust J Crop Sci 11:1425-1430

Nagashima H, Hikosaka K (2011) Plants in a crowded stand regulate their height growth so as to maintain similar heights to neighbours even when they have potential advantages in height growth. Ann Bot 108(1):207-214. doi: 10.1093/aob/mcr109

Nazarenko M, Lykholat Y, Grygoryuk I, Khromikh N (2018) Optimal doses and concentrations of mutagens for winter wheat breeding purposes. Part I. Grain productivity. J Cent Eur Agric 19(1):194-205. doi: 10.5513/JCEA01/19.1.2037

Negassa A, Shiferaw B, Koo J, Sonder K, Smale M, Braun HJ, Gbegbelegbe S, Guo Z, Hodson D, Wood S, Payne T, Abeyo BG (2013) The potential for wheat production in africa: analysis of biophysical suitability and economic profitability. CIMMYT, Mexico.

Nhemachena CR, Kirsten J (2017) A historical assessment of sources and uses of wheat varietal innovations in South Africa. S Afr J Sci 113:1-8

Oladosu Y, Rafii MY, Abdullah N, AbdulMalek M, Rahim HA, Hussin G, Abdul Latif M, Kareem I (2014) Genetic variability and selection criteria in rice mutant lines as revealed by quantitative traits. Sci World J Article ID 190531. doi: 10.1155/2014/190531

OlaOlorun BM, Shimelis H, Matthew I, Laing M (2019) Optimizing the dosage of ethyl methanesulphonate mutagenesis in selected wheat genotypes. S Afr J Plant Soil doi: 10.1080/02571862.2019.1610808

OlaOlorun BM, Shimelis H, Laing M, Mathew I (2020) Morphological variations of wheat (*Triticum aestivum* L. em. Thell.) under variable ethyl methanesulphonate mutagenesis. Cereal Research Communications. doi: 10.1007/s42976-020-00092-3

Onoda Y, Saluñga JB, Akutsu K, Aiba S, Yahara T, Anten NPR (2014) Trade-off between light interception efficiency and light use efficiency: implications for species coexistence in one-sided light competition. J Ecol 102:167-175 doi: 10.1111/1365-2745.12184

Osakabe Y, Osakabe K, Shinozaki K, Tran L-SP (2014) Response of plants to water stress. Front Plant Sci 5:86. doi: 10.3389/fpls.2014.00086

Palta JA, Chen X, Milroy SP, Rebetzke GJ, Dreccer MF, Watt M (2011) Large root systems: are they useful in adapting wheat to dry environments? Funct Plant Biol 38:347-354. doi: 10.1071/fp11031

Payne RW, Murray DA, Harding SA (2017) An introduction to the Genstat command language (18th Edition). VSN International Ltd, Hemel, Hempstead, UK, pp 1-137.

Porbeni JBO, OlaOlorun BM, Olalekun OJ, Oyetunde OA (2014) Agro-morphological effect of ethidium bromide on *Vigna unguiculata* (L.) Walp. Cv Ife Brown. Res J Agri 1(5):1-10

Prudhomme C, Giuntoli I, Robinson EL, Douglas B, Clark NW, Arnell R, Dankers BM, Fekete W, Franssen D, Gerten SN (2014) Hydrological droughts in the 21st century, hotspots and uncertainties from a global multimodel ensemble experiment. PNAS USA 111:3262-3267.

R Core Development Team (2020) R: A language and environment for statistical computing. R foundation for statistical computing (version 3.6.3). Vienna, Austria. www.cran.r-project.org/ (Accessed 13 January 2020).

Reddy AR, Chaitanya KV, Vivekanandan M (2004) Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. J Plant Physiol 161:1189-1202

Rehman A, Habib I, Ahmad N, Hussain M, Khan MA, Farooq J, Ali MA (2009) Screening wheat germplasm for heat tolerance at terminal growth stage. Plant Omics 2(1):9-19

Robbins II NE, Dinneny JR (2018) Growth is required for perception of water availability to patter root branches in plants. PNAS USA 115(4):E822-E831. doi: org/10.1073/pnas.1710709115

Sallam A, Alqudah AM, Dawood MFA, Baenziger PS, Borner A (2019) Drought stress tolerance in wheat and barley: advances in physiology, breeding and genetic research. Int J Mol Sci 20(13):31-37. doi: 10.3390/ijms20133137

Sanchez-Bragado R, Elazab A, Zhou B, Serret MD, Bort J, Nieto-Taladriz MT, Araus JL (2014) Contribution of the ear and the flag leaf to grain filling in durum wheat inferred from the carbon isotope signature: genotypic and growing conditions effects. J Integr Plant Biol 56(5):444-454

Shamuyarira KW, Shimelis H, Tapera T (2019) Genetic advancement of newly developed wheat populations under drought-stressed and non-stressed conditions. J Crop Sci Biotechnol 22:169-176. doi: 10.1007/s12892-018-0262-0

Shewry PR, Hey SJ (2015) The contribution of wheat to human diet and health. Food Energy Secur 4:178-202

Shlens J (2014) A tutorial on principal component analysis. Int J Remote Sens 51(2):1-12. doi: org/10.13140/2.1.1593. 1684

Singh K, Punia M, Singh V, Jagdale V (2017) Inter-generation correlation and regression analysis in F_2 and F_3 generations of wheat. Int J Pure Appl Biosci 5:809-816.

Singh NK, Balyan HS (2009) Induced mutations in bread wheat (*Triticum aestivum* L.) cv. 'Kharchia 65' for reduced plant height and improve grain quality traits. Adv Biol Res 3(5-6):215-221

Steele KA, Price AH, Witcombe JR, Shrestha R, Singh BN, Gibbons JM, Virk DS (2013) QTLs associated with root traits increase yield in upland rice when transferred through marker-assisted selection. Theor Appl Genet 126: 101-108.

Tadesse W, Inagaki M, Tawkaz S, Baum M, Van Ginkel M (2012) Recent advances and application of doubled haploids in wheat breeding. Afr J Biotechnol 11(89):15484-15492

Tambussi EA, Bort J, Guiamet JJ, Nogues S, Araus JL (2007) The photosynthetic role of ears in C3 cereals: Metabolism, water use efficiency and contribution to grain yield. Crit Rev Plant Sci 26:1-16

Tambussi EA, Nogué S, Araus JL (2005) Ear of durum wheat under water stress: water relations and photosynthetic metabolism. Planta 221:446-458

Tang L, Yin D, Chen C, Yu D, Han W (2019) Optimal design of plant canopy based on light interception: a case study with loquat. Front Plant Sci 10:364. doi: 10.3389/fpls.2019.00364

Tátrai ZA, Sanoubar R, Pluhár Z, Mancarella S, Orsini F, Gianquinto G (2016) Morphological and physiological plant responses to drought stress in *Thymus citriodorus*. Int J Agron 2016:1-8. Article ID 4165750. doi: org/10.1155/2016/4165750

Tsuji W, Inanaga S, Araki H, Morita S, An P, Sonobe K (2005) Development and distribution of root system in two grain sorghum cultivars originated from Sudan under drought stress. Plant Prod Sci. 8(5):553-562. doi: 10.1626/pps.8.553

Tuberosa R (2012) Phenotyping for drought tolerance of crops in the genomics era. Front Physiol 3: 347

van de Wouw M, Kik C, van Hintum T, van Treuren R, Visser B (2010) Genetic erosion in crops: concept, research results and challenges. Plant Genet Resour 8(1):1-15

van der Merwe JD, Cloete PC (2018) Financial impact of wheat quality standards on South African wheat producers: a dynamic linear programming (DLP) approach. Dev South Afr 35:53-69

Wasaya A, Zhang X, Fang Q, Yan Z (2018) Root phenotyping for drought tolerance: a review. Agron J 8:241. doi:10.3390/agronomy8110241

Wasson AP, Richards RA, Chatrath R, Misra SC, Prasad SVS, Rebetzke GJ, Kirkegaard JA, Christopher J, Watt M (2012) Traits and selection strategies to improve root systems and water uptake in water-limited wheat crops. J Exp Bot 63:3485-3498.

White CA, Sylvester-Bradley R, Berry PM (2015) Root length densities of UK wheat and oilseed rape crops with implications for water capture and yield. J Exp Bot 66:2293–2303. doi: .org/10.1093/jxb/erv077

Xie Q, Mayes S, Sparkes DL (2016) Optimizing tiller production and survival for grain yield improvement in a bread wheat × spelt mapping population. Ann Bot 117(1):51-6. doi: 10.1093/aob/mcv147

Yi XP, Zhang YL, Yao HS, Luo HH, Gou L, Chow WS, Zhang WF. 2016. Rapid recovery of photosynthetic rate following soil water deficit and re-watering in cotton plants (*Gossypium herbaceum* L.) is related to the stability of the photosystems. J Plant Physiol 193:23-34. doi: 10.1016/j.jplph.2016.01.016

Yu TF, Xu ZS, Guo JK, Wang YX, Abernathy B, Fu JD, Chen X, Zhou YB, Chen M, Ye XG, Ma YZ (2017) Improved drought tolerance in wheat plants overexpressing a synthetic bacterial cold shock protein gene SeCspA. Sci Rep 7:44050. doi: 10.1038/srep44050

Zhang GH, Xu Q, Zhu XD, Qian Q, Xue HW (2009) Shallot-Like1 is a kanadi transcription factor that modulates rice leaf rolling by regulating leaf abaxial cell development. Plant Cell 21:719-735. doi: 10.1105/tpc.108.061457

Zhang N, van Westreenen A, Anten NPR, Evers JB, Marcelis LFM (2019) Disentangling the effects of photosynthetically active radiation and red to far-

red ratio on plant photosynthesis under canopy shading: a simulation study using a functional-structural plant model. Ann Bot mcz197:1-12. doi: 10.1093/aob/mcz197

Zhang Z, Castelló A (2017) Principal components analysis in clinical studies. Ann Transl Med 5(17):351. doi: org/10.21037/atm.2017.07.12

Zhu L, Zhang DY (2013) Donald's ideotype and growth redundancy: a pot experimental test using an old and a modern spring wheat cultivar. PLoS One 8:e70006. doi: 10.1371/journal.pone.0070006

Zou KH, Tuncali K, Silverman SG (2003) Correlation and simple linear regression. Radiology, 227(3):617-628.

Zuśka Z, Kopcińska J, Dacewicz E, Skowera B, Wojkowski J, Ziernicka–Wojtaszek A (2019) Application of the principal component analysis (PCA) method to assess the impact of meteorological elements on concentrations of particulate matter (PM10): a case study of the Mountain Valley (the Sącz Basin, Poland). Sustainability 11(6740):1-12. doi: 10.3390/su11236740

Chapter 5

Development of wheat (*Triticum aestivum* L.) populations for drought tolerance and improved biomass allocation through ethyl methanesulphonate mutagenesis

Abstract

The narrow genetic variation in wheat (*Triticum aestivum* L.) for drought adaptive traits and biomass allocation presents a major bottleneck for breeding. Induced mutagenesis can enhance genetic variation and complements conventional breeding for drought tolerance improvement. The aim of this study was to induce mutations in wheat genotype LM43 using three ethyl methanesulphonate (EMS) treatments, and to develop mutant populations involving M_1 to M_4 generations for enhanced drought tolerance, biomass allocation and agronomic performance. Experiments were conducted under controlled environment and field conditions at the University of KwaZulu-Natal. The following data were collected: percentage germination (%G), days to 90% maturity (DTM), plant height (PH), shoot biomass (SB), root biomass (RB), root-shoot ratio (RSR), spike length (SL), spikelet count (SPS), thousand seed weight (TSW) and grain yield (GY) from M_1 to M_4 generations. Significant ($p<0.001$) differences across generations were observed for all assessed traits. The generation × population interaction effects were significant ($p<0.01$) for SB, TSW and GY. There were distinct genetic variation in performance among M_1 to M_4 populations derived from different EMS conditions. The differences among the generations showed that the mutagenic effects were cumulative and exhibited clear segregations at subsequent generations. The new selections with unique biomass allocation, drought response and agronomic performance will be useful for wheat improvement programs.

Keywords: agronomic performance, genetic variation, mutant generations, phenotypic variation, wheat, yield-related traits

5.1 Introduction

An estimated seven billion people across the world depend on bread wheat (*Triticum aestivum* L.; 2n=6x=42, AABBDD) for food, making it the second most important food crop globally (Tilman *et al*., 2011). Wheat is a source of fibre, carbohydrates and proteins (Mahajan and Tuteja, 2005). World production of wheat was approximately 218 million hectares with an output of 772 million tonnes of grain in the year 2017 (FAO, 2018). However, it is projected that a 70% increase in wheat production will be required to suffice human consumption by the year 2060 (Ortiz *et al*., 2008). Global data shows that wheat production and productivity has declined by 5.5% in the last few decades due to climate change-induced drought and heat stresses (Daryanto *et al*., 2016). There is a need to develop wheat cultivars with improved yield potential and enhanced resistance to biotic and abiotic constraints to meet the projected demand for wheat.

Drought stress is one of the major climate change-induced constraints to wheat production and productivity. Daryanto *et al*. (2016) estimated that 21% yield losses can be incurred in wheat on average when moisture availability decreases by 40%. The impact of drought on wheat production is influenced by genotype (Daryanto *et al*., 2016), intensity and duration of the stress (Park *et al.,* 2016; Sun *et al*., 2017), plant health and nutrition (Lobell *et al*., 2008; Yu *et al*., 2018) and genotype-by-environment interactions. Supplemental irrigation has been used as a coping strategy to mitigate the impact of drought stress. However, this option is not feasible due to population growth and scarcity of water for human consumption. Also, the low and erratic rainfall is inadequate to replenish water reservoirs to meet human, industrial and agricultural uses, which may create conflict on water management and use. Developing drought adapted cultivars is among the most sustainable strategies to reduce water demand for agriculture and minimize the impact of drought stress on wheat production.

Several wheat breeding programs spearheaded by the International Maize and Wheat Improvement Centre (CIMMYT), International Centre for Agricultural Research in Dry Areas (ICARDA) and various national organizations initiated the development of improved drought tolerant wheat varieties. The wheat genotypes reportedly exhibited high yield potential and adapted to water limited conditions

prevalent under dryland farming ecologies (Smale *et al*., 2002). The successful development of drought tolerant cultivars depends on identifying and exploiting wide genetic variation for drought adaptive traits in wheat. Drought adaptive traits include flowering and maturity periods, plant height and spike length, kernel weight, tillering capacity and biomass allocation (Abdolshahi *et al.,* 2013; Mehraban *et al.,* 2014; Hooshmandi, 2019). Most adaptive traits have been investigated extensively in studies on drought tolerance and yield in wheat, while biomass allocation has been less reported. Studies on biomass allocation involve quantifying biomass in the above and below ground plant parts. Assessment on root component traits has been neglected due to difficulties associated with root sampling and phenotyping (Den Herder *et al*., 2010; Fang *et al*., 2017). Conventional wheat varieties exhibit narrow genetic variation in root traits because most breeding programs primarily aim to improve harvest indices to increase yield potential. While this has led to increased grain yield production, it has narrowed genetic variation for rooting ability, lowered root to shoot ratios and increased susceptibility to drought stress in modern varieties (White *et al.,* 2015).

Genetic variation allows for selection of superior individuals. Breeding wheat populations for drought tolerance has been limited by a number of factors including large environmental variance encountered during phenotyping, lack of genetic variation and loss of genetic diversity in improved cultivars. The loss of genetic diversity has contributed to stagnant yields and high susceptibility of wheat to environmental stress (Keneni *et al*., 2012; Voss-Fels *et al*., 2015). The narrow genetic diversity in wheat is attributed to continuous directional selection within a narrow range of elite parental lines. A large number of spring wheat cultivars in developing countries were developed involving at least one elite parent bred by CIMMYT (Smale *et al*., 2002). Thus, there is a need to create new variation within a breeding population prior to selecting individuals and developing new cultivars with improved drought stress tolerance. Genetic diversity is enhanced after recombination of genes through controlled crosses. Recombination occurs through sexual reproduction when divergent and complementary parents are crossed. This process does not occur naturally in self-pollinating species such as wheat. Self-pollinating species require emasculation prior to crossing, which is tedious and expensive. Furthermore, conventional breeding by crossing of superior genotypes is a long-term

process that takes about 12 years to develop distinct, stable and uniform varieties (Shivakumar *et al.,* 2018). There is a need to rapidly create genetic variation and develop superior cultivars within a shorter possible period in order to respond to the rapidly changing environment.

Induced mutagenesis, which involves exposing biological material to chemical or physical agents that induce genetic modification through mutations in the DNA, has been used in widening genetic variation in self-pollinated species such as rice, sorghum and wheat (IAEA, 2020). The resultant mutant varieties created through mutagenesis have improved productivity and quality (Kenzhebayeva *et al*., 2014). The use of induced mutagenesis has the potential to create new genetic variation that may not be possible with conventional breeding strategies. For instance, the possible genetic recombination obtained by sexual reproduction after crossing is limited by the initial allelic diversity within the base breeding population (Voss-Fels *et al*., 2015). Mutagenesis broadens the possibilities of allelic diversity of the base population. The mutagenic agent can be manipulated to increase its efficacy by altering its dosage and treatment conditions. It is imperative to generate large mutant populations to enhance the efficiency of mutagenesis and increase the probability of obtaining superior mutant individuals. Various mutagens including ethyl methanesulphonate (EMS) have been used successfully to improve agronomic traits such as flowering and maturity period, reduced plant height, yield, grain quality and tolerance to abiotic and biotic stress (Maluszynski and Kasha, 2002; Kontz *et al*., 2009; Singh and Balyan, 2009; Dhaliwal *et al*., 2015; Nazarenko *et al*., 2018; Lethin *et al*., 2020).

The use of EMS mutagenesis requires less sophisticated equipment, which makes it appropriate for developing countries, and poses low health and environmental hazard risks (Anbarasan *et al*., 2013). However, mutations obtained in crops after exposure to EMS are random and some may not be useful in developing fit-for-purpose varieties. There is a need to develop various populations and select superior mutant genotypes or families after mutagenesis. The selected families can either be used as parental lines to develop breeding populations or released as mutant varieties. Early generation selection in mutant generations is important to advance desirable traits in wheat (OlaOlorun *et al*., 2020a). In a preliminary study

(OlaOlorun *et al*., 2019) established three ideal EMS treatment conditions in wheat genotype LM43. The three pre-determined EMS treatment conditions are suitable for induced mutation and to select ideotypes with high yield, improved drought tolerance and high root to shoot ratios. Biomass allocation to roots has been neglected in wheat breeding despite the importance of roots in nutrient cycling, water extraction, carbon retention to soil. Studies have reported that biomass allocation can be pivotal in drought tolerance (Griffiths and Paul, 2017; Mathew *et al.,* 2019). Therefore, the objectives of this study were to induce mutations in a wheat genotype LM43 using three predetermined ethyl methanesulphonate treatments, and to develop breeding populations involving M_1 to M_4 generations for enhanced drought tolerance, biomass allocation and agronomic performance.

5.2 Materials and methods

5.2.1 Plant materials

Bread wheat genotype designated as LM43, was selected from a panel of germplasm obtained from CIMMYT. The genotype was selected after prior evaluation for its drought tolerance and yield potential (Mwadzingeni *et al*., 2016). A preliminary study to establish optimal conditions for effective mutagenesis with minimum biological damage was conducted prior to embarking on a large-scale mutagenesis (OlaOlorun *et al*., 2019).

5.2.2 Selection procedure

The selection procedure across generations is illustrated in Figure 5.1. Preliminary phenotypic variation analyses showed that EMS mutagenesis was effective on genotype LM43 (OlaOlorun *et al.,* 2019). Hence this genotype was selected for large-scale mutagenesis under three EMS treatment conditions. Breeding populations were developed under four generations based on the three EMS treatment conditions (OlaOlorun *et al*., 2020b). Fresh EMS treated M_1 seeds were planted in the field between March and August 2018. The first breeding population (Population 1) was developed after the treatment of seeds at 0.1% v/v EMS for 1 hour at 25 ^{o}C. The second breeding population (Population 2) was derived after seeds were treated under 0.1% v/v EMS for 1 hour at 30 ^{o}C while the third breeding

population (Population 3) involved seeds exposed to 0.7% v/v EMS for 1.5 hour at 25 ^{o}C. In addition, an untreated seed of the genotype LM43 was included as Population 4 and as a comparative control. M_1 plants were grown to maturity and the grains were harvested and bulked according to their respective treatments and developed into populations. The M_2 seed harvested from M_1 plants were grown out as M_2 plants. During the M_2 generation, 180 individual plants were purposefully selected based on high biomass and yield potential and further evaluated at M_3 and M_4 generations. Selections made in the M_3 and M_4 generations were for improved agronomic performance, drought tolerance and biomass allocation under drought-stressed and non-stressed conditions.

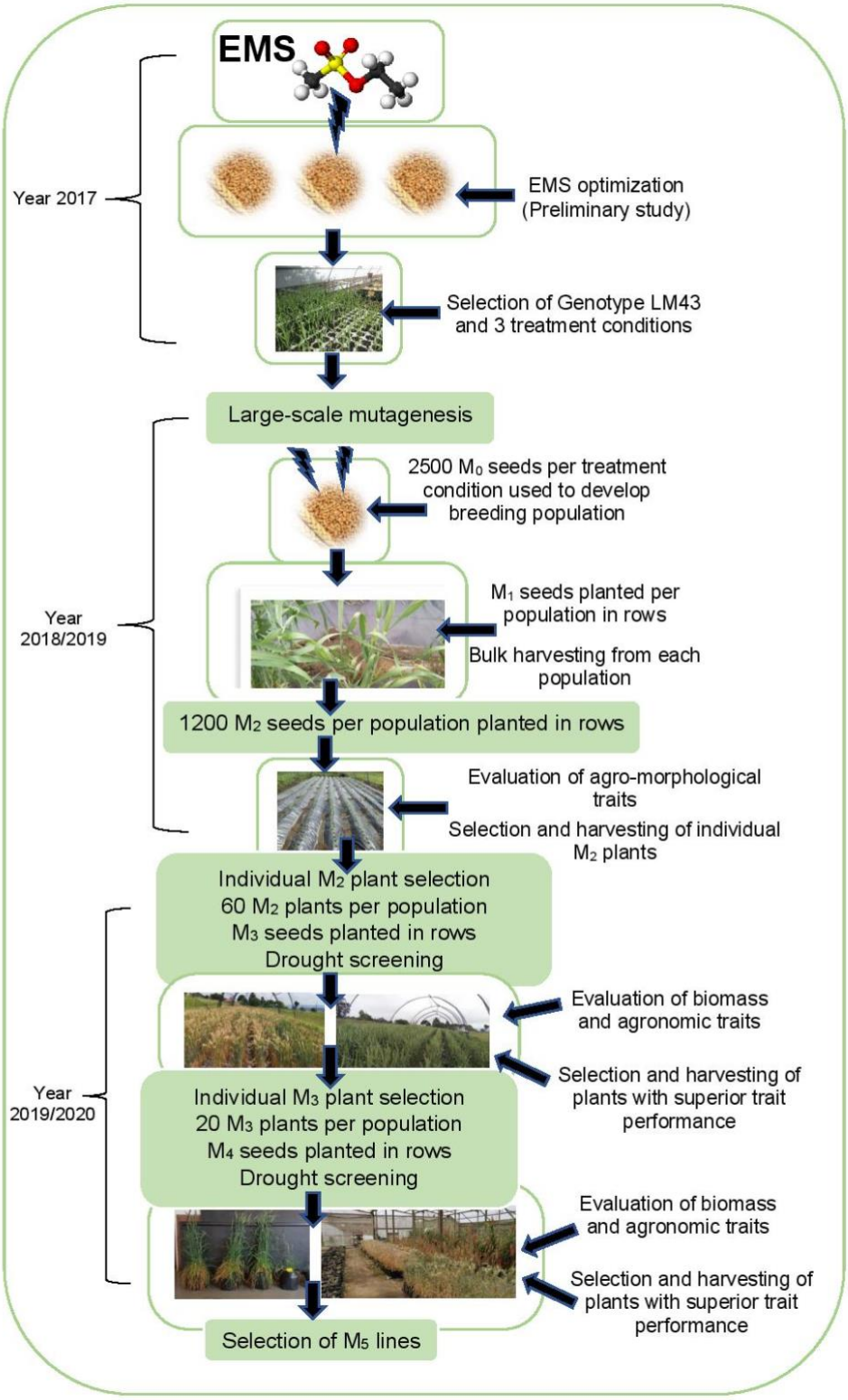

Figure 5.1: Development of wheat populations using three EMS treatments between 2017 and 2020

5.2.3 Planting sites and establishment

The M_1 and M_2 generations were evaluated at Ukulinga Research Farm of the University of KwaZulu-Natal (UKZN) (29^0 40'S, 30^0 24'E; 806m above sea level) during the 2018/2019 cropping season. The M_3 and M_4 generations were established both at Ukulinga Research Farm and under greenhouse condition at the Controlled Environment Facility (CEF) at UKZN during the 2019/2020 cropping season. The meteorological data during the growing period and soil physiochemical properties at both sites are provided in Tables 5.1 and 5.2, respectively. The M_1 and M_2 generations were planted under normal growing conditions with irrigation up to maturity, while the M_3 and M_4 were screened under drought-stressed and non-stressed conditions. Under field conditions, seeds were planted on a 2 m long rows with an intra- and inter-row spacing of 10cm and 60cm, respectively. In the greenhouse, seeds were planted in 10-litre capacity plastic pots filled with pine bark. All experiments were laid out in a randomized complete block design with two replications. For the drought tolerance assessments trails were conducted at both sites, drought was imposed by withholding irrigation water to 35% field capacity at anthesis, while the non-stressed treatment was well watered up to physiological maturity.

5.2.4 Data collection and analysis

Quantitative data from ten selected and tagged plants was collected during each generation to summarize the genetic variation and aid selection. The following data were collected during the M_1 through M_4: days to 90% maturity (DTM), plant height (PH), shoot biomass (SB), spike length (SL), 1000-seed weight (TSW) and grain yield (GY). In addition, percentage germination (%G) and number of spikelets per spike (SPS) were collected at M_1 and M_2 generations, while root biomass (RB) and root-shoot ratio (RSR) were measured at M_3 and M_4 generations. Data collection and measurements were adapted from Mathew *et al*. (2019). The data were subjected to analysis of variance (ANOVA) and vital descriptive statistics were computed using GenStat 18^{th} edition (Payne *et al*., 2017). The relationships among traits were quantified under each stress treatment using the Pearson correlations coefficient with the SPSS version 24 (IBM SPSS, 2016). Trait correlation strengths were categorized into weak, moderate and strong following Zou *et al.* (2003).

Table 5.5: Meteorological data recorded at the study sites during evaluation of the M_1 to M_4 generations of wheat

Planting Site	**Ukulinga**				**CEF**	
Generations	**M_1**	**M_2**	**M_3**	**M_4**	**M_3**	**M_4**
Meteorological variables						
Temp (^{0}C)	21.47	26.78	22.18	26.85	25.71	23.60
RH (%)	69.60	71.55	54.37	78.28	74.26	65.55
Rain (mm)	289	213	205	312	N/A	N/A
RS (MJ/m^2)	13.65	18.28	12.80	17.13	N/A	N/A
EvapT (mm)	78.83	115.50	78.50	105.50	N/A	N/A

Temp: average temperature, RH: average relative humidity, Rain: average total rainfall, RS: average radiation, EvapT: average total evapotranspiration, N/A= Not applicable.
Note: The controlled environment facility (CEF) and Ukulinga research farm are at the University of KwaZulu-Natal

Table 5.2: Physiochemical properties of soils used at the CEF and Ukulinga research farm

Soil Property	**Ukulinga**	**CEF**
Soil pH	4.60	5.10
Total Nitrogen (%)	0.20	0.50
Clay (%)	28.00	16.00
Organic Carbon (%)	2.60	5.50
Calcium (mg/L)	1453.00	1906.00
Electrical conductivity (cmol/L)	11.10	13.70
Potassium (mg/L)	241.00	289.00
Magnesium (mg/L)	369.00	404.00
Phosphorus (mg/L)	39.00	122.00
Bulk density (g/cm^3)	1.00	0.80

Note: The controlled environment facility (CEF) and Ukulinga research farm are at the University of KwaZulu-Natal

5.3 Results

5.3.1 Analysis of variance

The analysis of variance for M_1 and M_2 generations showed that the population × generation interaction effects were significant ($p<0.01$) for SB, TSW and GY (Table 5.3). Significant ($p<0.001$) differences across the mutant generations were observed for all traits measured, while the population main effect showed significant ($p<0.05$) impact on PH, RB and GY.

There were significant ($p<0.05$) differences in PH and SB in response to the three-way population × generation × water regime interaction effects at M_3 and M_4 generations (Table 5.4). The effects of the interaction involving generation and population were significant ($p<0.01$) for SB and TSW. The generation × water regime, and population × water regime interactions resulted in significant ($p<0.05$) differences in SB, SL, TSW and GY among the M_3 and M_4 mutants. Significant ($p<0.05$) differences were observed among the M_3 and M_4 mutants for most traits due to the main effect of mutant generation and water regime, while the breeding population had significant ($p<0.05$) effects on SB and GY only.

Table 5.3: Mean squares and significant tests for traits measured in three EMS-treated and control populations of wheat planted across two and four generations

Source of Variation	df	Traits				df	Traits					
		%G	SPS	RB	RSR		DTM	PH	SB	SL	TSW	GY
Replication	1	6.33	0.06	18.07***	0.001	1	37.41	27.31	446.11*	0.57	0.54	36.47*
Population (P)	3	122.16	1.97	2.23*	0.001	3	33.60	47.21*	56.86	0.18	11.95	0.65*
Generation (G)	1	641.86***	93.34***	62.82***	0.122***	3	2891.15***	338.56***	1054.01***	23.18***	656.90***	84.34***
P X G	3	170.97	1.95	0.29	0.001	9	26.58	8.60	85.09**	0.19	22.07**	5.40**
Error	7	54.25	1.03	0.51	0.001	15	58.14	16.71	94.39	0.35	5.22	6.62

df: degree of freedom, %G: percentage germination, SPS: number of spikelets per spike, RB: root biomass, RSR: root-shoot ratio, DTM: days to 90% maturity, PH: plant height, SB: shoot biomass, SL: spike length, TSW: 1000-seed weight, GY: grain yield, * significant at P≤0.05 probability level; ** significant at P≤0.01 probability level, *** significant at P≤ 0.001 probability level

Table 5.4: Mean squares and significant tests for traits measured in three EMS-treated and control populations of wheat under two water regimes at M_3 and M_4 generations

Source of Variation	df	Traits							
		DTM	**PH**	**SB**	**RB**	**RSR**	**SL**	**TSW**	**GY**
Replication	1	92.73	13.02	346.20	61.28***	0.011*	0.14	0.69	9.24
Population (P)	3	105.74	8.33	0.10*	3.38	0.003	0.09	2.83	0.60*
Generation (G)	1	7397.84***	107.69**	6679.10***	120.70***	0.219***	0.44	211.75***	134.06***
Water Regime (WR)	1	158.17*	63.37*	15.90**	3.02**	0.001***	5.67**	707.16***	124.93***
P X G	3	102.69	23.28	47.70**	4.04	0.003	0.01	34.16***	0.68
G X WR	1	57.45	20.75	246.00*	0.05	0.001	6.44***	6.43*	15.04*
P X WR	3	1.72	25.37	71.40*	7.00	0.001	0.31	11.53*	6.47*
P X G X WR	3	0.63	30.02*	18.80*	5.92	0.001	0.11	0.83	2.08
Error	15	44.08	11.92	114.50	3.33	0.003	0.53	4.15	14.01

df: degree of freedom, DTM: days to 90% maturity, PH: plant height, SB: shoot biomass, RB: root biomass, RSR: root-shoot ratio, SL: spike length, TSW: 1000-seed weight, GY: grain yield, * significant at P≤0.05 probability level; ** significant at P≤0.01 probability level, *** significant at P≤ 0.001 probability level

5.3.2 Quantitative traits measured during M_1 to M_4 generations

Summaries of quantitative traits measured at each generation and from various breeding populations were presented in Tables 5.5 to 5.8. M_1 mutants from Population 2 recorded the shortest plant height (96.23cm), highest shoot biomass (66.06g/m^2) and grain yield (21.75g) compared with other breeding populations (Table 5.5). At the M_2, the mutants from Population 3 recorded the highest SB (61.82g/m^2) while mutant plants developed in Population 2 maintained the highest GY (19.48g). Mutants from Population 1 recorded the shortest PH (83.71cm) and highest TSW (47.29g) (Table 5.6).

At M_3, mutant plants developed in population 2 produced the highest grain yield of 11.58 g under drought-stress condition (Table 5.7). The highest shoot biomass was produced under non-stress and water stressed conditions at 80.04 and 71.51 g/m^2, respectively for mutants in Population 1. Mutants from population 2 recorded the highest root biomass under non-stress and water stress conditions at 14.36 and 13.37 g/m^2, respectively. During the M_4 generation, mutant plants established in population 1 produced the highest root biomass (9.38 g/m^2) under non-stressed condition, while population 2 recorded the highest RB (7.87 g/m^2) under water stress. The highest GY (23.51 g) under non-stressed condition was recorded for mutants from population 3 while mutants from population 1 had the highest GY of 14.53 g under water stressed conditions. Under water stress, mutants from population 2 had the highest SB (32.93 g/m^2) while mutant plants from population 3 recorded the shortest PH of 87.33 cm (Table 5.8). Figure 5.2 summarizes the differences among the M_4 wheat populations under water stressed and non-stressed conditions in two planting sites.

The mean performance of the three EMS-treated populations and the untreated control across four generations are presented in Figure 5.3. Mutants developed from population 3 had the highest SB of 55.43 g/m^2 while the highest GY (18.39 g) was recorded for mutant plants in population 2. The SL and TSW were the highest for mutants from population 2 (13.64 cm and 61.61 g, respectively) across the four generations.

Table 5.5: Mean trait performance of three EMS-treated and control populations of wheat at M_1 generation

Breeding population	Statistics	Traits							
		%G	DTM	PH	SB	SL	SPS	TSW	GY
Population 1	Min	90.33	117.00	65.50	47.30	12.00	19.00	45.02	17.77
	Max	97.00	133.00	115.50	83.60	16.00	28.00	63.22	24.00
	Mean	94.42	123.00	99.84	62.37	13.96	11.92	54.15	20.98
Population 2	Min	91.13	115.00	69.00	46.17	12.00	21.00	56.08	19.87
	Max	97.00	133.00	114.00	84.54	16.50	27.00	75.45	26.14
	Mean	94.95	123.00	96.23	66.06	13.85	24.73	63.14	21.75
Population 3	Min	92.13	112.00	81.50	43.34	11.50	18.00	57.22	16.19
	Max	97.33	132.00	123.00	86.81	16.00	26.00	71.66	27.24
	Mean	93.80	121.00	101.55	63.70	13.55	21.50	63.56	21.30
Population 4 (Control)	Min	80.13	115.00	86.50	25.20	11.00	18.00	59.16	15.44
	Max	95.98	133.00	113.00	30.93	16.00	27.00	77.18	28.25
	Mean	91.68	121.75	102.43	49.34	13.20	22.39	65.60	21.69

%G: percentage germination, DTM: days to 90% maturity, PH: plant height, SB: shoot biomass, SL: spike length, SPS: number of spikelets per spike, TSW: 1000-seed weight, GY: grain yield

Table 5.6: Means of agronomic traits for three EMS-treated and control populations of wheat at M_2 generation

Breeding population	Statistics	Traits							
		%G	DTM	PH	SB	SL	SPS	TSW	GY
Population 1	Min	58.00	84.00	72.00	38.10	8.00	12.00	44.70	12.29
	Max	78.40	85.00	98.00	84.00	12.00	22.00	50.80	21.72
	Mean	68.20	84.50	83.71	58.79	10.75	17.78	47.29	19.31
Population 2	Min	78.64	83.00	75.00	55.72	7.00	15.00	40.90	16.97
	Max	90.40	86.00	101.00	72.43	12.00	21.00	47.10	20.02
	Mean	84.52	84.5	89.76	61.25	10.30	18.15	44.28	19.48
Population 3	Min	70.24	82.00	72.00	31.74	8.00	13.00	42.40	14.38
	Max	80.80	84.00	97.50	86.86	12.50	22.00	47.80	21.02
	Mean	75.52	83.00	85.62	61.82	10.69	18.34	44.91	18.82
Population 4 (Control)	Min	95.95	83.00	86.00	27.64	8.00	13.00	37.50	12.36
	Max	96.20	89.00	95.00	64.40	18.00	21.00	46.20	14.98
	Mean	96.08	86.00	91.25	44.91	11.06	17.68	42.90	14.83

%G: percentage germination, DTM: days to 90% maturity, PH: plant height, SB: shoot biomass, SL: spike length, SPS: number of spikelets per spike, TSW: 1000-seed weight, GY: grain yield

Table 5.7: Mean agronomic performance of three EMS-treated and control populations of wheat at M_3 generation under two water regimes

Breeding population	Statistics	DTM		PH		SB		RB		RSR		SL		TSW		GY	
		NS	WS	NS	WS	NS	WS	NS	WS	NS	WS	NS	WS	NS	WS	NS	WS
Population 1	Min	88.00	95.00	47.00	41.50	4.38	3.50	1.25	1.00	0.068	0.070	5.25	6.00	10.00	10.00	1.42	1.00
	Max	173.00	169.00	129.00	122.50	274.08	196.00	74.38	44.58	0.622	1.400	15.33	15.17	80.00	60.00	69.79	42.30
	Mean	130.47	121.30	96.18	94.81	80.04	71.51	13.72	13.25	0.217	1.280	11.55	11.52	47.11	35.65	13.42	11.54
Population 2	Min	91.00	84.00	43.00	47.00	4.25	4.17	1.63	1.25	0.068	1.023	5.50	5.05	5.00	0.50	0.50	0.21
	Max	172.00	174.00	125.00	124.00	242.92	194.54	77.08	55.36	0.442	1.694	15.50	13.00	70.00	60.00	59.58	37.08
	Mean	131.40	120.29	94.43	94.25	74.29	69.08	14.36	13.37	0.210	0.268	11.02	10.67	46.34	36.35	13.39	11.58
Population 3	Min	92.00	77.00	61.00	51.00	4.29	1.79	2.50	0.63	0.055	0.071	4.40	3.67	15.00	10.00	0.21	0.21
	Max	174.00	176.00	124.00	123.00	259.29	193.13	55.00	48.33	0.926	1.472	15.67	13.17	150.00	150.00	76.67	58.96
	Mean	130.98	121.51	94.19	94.07	71.44	66.57	13.66	11.65	0.212	0.235	10.73	10.32	46.81	37.27	13.76	11.03
Population 4 (Control)	Min	125.00	132.00	85.00	62.50	49.75	33.75	7.78	2.50	0.245	0.239	9.00	6.33	45.00	35.00	6.88	2.19
	Max	150.00	144.00	112.00	102.50	83.25	73.79	18.50	17.00	0.271	0.341	11.67	10.50	55.00	40.00	20.83	17.00
	Mean	137.00	135.75	92.50	85.88	66.36	58.42	11.88	7.05	0.258	0.360	10.21	11.04	50.00	38.75	14.09	10.30

NS: non-stressed condition, WS: water stressed condition, DTM: days to 90% maturity, PH: plant height, SB: shoot biomass, RB: root biomass, RSR: root-shoot ratio, SL: spike length, TSW: 1000-seed weight, GY: grain yield

Table 5.8: Mean agronomic performance of three EMS-treated and control populations of wheat at M_4 generation under two water regimes

Breeding population	Statistics	DTM		PH		SB		RB		RSR		SL		TSW		GY	
		NS	WS	NS	WS	NS	WS	NS	WS	NS	WS	NS	WS	NS	WS	NS	WS
Population 1	Min	86.00	69.00	70.33	69.33	15.42	10.36	1.73	1.55	0.013	0.019	8.80	8.60	30.00	15.00	6.25	2.14
	Max	108.00	118.00	98.00	97.00	65.07	57.37	16.83	15.83	0.207	0.249	21.10	13.00	48.30	41.70	36.50	30.70
	Mean	98.41	92.09	88.09	85.02	34.23	31.67	9.38	7.31	0.061	0.076	10.87	10.76	42.17	32.61	19.07	14.53
Population 2	Min	89.00	82.00	66.17	65.33	16.93	14.78	3.48	1.25	0.005	0.012	8.70	8.50	28.30	15.00	3.52	3.18
	Max	110.00	118.00	99.00	96.71	58.13	52.86	19.83	14.167	0.261	0.237	12.90	12.50	49.30	46.70	35.90	31.20
	Mean	100.02	91.82	89.51	86.32	34.36	32.93	9.30	7.87	0.071	0.081	10.91	10.88	40.75	31.23	18.30	14.21
Population 3	Min	84.00	82.00	68.17	64.67	12.02	8.38	2.65	1.71	0.011	0.014	9.60	8.50	28.30	16.00	1.71	1.13
	Max	109.00	102.00	99.83	97.17	69.73	58.68	20.33	12.50	0.141	0.276	13.80	13.40	48.30	46.70	46.50	37.50
	Mean	98.07	90.57	87.33	85.67	32.36	31.71	9.29	7.09	0.062	0.079	10.66	10.66	41.73	34.16	23.51	14.40
Population 4 (Control)	Min	90.00	88.00	82.83	81.17	37.80	27.63	6.29	5.92	0.022	0.071	9.80	9.20	30.00	16.70	17.75	5.00
	Max	107.00	97.00	95.67	91.83	50.26	36.30	13.67	11.17	0.102	0.103	12.20	12.20	48.30	30.00	26.10	22.30
	Mean	98.50	92.00	89.54	81.29	43.39	31.60	8.35	7.83	0.058	0.082	10.98	10.70	40.40	25.85	21.70	12.26

NS: non-stressed condition, WS: water stressed condition, DTM: days to 90% maturity, PH: plant height, SB: shoot biomass, RB: root biomass, RSR: root-shoot ratio, SL: spike length, TSW: 1000-seed weight, GY: grain yield

Figure 5.2: Differences between drought-stressed and non-stressed M_4 wheat populations at (A) the controlled environment facility and (B) Ukulinga research farm of University of KwaZulu-Natal

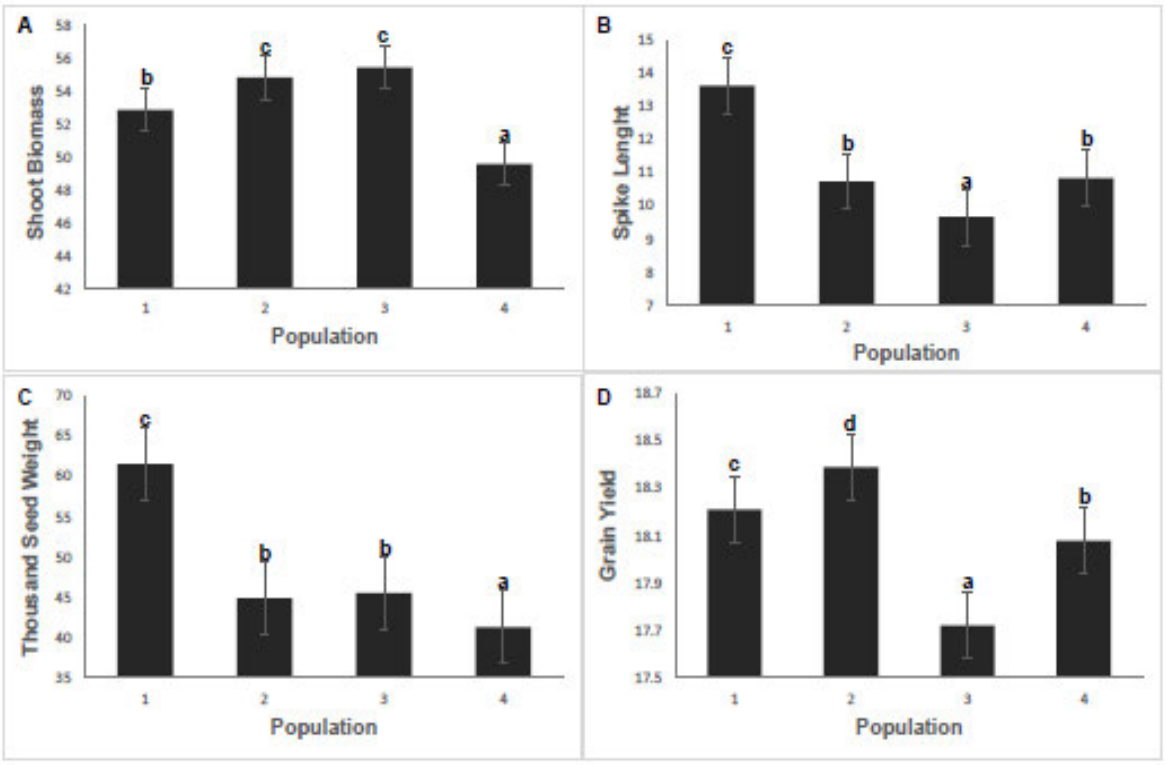

Figure 5.3: Mean performance of (A) shoot biomass, (B) spike length, (C) thousand seed weight and (D) gain yield for three EMS-treated and control populations of wheat during four selection generations. Different letters on error bars represent significant differences at the 0.05 probability level

5.3.3 Variation observed at M_3 generation

During the M_3 generation a large number of individual plants were available for selection based on their breeding population and observed variation in spike and awn morphology (Figure 5.4). Individual plants with variable tiller number (Figure 5.5), plant height and shoot biomass production (Figure 5.6) and, biomass partitioning into roots and shoots (Figure 5.7) were also observed. Qualitative traits had limited variation in M_3 generation when compared with the M_2. However, segregation at M_3 generation produced a wider range of variation (Figures 5.6 and 5.7) making selection more efficient. Various spike mutants with high number of seeds from each breeding population were selected. Subsequently, abnormal and deformed spikes with low number of seeds were discarded. Mutants with high root and shoot biomass and number of tillers were identified and advanced to M_4 generation.

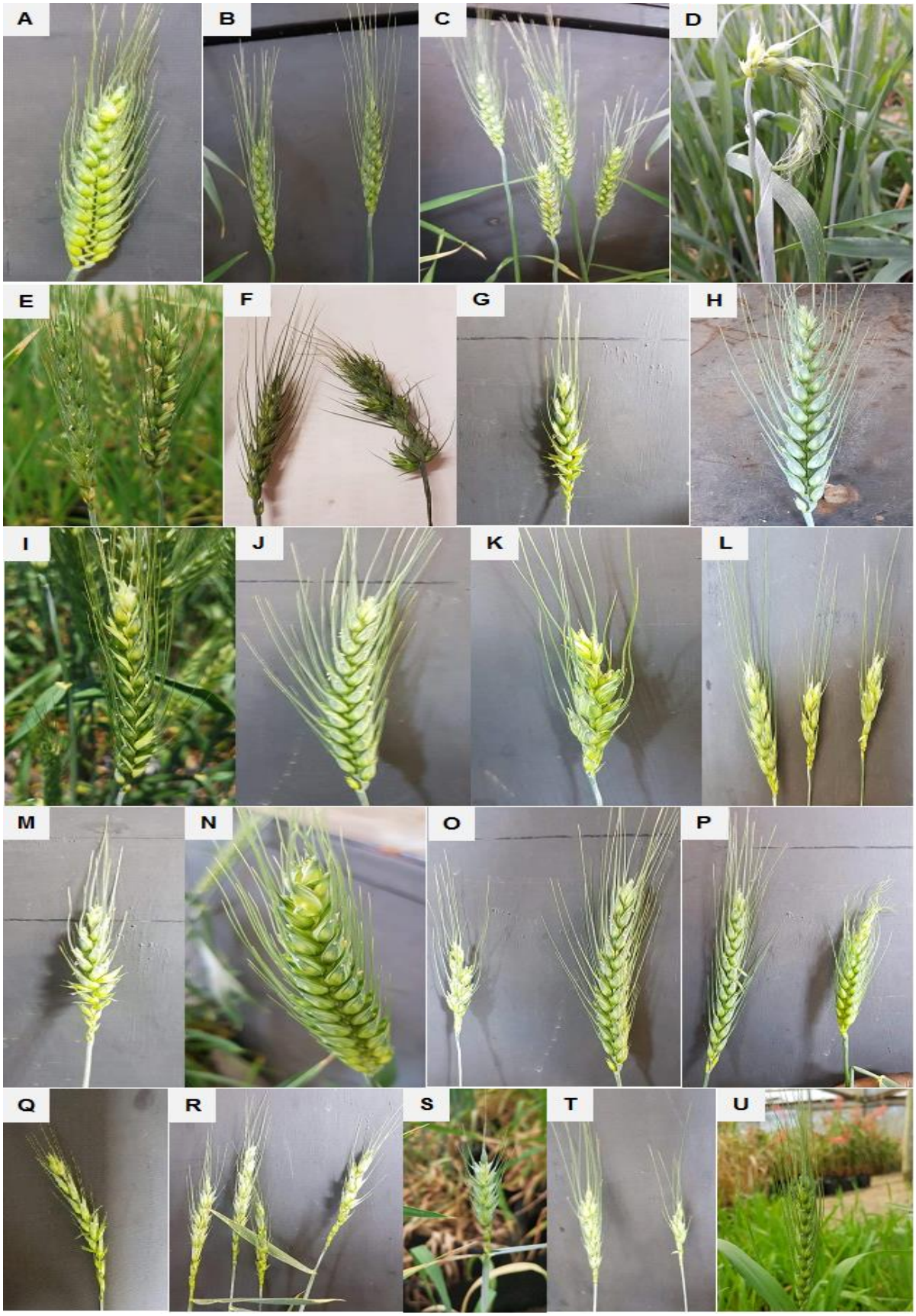

Figure 5.4: Figures A to T show variations in spike and awn morphology in wheat mutant populations during the M_3 generation under the controlled environment facility, Note: A-E (Population 1), F-M (Population 2), N-T (Population 3) and U (Control)

Figure 5.5: Differences in tiller formation in wheat mutants during the M_3 generation (A-F) at the controlled environment facility. Note: A and B (Population 1), C and D (Population 2), and E and F (Population 3)

Figure 5.6: Variation in plant height and shoot biomass production among M_3 wheat populations. Note: B and G (Population 1), C and D (Population 2), E, F and H (Population 3) and A (Control)

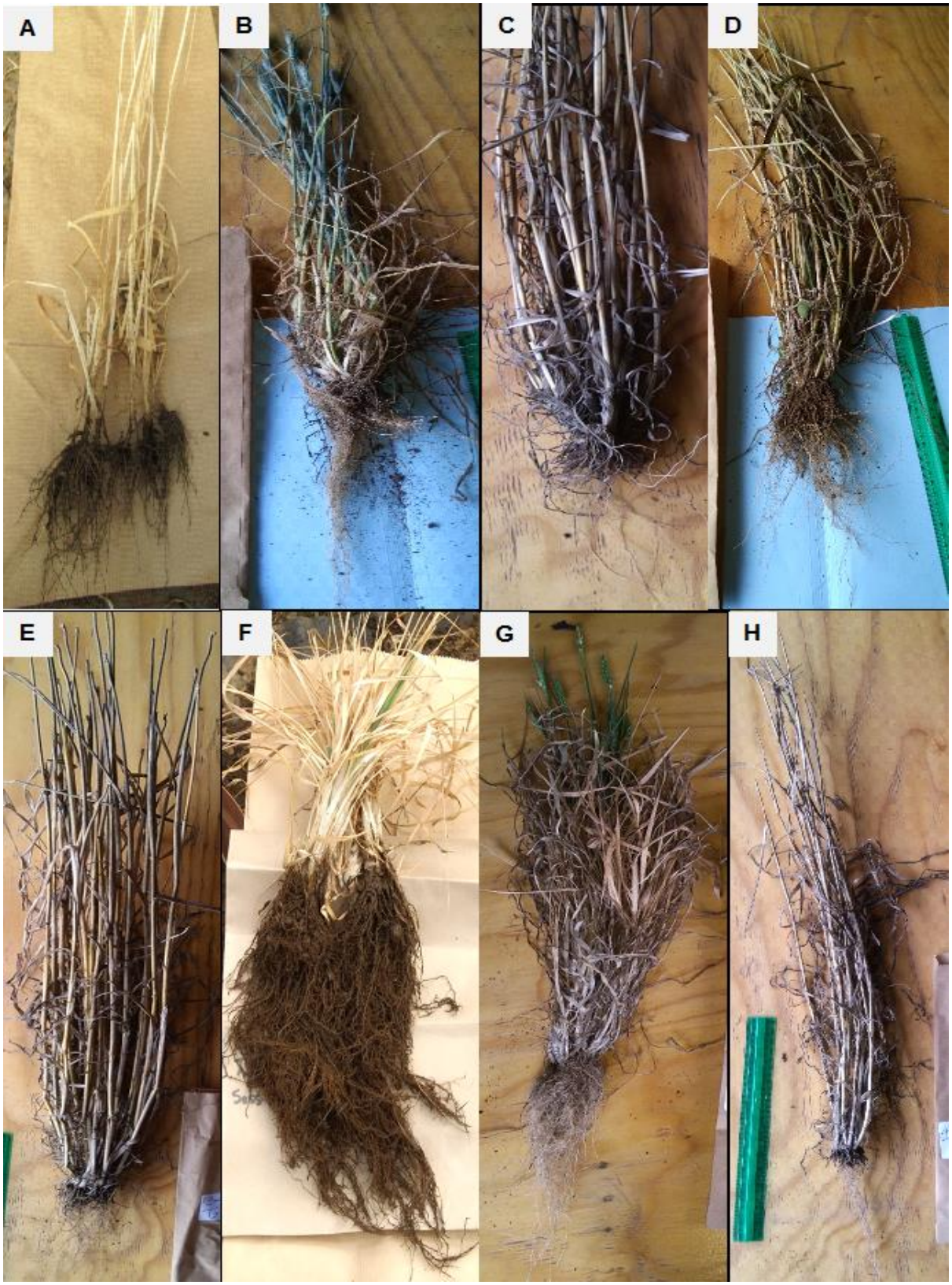

Figure 5.7: Variation in biomass partitioning between roots and shoots among M_3 wheat populations. Note: A and B (Population 1), C and D (Population 2), E, F and G (Population 3) and H (Control)

5.3.4 Quantitative traits association

Grain yield showed positive and significant associations with SL (*r*= 0.71; p<0.001), TSW (*r*= 0.41; p<0.05) and PH (*r*= 0.49; p<0.01). Plant height was positively associated with all traits measured in all the four mutant generations (Table 5.9). Shoot biomass exhibited positive and moderate correlations with DTM (*r*= 0.46; p<0.01), PH (*r*= 0.43; p<0.05) and TSW (*r*= 0.40; p<0.05). Strong and positive correlations existed between TSW and PH (*r*= 0.79; p<0.001), and between TSW and SL (*r*= 0.77; p<0.001). Likewise, DTM had moderate correlation with TSW (*r*= 0.46; p<0.01).

In Table 5.10, the upper diagonal shows correlations recorded under non-stressed conditions. There were strong correlations between GY and RSR (*r*=-0.72, p<0.001), SL (*r*=0.77, p<0.001) and TSW (*r*=0.65, p<0.01). The secondary traits also exhibited interdependent associations. The RSR exhibited a negative and strong association with SL (*r*=-0.72, p<0.001) while SB and RB (*r*=0.83), SB and RSR (*r*=0.62) and, RB and RSR (*r*=0.79) were significantly (p<0.05) correlated. The correlations among traits measured under water stressed conditions were different. Root biomass exhibited stronger correlation with GY (r=0.55, p<0.05) than the correlation between SB and GY (r=0.30, p<0.05) under water stressed conditions (Table 5.10, lower diagonal). SB was correlated to all the other traits, while RB was only correlated to RSR, SB and GY. Grain yield exhibited significant association (p<0.05) with all traits except PH. The RSR exhibited moderately to strong correlations with GY (*r*=0.67, p<0.05), SB (*r*=0.74, p<0.001) and RB (*r*=0.94, p<0.001).

Table 5.9: Pairwise correlation coefficients among agronomic traits measured in three EMS-treated populations of wheat and control during four generations

Traits	DTM	PH	SB	SL	TSW	GY
DTM	-					
PH	0.50**	-				
SB	0.46**	0.43*	-			
SL	0.26	0.82***	0.24	-		
TSW	0.46**	0.79***	0.40*	0.77***	-	
GY	-0.05	0.49**	0.212	0.71***	0.41*	-

DTM: days to 90% maturity, PH: plant height, SB: shoot biomass, SL: spike length, TSW: 1000-seed weight, GY: grain yield, * significant at P≤0.05 probability level; ** significant at P≤0.01 probability level, *** significant at P≤ 0.001 probability level

Table 5.10: Pair-wise correlation coefficients among agronomic traits measured in three EMS-treated and control populations of wheat evaluated under water-stressed (lower diagonal) and non-stressed (upper diagonal) conditions during the M_3 and M_4 generations

Traits	DTM	PH	SB	RB	RSR	SL	TSW	GY
DTM	-	0.53*	0.88***	0.90***	0.75***	-0.35	0.66**	-0.31
PH	0.41	-	0.60*	0.58*	0.14	0.47	0.29	0.26
SB	0.85***	0.77***	-	0.83***	0.62**	0.19	0.36	0.01
RB	0.35	0.48	0.57*	-	0.79***	-0.25	0.55*	0.27
RSR	0.63**	0.51*	0.74***	0.94***	-	-0.72***	0.61**	-0.72***
SL	0.45	0.69***	0.73***	0.15	-0.22	-	0.46	0.77***
TSW	0.70**	0.10	0.69***	0.41	0.57**	0.37	-	0.65**
GY	-0.55*	0.06	0.30*	0.55*	-0.67**	0.33*	0.23**	-

NS: non-stressed condition, WS: water-stressed condition, DTM: days to 90% maturity, PH: plant height, SB: shoot biomass, RB: root biomass, RSR: root-shoot ratio, SL: spike length, TSW: 1000-seed weight, GY: grain yield, * significant at P≤0.05 probability level; ** significant at P≤0.01 probability level, *** significant at P≤ 0.001 probability level

5.4 Discussion

5.4.1 Genotypic variation for phenotypic traits

The significant ($p<0.05$) effects of generations, breeding populations and their interaction for most agronomic traits (Tables 5.3 and 5.4) were probably a result of genetic segregation or cumulative mutagenic effects in subsequent generations. Each generation was self-pollinated to generate the subsequent generation and the variation in subsequent generations could be due to segregation at heterozygous loci caused by mutations in M_1 generation. Similarly, Shorinola *et al.* (2019) found both superior and inferior mutants in later generations of wheat and supposed that the variation emanated from segregating heterozygous mutant phenotypes from the initial population. In other studies, the phenotypic variation between early and subsequent populations was attributed to the cumulative effects of the EMS. Hussain *et al.* (2018) asserted that the variation in subsequent generations is induced by non-lethal cumulative mutagenic effects. Singh *et al*. (2006) reported significant variation between M_1 and M_2 generations with reduced variation in M_3 generation, which was attributed to homozygosity even at mutated loci in advanced generations. Expectedly, phenotypic expression in mutant generations was significantly ($p<0.05$) affected by drought-stress. Traits such as SB, SL, TSW and GY were significantly reduced under drought stress, which corroborated previous studies (Marchin *et al*., 2020). Soil water is vital for biological process and nutrient transport, and inadequate water supply interferes with essential processes leading to poor growth and development (Daryanto *et al*., 2016). Grain yield production under drought condition was likely supported by families that were able to maintain high shoot biomass production. It is reported that agro-morphological shoot-related traits influence grain production under water-limiting environments by translocation of assimilates previously synthesized in the shoot before the onset of detrimental drought stress (Abdolshahi *et al.,* 2015).

5.4.2 Mean performance of EMS treated population

The lack of definite trends in the pattern of variation among the EMS-treated wheat populations point to the random nature of mutations induced by EMS and the wide variation created in subsequent segregating generations. The superior agronomic

performance of EMS mutagenized populations compared to the untreated control for biomass, yield and yield-related traits measured under water stress during M_3 and M_4 generation indicates that EMS is efficient in creating potentially useful variation. It can be assumed that genetic modification through mutations induced by EMS improved drought tolerance. EMS is a potent mutagen and widely used in plant breeding programs (Talabi *et al*., 2012; Luz *et al*., 2016). Mutagenesis has potential to create genetic variation for exploitation in breeding for improved biomass and yield-related traits under water-limiting environments (Addai and Salifu, 2016; Luz *et al*., 2016).

5.4.3 Morphological traits of M_3 mutants

Morphological variations reported in this study revealed the usefulness of chemical mutagenesis in wheat breeding. Detectable mutations result in traits that are morphologically distinct showing that such traits would be underpinned by inheritable genetic changes (Gnanamurthy *et al*., 2012). The various types of spikes observed at the M_3 generation suggested that genetic changes in the spikes were attributable to EMS mutagenesis. Mutations can occur as chromosomal breakage, disturbed auxin synthesis, disruption of mineral metabolism and accumulation of free amino acids leading to variation in spike morphology (Goyal and Khan, 2010). Plants with longer spikes are useful variants that can be exploited to improve the number of seeds per plant, thereby increasing the genetic yield potential. Variations in spike mutants generated from an EMS mutagenized wheat population study were reported by Dhaliwal *et al.* (2015). The positive effect of EMS mutagen was also confirmed by the wide range of variation in biomass traits. Variation in biomass is important to develop a larger breeding parental population for subsequent drought improvement programs, since evaluating and optimizing biomass partitioning will indirectly improve yield especially for water-limited environments.

5.4.4 Trait associations

The significant ($p<0.05$) correlations observed among the measured traits suggest that the traits were interdependent and provide opportunities for simultaneous selection. The positive and significant association exhibited by GY and SB with the other yield related traits indicate the strong linkage between above ground traits. These traits can easily be selected simultaneously during yield improvement. Taller

plants may be able to accumulate adequate photosynthates for attaining higher above ground biomass, which can directly increase grain yield (Zhang *et al*., 2009). Previously, the influence of above ground traits such as biomass production, spike morphology and kernel weight on grain yield was established (Reynolds *et al.,* 2007; Kandić *et al.,* 2009; Rahman *et al.,* 2016). However, genotypes that accumulate excessive above ground biomass at the expense of developing extensive root systems may be susceptible to drought stress, especially in sub-Sahara Africa where wheat is grown under residual moisture and the rainfall is increasingly becoming erratic and inadequate (Haque *et al*., 2016). The stronger associations between the biomass traits and grain yield under water stressed conditions shows that biomass partitioning under drought is more critical for plant survival and attaining reasonable yield. For instance, a slight decrease in rooting capacity is likely to have higher influence on grain yield under drought stressed conditions compared to non-stressed conditions. Genotypes with potential to accumulate higher above ground biomass before the onset of drought stress have comparative advantage under terminal drought as they can translocate assimilates from shoot biomass to grains during grain filling (Kandić *et al.,* 2009). The positive and significant correlations of RB and SB are favourable to develop cultivars with high extensive root biomass for water and nutrient extraction and shoot biomass for building adequate above ground biomass to support grain filling. Palta *et al*. (2011) asserted that a direct and positive relationship between root and shoot biomass is necessary for grain yield improvement. The negative association between RSR and GY regardless of moisture availability conditions indicates that there should be a balance between biomass allocation to above and below ground parts to avoid compromising grain production. Excessively large root systems have high maintenance costs that will limit amount of assimilates available for biomass accumulation in shoots or grain. On the other hand, shallow rooted plants with disproportionately large shoots have higher risk for lodging at anthesis, which increases chances of susceptibility to diseases and pests and reduces grain quantity and quality (Berry, 2013; Dahiya *et al.,* 2018).

5.5 Conclusion

This study established the importance of EMS mutagenesis in creating genetic variation within and among wheat breeding populations. Wide phenotypic variation in

mutants under each breeding population were identified for improving drought tolerance, biomass, yield and yield-related traits. The differences in agronomic performance among the generations exhibited that segregation and cumulative mutagenic effects contributed to the genetic variation. There is a need to ensure that the favourable mutations are fixed in homozygous and homogenous states before cultivar release. Mutants with favourable agronomic performance can be selected as parental populations for crop improvement. Identified mutants need further screening for biomass and yield stability in diverse environments especially in drought stressed areas. Also, further research is recommended to explore molecular techniques to evaluate the genetic basis of the mutations for marker-assisted selection.

5.6 References

Abdolshahi R, Safarian A, Nazari M, Pourseyedi S, Mohamadi-Nejad G (2013) Screening drought-tolerant genotypes in bread wheat (*Triticum aestivum* L.) using different multivariate methods. Arch Agron Soil Sci 59:685-704. doi: 10.1080/03650340.2012.667080

Addai IK, Salifu B (2016) Selection of mutants with improved growth and total grain yield in the M_2 generation of pearl millet (*Pennicetum glaceum* L.) in the Northern region of Ghana. J Agron 15:88-93. doi: 10.3923/ja.2016.88.93

Anbarasan K, Sivalingam HD, Rajendran R, Anbazhagan M, Chidambaram AA (2013) Studies on the mutagenic effect of EMS on seed germination and seedling characters of sesame (*Sesamum indicum* L.) Var.T MV3. Int J Biol Sci 3(1):68-70

Berry PM (2013) Lodging resistance in cereals. In: Christou P, Savin R, Costa-Pierce BA, Misztal I, Whitelaw CBA (eds) Sustainable food production. Springer, New York. doi: 10.1007/978-1-4614-5797-8

Dahiya S, Kumar S, Harender, Chaudhary C (2018) Lodging: significance and preventive measures for increasing crop production. Int J Chem 6(1):700-705

Daryanto S, Wang L, Jacinthe PA (2016) Global synthesis of drought effects on maize and wheat production. PLoS One 11(5):e0156362. doi: 10.1371/journal.pone.0156362

Den Herder G, Van Isterdael G, Beeckman T, De Smet I (2010) The roots of a new green revolution. Trends Plant Sci 15:600-607. doi: 10.1016/j.tplants.2010.08.009

Dhaliwal AK, Mohan A, Sidhu G, Maqbool R, Gill KS (2015) An ethylmethane sulfonate mutant resource in Pre-Green revolution hexaploid wheat. PLoS One 10(12):e0145227. doi: 10.1371/journal.pone.0145227

FAO (Food and Agricultural Organization) (2018) World food and agriculture-statistical pocketbook. Rome, pp 1-254

Fang Y, Du Y, Wang J, Wu A, Qiao S, Xu B, Zhang S, Siddique KHM, Chen Y (2017) Moderate drought stress affected root growth and grain yield in old, modern and newly released cultivars of winter wheat. Front Plant Sci doi:10.3389/fpls.2017.00672

Gnanamurthy S, Mariyammal S, Dhanavel D, Bharathi T (2012) Effect of gamma rays on yield and yield components characters R_3 generation in cowpea (*Vigna unguiculata* (L.). Walp.). Int J Res Plant Sci 2:39-42

Goyal S, Khan S (2010) Cytology of induced morphological mutants in *Vigna mungo* (L.) Hepper. Egypt J Biol 12:81-85

Griffiths CA, Paul MJ (2017) Targeting carbon for crop yield and drought resilience. J Sci Food Agric 97(14):4663-4671

Haque E, Osmani AA, Ahmadi SH, Ban T (2016) Development of pre-breeding technology for root system study and selection of Kihara Afghan wheat landraces (KAWLR) to enhance wheat breeding in the rain-fed region. Breed Sci 66(5):808-822

Hooshmandi B (2019) Evaluation of tolerance to drought stress in wheat genotypes. Res Paper 37(2):37-43

Hussain M, Iqbal MA, Till BJ, Rahman MU (2018) Identification of induced mutations in hexaploid wheat genome using exome capture assay. PLoS One 13(8):e0201918. doi: 10.1371/journal.pone.0201918

IAEA (International Atomic Energy Agency) (2020) IAEA mutant database. IAEA, Vienna. http://mvd.iaea.org/ (Accessed June 15, 2020).

IBM SPSS I (Statistical Package for the Social Sciences) (2016) IBM corporation released. IBM SPSS Statistics for Windows, Version 24.0. Armonk, New York.

Kandić V, Dodig D, Jović M, Nikolić B, Prodanović S (2009) The importance of physiological traits in wheat breeding under irrigation and drought stress. Genetika 41(1):11-20. doi: 10.2298/GENSR0901011K

Keneni G, Bekele E, Imtiaz M, Dagne K (2012) Genetic vulnerability of modern crop cultivars: causes, mechanism and remedies. Int J Plant Res 2:69–79

Kenzhebayevaa S, Turashevaa S, Doktyrbaya G, Buerstmayrb H, Atabayevaa S, Alybaeva R (2014) Screening of mutant wheat lines to resistance for fusarium head blight and using SSR markers for detecting DNA polymorphism. Proceeding International Conference. Agricultural and Biosystem Engineering. IERI Procedia. 8:66-76

Kontz B, Franklin S, Brunel C (2009) Selection of winter wheat mutant lines resistant to drought stress. J Undergrad Res 7(9). openprairie.sdstate.edu/jur/vol7/iss1/9

Lethin J, Shakil SSM, Hassan S, Sirijovski N, Töpel M, Olsson O, Aronsson H (2020) Development and characterization of an EMS-mutagenized wheat population and identification of salt-tolerant wheat lines. BMC Plant Biol 20:18. doi: 10.1186/s12870-019-2137-8

Lobell DB, Burke MB, Tebaldi C, Mastrandrea MD, Falcon WP, Naylor RL (2008) Prioritizing climate change adaptation needs for food security in 2030. Science 319(5863):607-610. doi: 10.1126/science.1152339

Luz KV, Silveira SF, Magalhães da Fonseca G, Groli EL, Figueiredo RG, Baretta D, Kopp MM, Junior AM, Carlos da Maia L, Costa de Oliveira A (2016) Identification of variability for agronomically important traits in rice mutant families. Plant Breed 75(1):41-50. doi: 10.1590/1678-4499.283

Mahajan S, Tuteja N (2005) Cold, salinity and drought stresses: an overview. Arch Biochem Biophys 444(2):13-58

Maluszynski M, Kasha KJ (Eds) (2002) Mutations, *in vitro* and molecular techniques for environmentally sustainable crop Improvement. Kluwer Academic Publishers. Dordrecht, Boston, London. ISBN 1- 4020-0602-0

Marchin RM, Ossola A, Leishman MR, Ellsworth DS (2020) A simple method for simulating drought effects on plants. Front Plant Sci 10:1715. doi: 10.3389/fpls.2019.01715

Mathew I, Shimelis, H, Mutema M, Clulow A, Zengeni R, Mbava N, Chaplot V (2019) Selection of wheat genotypes for biomass allocation to improve drought tolerance and carbon sequestration into soils. J Agron Crop Sci 205:385-400. doi:10.1111/jac.12332

Mehraban SA, Tobe A, Gholipouri A, Amiri E, Ghafari A, Rostaii M (2018) Evaluation of drought tolerance indices and yield stability of wheat cultivars to drought stress in different growth. World J Environ Biosci 7(1):8-14

Mwadzingeni L, Shimelis H, Tesfay S, Tsilo TJ (2016) Screening of bread wheat genotypes for drought tolerance using phenotypic and proline analyses. Front Plant Sci 7(1276):1-12.

Nazarenko M, Lykholat Y, Grygoryuk I, Khromikh N (2018) Optimal doses and concentrations of mutagens for winter wheat breeding purposes. Part I. Grain productivity. J Cent Eur Agric 19(1):194-205. doi: /10.5513/JCEA01/19.1.2037

OlaOlorun BM, Shimelis H, Matthew I, Laing M (2019) Optimizing the dosage of ethyl methanesulphonate mutagenesis in selected wheat genotypes. S Afr J Plant Soil. doi: 10.1080/02571862.2019.1610808

OlaOlorun BM, Shimelis H, Mathew I (2020a) Variability and selection among mutant families of wheat for biomass allocation, yield and yield-related traits under drought-stressed and non-stressed conditions. Journal of Agronomy and Crop Science (Wiley). doi: 10.1111/jac.12459

OlaOlorun BM, Shimelis H, Laing M, Mathew I (2020b) Morphological variations of wheat (*Triticum aestivum* L. em. Thell.) under variable ethyl methanesulphonate mutagenesis. Cereal Research Communications. doi: 10.1007/s42976-020-00092-3

Ortiz R, Sayre KD, Govaerts B, Gupta R, Subbarao GV, Ban T, Hodson D, Dixon JM, Iván Ortiz-Monasterio J, Reynolds M (2008) Climate change: can wheat beat the heat? Agric Ecosyst Environ 126(1-2):46-58

Palta JA, Chen X, Milroy SP, Rebetzke GJ, Dreccer MF, Watt M (2011) Large root systems: are they useful in adapting wheat to dry environments? Funct Plant Biol 38:347-354. doi: 10.1071/fp11031

Park S, Im J, Jang E, Rhee J (2016) Drought assessment and monitoring through blending of multi-sensor indices using machine learning approaches for different climate regions. Agr Forest Meteorol 216:157-169

Payne RW, Murray DA, Harding SA (2017) An introduction to the Genstat command language (18 th Edition). VSN International Ltd., Hemel, Hempstead, UK, pp 1-137.

Rahman M, Barma NCD, Biswas BK, Khan AA, Rahman J (2016) Study on morpho-physiological traits in spring wheat (*Triticum aestivum* L.) under rainfed condition. Bangladesh J Agr Res 41(2):235-250

Reynolds M, Calderini D, Condon A, Vargas M (2007) Association of source/sink traits with yield, biomass and radiation use efficiency among random sister lines from three wheat crosses in a high-yield environment. J Agric Sci 145:3-16

Shivakumar M, Nataraj V, Kumawat G, Rajesh V, Chandra S, Gupta S, Bhatia VS (2018) Speed breeding for Indian agriculture: a rapid method for development of new crop varieties. Curr Sci 115(7):1241

Shorinola O, Kaye R, Golan G, Peleg Z, Kepinski S, Uauy C (2019) Genetic screening for mutants with altered seminal root numbers in hexaploid wheat using a high-throughput root phenotyping platform. G3 (Bethesda) 9(9):2799-2809. doi: 10.1534/g3.119.400537

Singh NK, Balyan HS (2009) Induced mutations in bread wheat (*Triticum aestivum* L.) cv. 'Kharchia 65' for reduced plant height and improve grain quality traits. Adv Biol Res 3(5-6):215-221

Singh SP, Singh RP, Prasad JP, Agrawal RK, Shahi JP (2006) Induced genetic variability for protein content, yield and yield components in microsperma lentil (*Lens culinaris* Medik). Madras Agric J 93(7-12):155-159

Smale M, Reynolds MP, Warburton M, Skovmand B, Trethowan R, Singh RP, Ortiz-Monasterio I, Crossa J (2002) Dimensions of diversity in modern spring bread wheat in developing countries from 1965. Crop Sci 42:1766–1779

Sun P, Zhang Q, Cheng C, Singh VP, Shi PJ (2017) ENSO-induced drought hazards and wet spells and related agricultural losses across Anhui province, China. Nat Hazards 89(2):963-983.

Talebi AB, Talebi AB, Shahrokhifar B (2012) Ethyl methane sulphonate (EMS) induced mutagenesis in malaysian rice (cv.MR219) for lethal dose determination. Am J Plant Sci 3:1661-1665. doi: 10.4236/ajps.2012.312202.

Tilman D, Balzer C, Hill J, Befort BL (2011) Global food demand and the sustainable intensification of agriculture. Proc Natl Acad Sci USA 108(50):20260-20264

Voss-Fels K, Frisch M, Qian L, Kontowski S, Friedt W, Gottwald S, Snowdon RJ (2015) Sub-genomic diversity patterns caused by directional selection in bread wheat gene pools. Plant Genome 8(2):1-13. doi: 10.3835/plantgenome2015.03.0013

Yu H, Zhang Q, Sun P, Song C (2018) Impact of droughts on winter wheat yield in different growth stages during 2001–2016 in Eastern China. Int J Disaster Risk Sci 9:376-391. doi: org/10.1007/s13753-018-0187-4

Zhang GH, Xu Q, Zhu XD, Qian Q, Xue HW (2009) Shallot-Like1 is a kanadi transcription factor that modulates rice leaf rolling by regulating leaf abaxial cell development. Plant Cell 21:719-735. doi: 10.1105/tpc.108.061457

Zou KH, Tuncali K, Silverman SG (2003) Correlation and simple linear regression. Radiology. 227(3):617-628

Chapter 6

An overview of research findings and implications for breeding

6.1 Introduction and objectives of the study

Wheat (*Triticum aestivum* L.; 2n=6x=42, AABBDD) is one of the most important cereal crops globally. It has diverse economic importance along its value chains. However, the production and productivity of wheat is constrained by recurrent drought and heat stress, especially in the Sub-Saharan Africa. Improving the productivity of wheat under dry-land farming systems is imperative to meet food demand for the rapidly growing population. Creating and assessing genetic variation through induced mutagenesis is a prerequisite to widen genetic diversity in wheat and develop highly productive and climate-resilient cultivars. Therefore, the aim of this research was to improve drought tolerance and grain yield, and to enhance biomass allocation in wheat under water-limited conditions through mutation breeding. The specific objectives of the study included:

a. To determine the optimum dosage and treatment conditions of ethyl methanesulphonate (EMS) for effective mutagenesis to induce genetic variation for drought tolerance and enhanced biomass allocation in selected wheat genotypes.
b. To evaluate agro-morphological variation induced through mutagenesis using three pre-determined EMS treatments for a specific wheat genotype to develop breeding populations.
c. To evaluate genetic variation present in the third mutation generation (M_3), and to select families with superior biomass allocation, grain yield and agronomic performance evaluated in the controlled and field environments under non-stressed and drought-stressed conditions
d. To induce mutations in a selected wheat genotype using three EMS treatments and develop breeding populations involving M_1 to M_4 generations for enhanced drought tolerance, biomass allocation and agronomic performance

6.2 Research findings in brief

6.2.1 Optimizing the dose of ethyl methanesulphonate mutagenesis in selected wheat genotypes

Seeds of three genotypes (LM29, LM43 and LM75) were treated with three EMS doses (0.1, 0.4 and 0.7% v/v) at three temperatures (25, 30 and 35 °C) for three exposure periods (1hr, 1.5hrs and 2hrs). Seedling parameters were collected under greenhouse conditions after mutagenesis to establish a suitable lethal dose (LD_{50}). The main outcomes of this study were:

a. The estimated lethal doses (LD_{50}) using simple linear regression model for LM43, LM29 and LM75 were 0.32, 1.07, and 1.81%v/v EMS, respectively, indicating differential response of the test genotypes.
b. The ideal treatment conditions for effective mutagenesis were 0.7% EMS for 2 hours at 35 °C for genotypes LM29 and LM43 and 0.4% EMS for 2 hours at 25 °C for genotype LM75.

6.2.2 Agro-morphological variations of wheat (*Triticum aestivum* L.) under variable ethyl methanesulphonate mutagenesis

A prototype wheat genotype LM43 was subjected to EMS mutagenesis under three pre-determined treatment conditions (0.1% v/v for 1 hour at 25 °C, 0.1% v/v for 1 hour at 30 °C and 0.7% v/v for 1.5 hours at 25 °C). After mutagenesis, the treated seeds were planted, and treatments evaluated under field conditions for two generations. The following agronomic traits were assessed: percentage germination (%G), number of days to heading (DTH), number of days to maturity (DTM), number of tillers (TN), productive tillers (PTN), plant height (PH), spike length (SL), spikelets per spike (SPS), kernels per spike (KPS), thousand seed weight (TSW), grain yield (GY) and above ground biomass (AGB). Descriptive statistics and analysis of variance were calculated. Lethality, mutation frequency, efficiency and effectiveness were calculated at M_2. The core findings of this study were:

a. There were significantly ($p< 0.05$) higher SPS, KPS and GY at the M1 generation. TN, KPS and GY increased significantly at M_2 implying significant genetic differences between the test generations.

b. EMS treatment with 0.1% v/v for 1 hour at 30 °C was the most effective and efficient in inducing mutation with minimum amount of biological damage in this population.
c. Plants treated with 0.1% v/v EMS for 1 hour at 25 °C recorded the highest rate of lethality.
d. Macro-mutations were also exhibited as abnormalities in spike, peduncle, awn and flag leaf morphology.

6.2.3 Variability and selection among mutant families of wheat for biomass allocation, yield and yield-related traits under drought-stressed and non-stressed conditions

Hundred and eighty M_3 mutant families of wheat developed from three above pre-determined EMS treatment conditions were evaluated in greenhouse and field environments under drought-stressed and non-stressed conditions. Data were collected on days to 50% heading (DTH), days to 90% maturity (DTM), plant height (PH), number of productive tillers (PTN), shoot biomass (SB), root biomass (RB), total biomass (TB), root-shoot ratio (RSR), spike length (SL), spikelet per spike (SPS), thousand seed weight (TSW) and grain yield (GY), and subjected to analysis of variance, Pearson correlation, principal component and cluster analyses using the R software version 3.6.3. The core findings of the study were:

a. Significant ($p<0.05$) differences in biomass, yield and agronomic traits were found among genotypes, environments and their interactions, suggesting that genotypic and environmental factors were crucial determinants of biomass allocation and yield improvement.
b. Superior families designated as 52, 159, 103, 126, 145 with improved drought tolerance and high biomass allocation to roots were recommended for developing breeding populations with high grain yield potential, improved drought tolerance and increased biomass allocation to roots
c. Selected mutant families from each cluster were considered for genetic advancement due to their genetic dissimilarities and high mean performance in grain yield and total biomass production.
d. The significant and positive correlations between GY and yield-related traits under both water regimes indicate that these traits can be used for genotype selection with enhanced GY.

6.2.4 Development of wheat (*Triticum aestivum* L.) populations for drought tolerance and improved biomass allocation through ethyl methanesulphonate mutagenesis

Three breeding populations of wheat developed using the above three pre-determined EMS treatment conditions were evaluated for drought tolerance, biomass allocation and agronomic performance. Evaluation of mutant populations was carried out in greenhouse and field environments under drought-stressed and non-stressed conditions during M_1 to M_4 generations. Data were collected on percentage germination (%G), days to 90% maturity (DTM), plant height (PH), shoot biomass (SB), root biomass (RB), root-shoot ratio (RSR), spike length (SL), spikelet per spike (SPS), thousand seed weight (TSW) and grain yield (GY). Descriptive statistics, analysis of variance and Pearson correlation analysis were calculated using Genstat 18th edition and SPSS version 24. The core findings of the study were:

a. Significant ($p<0.001$) differences across generations were observed for all traits suggesting that EMS mutagenesis provided adequate genetic variation for selection across generations.
b. The significant ($p<0.01$) interaction effects found between generations and breeding populations for SB, TSW and GY indicated that there were distinct genetic variation in performance among M_1 to M_4 populations derived from different EMS conditions.

6.3 Implications of the research findings for wheat breeding to improve yield and drought tolerance, and enhance biomass allocation using chemical mutagenesis

The following implications for breeding were noted:

a. The information generated from the optimization study can be used as a guide for large-scale wheat mutagenesis to create new genetic variation for drought tolerance and biomass improvement.
b. The selected superior families are recommended for genetic advancement and genetic analysis to identify genomic regions controlling biomass allocation and yield gains under drought stress.
c. Significant variation across generations were observed for biomass, yield and yield-related traits suggesting that the genetic effects after mutagenesis were

cumulative and mutants can be selected in subsequent generations until desirable phenotypes are obtained.

d. This is the first study that reported novel mutants specifically selected for enhanced biomass allocation as a strategy to improve yield and drought tolerance in wheat.

6.4 Research recommendations

a. There is a need to test the recommended populations in multiple sites to assess their stability and ensure that the favorable mutations are fixed in homozygous and homogenous states.
b. Mutants with unique biomass allocation, drought response and agronomic performance can be selected as parental populations for future genetic enhancement and crop improvement programs.
c. Molecular analysis is recommended to evaluate the genetic basis of the mutations for marker-assisted selection. Recommended populations can be useful resources in functional mutagenomics and cytogenetics.