EFFECTS OF MUTAGENESIS ON DROUGHT TOLERANCE AND AGRONOMIC TRAITS OF SELECTED BREAD WHEAT (*Triticum aestivum* L.)

BY

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DECLARATION

Declaration by the Candidate

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DEDICATION

This thesis is dedicated to my parents, Mr. Douglas Githinji and Dr. Felicity Githinji, for their continued prayers and supports both morally and financially without which I would not have made it.

ABSTRACT

Wheat (*Triticum aestivum* L) is an annual crop widely cultivated as small-grain cereal. It is among the most important cereal crops grown that contribute significantly to food security and is ranked second after maize. Abiotic stress conditions such as drought cause extensive losses in wheat production worldwide. In Kenya, arid and semiarid lands (ASALs) represent 83% of total land area, and experience crop failure due to drought stress. With very few drought tolerant varieties that have been recommended and released for commercial production, there is need to develop more drought tolerant wheat varieties. The objectives of this study were to screen for drought tolerance in some Mutant wheat lines *in vitro* using Polyethylene Glycol (PEG); to screen for drought tolerance at seedling stage and to morphologically characterize the Mutant wheat lines. For the *in vitro* test, a completely randomised design with three replicates was used in which four wheat varieties, that is, Mutant 1, Mutant 2, Chozi and Duma were placed in petri dishes on moistened filter paper. Drought stress was induced by creating different water potential of PEG that is 0, -3, -9 and -15 bars. Data was recorded on root length, shoot length and root length/shoot length ratio. For the seedling test, the seeds were sown in polythene bags in a complete randomised design and at the early stages they were screened for several parameters including emergence percentage (EP), emergence index (EI), emergence rate index (ERI), energy of emergence (EE), mean emergence time (MET), desiccation tolerance index (DTI) and percentage seedling recovery (PSR). The morphological characterization was done using a randomized complete block design where the four wheat varieties were grown and normal agronomic practices carried out. Data on plant height, spike length, number of tillers, days to 50% heading and yield was collected. Data was subjected to analysis of variance using GENSTAT 12th edition and means separated using Duncan multiple range test. Correlation was done by Pearson Correlation Coefficients. Results indicated that there was a significant difference ($p \leq 0.05$) between Mutant 1 and Mutant 2 having longer roots, shoots and a better root to shoot ratio compared to Chozi and Duma in the different PEG concentrations used. Mutant 1 and Mutant 2 had a better performance in terms of EP, EI, ERI, EE, MET, DTI and PSR compared to the other wheat varieties (Chozi and Duma) evaluated. Morphological characterizations revealed significant difference in terms of yield, Mutant 2 had the highest mean grains per spike followed by Chozi then Mutant 1. Mutant 2 and Mutant 1 had shorter days to heading compared to Chozi and Duma varieties which means they mature earlier which is a good trait for drought tolerance. Hence, the two Mutant varieties can be candidates for commercial production in ASALs regions. It is recommended that the Mutant wheat be screened for other biotic and abiotic stresses that affect wheat production in Kenya.

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LIST OF ACRONYMNS AND ABBREVIATIONS

ABA	Abscisic acid
ASALs	Arid and Semi-Arid Lands
CAN	Calcium Ammonium nitrate
CIMMYT	International Maize And Wheat Improvement Centre
CMS	Cell membrane stability
CIAT	International Centre for Tropical Agriculture
CRD	Complete randomised design
DNA	Deoxyribonucleic Acid
DT	Desiccation tolerance
DSI	Drought Susceptibility Index
EI	Emergence index
EP	Emergence percentage
ERI	Emergence rate index
EPZA	Export processing zone authority
FAO	Food **a**nd Agriculture Organization
HI	Harvest Index
IAEA	International Atomic Energy Agency

IBPGR	International Board for Plant Genetic Resources
ICARDA	International Centre for Agricultural Research In The Dry Areas
ISTA	International Seed Testing Association
KALRO	Kenya Agriculture and Livestock Research Organization
KARI	Kenya Agricultural Research Institute
LSD	Least significant difference
MET	Mean emergence time
MW	Molecular weight
PEG	Polyethylene Glycol
QTL	Quantitative Trait Locus
WU	Water use
WUE	Water Use Efficiency

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CHAPTER ONE

INTRODUCTION

1.1 Background Information

Wheat (*Triticum aestivum* L.) is an annual crop that is widely cultivated as a small-grain cereal. Botanically, wheat belongs to the genus *Triticum* in the tribe *Hordeae* of the family *Poaceae*. *Triticum* species fall into three natural groups based on chromosome number: diploid (2n=14), tetraploids (2n=4x=28) and hexaploid (2n=6x=42). The term wheat is normally used to refer to the cultivated species of the genus *Triticum* (Acquaah, 2012).

Wheat is the major food crop in the world and sustains the majority of the world population. It is grown on about 225 million hectares worldwide from the equator to latitudes of 60°N and 44°S and at altitudes ranging from sea level to more than 3000 m. Approximately 600 million tons of wheat is produced annually, roughly half of which is in developing countries (Goyal and Manoharachary, 2014).

Roughly 95% of the wheat crop is common wheat (*Triticum aestivum* L.), used for making bread, cookies, and pastries, whereas the remaining 5% is durum wheat (*T. turgidum* ssp. durum), used for making pasta and other semolina products (Dubcovsky and Dvorak, 2007). In Kenya, wheat is among the most important cereal crops grown that contribute significantly to food security in the country. It is ranked second after maize in Kenya (CIMMYT, 2015).

Wheat yield is significantly influenced by global climate change and water resources scarcity in the environment (Al-Ghamdi, 2009). Drought is one of the environmental stresses seriously limiting crop production in the majority of agricultural fields of the world (Abedi and Pakniyat, 2010) and recent global climate change has made this situation more adverse (Anand *et al.,* 2003).

Wheat is highly adapted to diverse ranges of environments from tropical to temperate. Although wheat has a wide range of climatic adaptability, its productivity is limited by several abiotic stresses. Among these stresses, heat and drought is the most widespread limitation to wheat productivity under dry-land conditions (Narayanan *et al.,* 2014). Other abiotic stresses include low nitrogen content and salinity. Abiotic stress conditions cause extensive losses to agricultural production worldwide (Sávio *et al.,* 2012).

About one fifth of the developing world's wheat is grown in the arid and semi-arid lands (ASALs) (Ndiema *et al.,* 2011). Despite these limitations the world's ASALs and cropping environment are increasingly becoming crucial for food security in developing world. Worldwide, land with inherent characteristics for arable crop production continues to decline, while population growth and demand for wheat are rising. Therefore, gains in wheat production in ASAL environments are important because it is unlikely that increased production in the favourable environments will be sufficient to meet the projected growth demand for wheat from the present to 2020 (Geleta *et al.,* 2015).

In Kenya, wheat has been grown since the turn of the 20th century at first by large-scale farmers and later by small-scale producers (Kinyua, 1997). It was traditionally cultivated in the high attitudes ranging from 1, 800 meters above sea level to 3,000 meters above sea level. Recently wheat has been introduced into lower dry lands areas of Machakos, Naivasha, Koibatek and Lower Narok among others (Kinyua, 1997).

Eastern Africa has recently experienced periodic food shortages partly attributable to intermittent but severe droughts (AFREPREN, 2012). In Kenya, ASALs represent 83% of total land area (56.9 million ha), which experience frequent crop failure due to drought stress. Approximately 300,000 hectares of these areas are arable land and are not fully utilised because annual rainfall is low (200-400mm), unreliable (40-50 days) and highly erratic (Kinyua *et al.*, 2000). Lack of widely adapted drought resistant wheat varieties and unfavourable weather patterns pose a major problem to wheat production in these areas (Kimurto *et al.*, 2003). Consequently, there is need to develop improved plant materials that have drought tolerance and allow efficient utilisation of limited rain water.

1.2 Statement of the Problem

Drought stress is a major cause of decreased yield in food crops. Drought can affect any crop in any part of the world and the consequences can be disastrous. Increasing food demand and declining water availability are the major threats to world food security. It is obviously true that present and future wheat food security will depend on water scarce environments. Farmers and researchers are striving hard to produce drought tolerant wheat varieties (Mafakheri *et al.*, 2010).

Biotechnology techniques such as mutation breeding are being used to improve local varieties of basic food crops for yield and quality, early maturity and tolerance to biotic and abiotic stresses (IAEA, 2013). This is essential especially in Kenya where only three varieties, Chozi, Duma and Ngamia, that had been recommended and released for commercial production in the marginal rainfall areas of Kenya

(Kinyua *et al*., 1998; Ndiema, 2010). There is need to develop more drought tolerant wheat varieties for the ASALS.

1.3. Justification of the Study

Drought is one of the most important abiotic stress limiting plant growth and crop productivity globally. The imperative to develop drought-resistant crops is intensifying due to increasingly limited water supplies for crop irrigation. In addition, global climate change, such as elevated temperatures, changing precipitation patterns and increased water deficit in arid and semi arid areas (White *et al*., 2004; Misra, 2014).

Screening for drought tolerance under field conditions involves considerable resources (land, people, and power) and requires suitable environmental conditions for the effective and repeatable phenotypic expression of drought tolerance that is due to the genotype, hence there is a need to use simple but effective early screening methods that relate to the field phenotypes (Kim *et al*., 2001).

An alternative may be to screen material under laboratory or green-house conditions using seedlings as test material. The use of *in vitro* screening using polyethylene glycol (PEG) is a fast and effective method for screening plants for drought tolerance. Screening genotypes at seedling stages have several benefits, such as low cost, ease of handling, less laborious and getting rid of susceptible genotypes at earliest (Meeta *et al*., 2013). Seedling trait is an important aspect of any crop breeding programme, since the final stand of a crop mostly depends on seedling characteristics. Various factors like seed germination, seedling vigour, growth rate, mean emergence time and desiccation tolerance affect the yield of a crop (Crosbie *et al*., 1980; Noorka and Khaliq, 2007).

1.4 General Objective

To determine the effect of mutation on agronomic traits of selected wheat and potential use of Mutants in ASALs for food security.

1.4.1 Specific Objectives

1. To determine drought tolerance in the Mutant wheat *in vitro* using Polyethylene Glycol (PEG)
2. To screen for drought tolerance in the selected Mutant wheat at seedling stage.
3. To determine the effect of induced mutagenesis on agronomic traits of selected Mutant wheat varieties

1.5 Hypotheses

i. Mutation has an effect on the agronomic traits of wheat

ii. Mutant wheat are drought tolerant at seedling stage and *in vitro*

CHAPTER TWO

LITERATURE REVIEW

2.1 Wheat Production in Kenya

Wheat growing areas in Kenya include the scenic Rift Valley regions of Uasin Gishu, Narok, Elgeyo Marakwet, Nakuru and Timau areas. These areas have altitudes ranging between 1200m and 1,500m above sea level, with annual rainfall varying between 800 mm and 2,000 mm, with up to 2,500 mm on higher grounds (EPZA, 2005). Wheat varieties grown in Kenya include the high-yielding and drought-resistant Njoro BW1 and Njoro BW2. Njoro BW1 is grown in dry parts such as Lower Narok, Machakos and Laikipia. Njoro BW2 performs well in acidic soils, like parts of Uasin Gishu and Nakuru districts. Other varieties are the Duma and Chozi, mainly grown in dry areas, and Kenya Heroe and Kenya Yombi (EPZA, 2005).

2.2 Breeding for Drought Resistance

Drought tolerance was defined by Hall (1993) as the relative yield of a genotype compared to other genotypes, subjected to the same drought stress. Drought susceptibility of a genotype is often measured by reduction in yield under drought stress (Farshadfar and Elyasi, 2012). Developing drought-tolerant wheat genotypes has been the focus of many wheat improvement programs. Three major approaches for improving drought tolerance in wheat have been used for many years.

1) The empirical selection for yield under water-limited condition. This has been widely used and the good performance of modern cultivars is testimony to the success of this approach. However, there are clear signs that the rates of gain are declining and are insufficient to meet demand (Tester and Langridge, 2010).

2) Define physiological ideotypes for improved yield under water-limited conditions, identify sources of variation for these traits and introduce these traits into elite varieties (Richards *et al*., 2010).

3) Marker assisted selection based on screening for desirable alleles at Quantitative Trait Locus (QTL) for drought tolerance (Gupta *et al*., 2010).

Conventional breeding needs the detection of genetic variability under drought between plant genotypes, or between sexually compatible cultivars, and introduction of tolerance line with proper agronomic traits. Although conventional breeding for water stress resistance has had some prosperity, it is a slow process which is limited by the availability of proper genes for breeding. In traditional breeding, crosses are partially uncontrolled and breeders select parents to cross, but at the genetic approach, the outcomes are unpredictable (Wieczorek, 2003).

Conventional breeding strategies are labour-intensive which requires great efforts to separate undesirable traits from desirable traits, and this is not economically suitable. For instance, crops must be back-crossed again over lots of growing seasons to breed undesirable traits generated by random mixing of genomes (Nezhadahmadi *et al.*, 2013). On the other hand, the improvement of resistant plants through genetic engineering needs detection of important genes to respond as stress resistance crops by transferring novel genes into plants (Nezhadahmadi *et al.*, 2013). Drought affects the activity of a vast number of genes, and gene expression experiments have detected various genes that are induced and repressed under drought stress (Sahi *et al.*, 2006). The nature of drought tolerance makes the management difficult in traditional breeding techniques.

The best strategy for crop productivity, yield improvement and yield stability under soil moisture deficient conditions is to develop drought-tolerant crop varieties. Understanding the plant response in dry conditions is a fundamental part of producing drought-tolerant cultivars (Zhao *et al.,* 2008). Selection of wheat genotypes with better adaptation to drought stress leads to an increased productivity of the wheat (Sayyah, *et al*., 2012). Comparison of relative performance of genotypes in drought stress and non-stress conditions can be considered as a favorable index for making a decision about selection of the tolerant genotypes in breeding plants for dry environments (Sayyah, *et al.,* 2012).

Breeding for drought tolerance by selecting solely for grain yield is difficult because the heritability of yield under drought conditions is low due to small genotypic variance or due to the large variances in the genotype-environment interaction (Farshadfar *et al*., 2015). Improvement of the wheat plant itself gives a long-term avenue for raising its yield in the field. Thus, under stressful environments, yield per se is not always the most suitable or easiest selection trait and an approach based on the evaluation and incorporation of physiological traits into a potentially high-yielding genotype may improve its adaptability and thus its response to environmental variability (Farshadfar *et al*., 2015).

2.3 Measurement of Drought Resistance in Plant Breeding

Various procedures are used for measuring drought resistance in crop plants. The most commonly used procedures include: leaf water retention, photosynthesis, yield performance, and root lengths of seedlings (Agri Info, 2011). These techniques can be used for large scale screening of segregating populations in breeding programme.

2.3.1 Leaf Water Retention

In this method leaves are excised from the plant and are allowed to dry. The slower drying genotypes are considered as drought tolerant. In other words, the high water retainer genotypes are considered as drought tolerant. The cut leaf method was used in wheat, barley and oats (Agri Info, 2011). In wheat, two varieties Pelissier and Pitic were significantly better water retainers when leaves were excised from three week old plants. These two varieties are highly resistant to drought. Some investigators use tissue water potential as an index of water stress under drought conditions. The tissue water potential is measured with the help of thermocouple psychrometer. The portable field psychrometer is widely used for measuring drought resistance in segregating populations (Agri Info, 2011).

2.3.2 Rate of Photosynthesis

The rate of photosynthesis during and after moisture stress is an important index of drought resistance. In wheat, Pitic 62 (a drought resistant variety) exhibited high photosynthetic rate under drought conditions in several tests. Now, portable non-destructive photosynthesis analyzers are available which can be used in the fields for large scale screening of germplasm as well as segregating populations in standing crops. The genotypes which have high photosynthetic rate under moisture stress are considered as drought resistant, because such genotypes give higher yield than those having low photosynthetic rate. A simple portable photosynthesis analyzer makes it possible to measure photosynthesis of many plants within a short time (Agri Info, 2011).

2.3.3 Yield Performance

Superior yield performance under moisture stress conditions is an important and reliable index of drought tolerance. The yield tests should be conducted in drought prone areas at several locations or for several years. These will help in identification of genotypes with drought tolerance and also in the elimination of drought susceptible lines. The yield test should be conducted under both fields as well as glass house conditions. Moreover, large number of populations should be grown. This will enhance chances of obtaining superior drought resistant genotype (Agri Info, 2011).

2.3.4 Root Length of Seedlings

The root length during seedling stage is also used a measure of drought tolerance. In wheat during root length of 5-7 days old seedlings grown in sand was related to root mass at maturity. In a more recent study it was observed that root mass after 30 days was reliable index of root mass at maturity (Agbicodo *et al.,* 2009). Thus those genotypes which have longest root during seedlings stage also exhibit extensive root system at maturity. This is a simple and quick method of measuring drought tolerance in each crop season. Moreover, after screening, superior plants can be replanted and grown to maturity. Some workers use hydroponics tank to measure the root growth of seedlings (Agbicodo *et al.,* 2009).

Breeding for drought tolerance is complicated by the lack of fast, reproducible screening techniques and the inability to routinely create defined and repeatable water stress conditions when a large amount of genotypes are to be evaluated efficiently (Ghasemi and Farshadfar, 2015) . Achieving a genetic increase in yield under these environments has been recognized to be a difficult challenge for plant breeders while progress in yield has been much higher in favorable environments (Richards *et al.*, 2002). Thus, drought indices which provide a measure of drought based on yield loss under drought conditions in comparison to normal conditions have been used for screening drought-tolerant genotypes (Mitra, 2001).

In recent years, interest in crop response to environmental stresses has greatly increased because severe losses may result from heat, cold, drought and high concentrations of toxic mineral elements (Rabiei *et al.,* 2012). Wheat grows as a rain-fed crop in semi-arid areas, where large fluctuations occur in the amount and frequency of events from year to year and insufficient water is the primary limitation to wheat production worldwide (Ashraf and Harris, 2005). Generally, different strategies have been proposed for the selection of relative drought tolerance and resistance, so some researchers have proposed selection under non-stress conditions (Betran *et al.*, 2003), others have suggested selection in the target stress conditions (Ceccarelli and Grando, 2000) while, several of them have chosen the mid-way and believe in selection under both non-stress and stress conditions (Rajaram and Van Ginkle, 2001).

Drought stress at the grain filling period dramatically reduces grain yield (Noorifarjam *et al*., 2013). Various quantitative criteria have been proposed for selection of genotypes based on their yield performance in stress and non-stress environments. Based on these indicators, genotypes are compared in irrigated and rain fed conditions or in different levels of irrigations (Taghian and Abo-Elwafa, 2003).

2.4 Screening Wheat for Drought

In rain fed agricultural conditions, water limitation may be a critical constraint to primary productivity under future scenarios or more arid climates due to global environmental changes (Fischer *et al.*, 2001). In meteorological terms, drought implies a relatively prolonged dry spell (absence of rains) resulting in moisture stress in the soil detrimental to crop growth especially in rain fed agriculture.

Physiologists refer drought as water stress irrespective of length of period. Water deficit occurs in the plant whenever transpiration exceeds absorption. It may be due to reduced absorption of water from soil, excessive water loss through evaporation or both. In other words, drought may be defined as the inadequacy of water availability including precipitation and soil moisture storage capacity and distribution during the lifecycle of the crop to restrict expression of its full genetic potentials (Trivedi, 2006).

Drought is one of the most common environmental stresses that affect growth and development of plants. Drought continues to be an important challenge to agricultural researchers and plant breeders. It is assumed that by the year 2025, around 1.8 billion people will face absolute water shortage and 65% of the world's population will live under water-stressed environments. Tolerance to water stress is a complicated parameter in crops' performance because it can be influenced by several characteristics (Nezhadahmadi *et al.*, 2013). Tolerance can be divided into two parts; drought avoidance and dehydration tolerance (Kuol, 2004). Drought avoidance includes changes in root depth, water use efficiency and changes in plants' mechanism to use water.

Dehydration tolerance consists of plants' capability to partially dehydrate and grow again when rainfall continues (Salekdeh, *et al.,* 2002). Adaption of plants to drought stress is a vital issue to develop new improve methods for increasing stress tolerant plants (Rizhsky *et al.*, 2002). Many factors can affect plants' responses to drought stress such as plant genotype, growth stage, severity and duration of stress, physiological process of growth (Chaves *et al*., 2003), different patterns of genes expression (Denby and Gehring, 2005), different patterns of the activity of respiration (Ribas-Carbo *et al*., 2005), activity of photosynthesis machinery (Flexas *et al*, 2004), and environmental factors. Drought stress can have effects on genes expression, and detection of genes during water stress is crucial to observe their responses (Rizhsky *et al*., 2002).

Dehydration avoidance refers to the capacity of the plant to maintain an adequate plant water status under the soil water deficit. The plant 'avoids' the stress by different strategies that include deep rooting (Lopes and Reynolds, 2010), reduced leaf area, reduced growth duration (early flowering), and mechanisms related to increased water use efficiency (WUE) (Araus *et al.*, 2002). Water Use Efficiency has been defined as the ratio of total biomass or above-ground biomass or grain yield against water used during crop growth or evapotraspiration (Angus and van Herwarden, 2001). Under drought, grain yield can be expressed as a function of water use (WU), WUE, and harvest index (HI) (Salekdeh *et al*., 2009).

Dehydration tolerance, on the other hand, refers to the ability to remain viable and grow after a dehydration–hydration cycle. Therefore, dehydration tolerance indicates the tissue capacity to withstanding desiccation. It is generally measured as the ability of the plant to recover from stress. The range of tolerance to dehydration would depend on the species and stage of development. Further progress in breeding for drought tolerance will depend on our ability to identify suitable cultivar level differences in expression of the gene networks involved in stress adaptation (Pierre, *et al*., 2012).

Water stress affects almost every developmental stage of the plant. However, damaging effects of this stress was more noted when it coincided with various growth stages such as germination, seedling shoot length, root length and flowering (Khayatnezhad *et al*., 2010). Among these critical stages, water stress induced during seedling stage has been exploited in various crop species to screen germplasm i.e. wheat (Dhanda *et al*. 2004), sorghum (Bibi *et al*., 2010), maize (Khayatnezhad, 2011) and sunflower (Rauf *et al*., 2008).

Screening genotypes at seedling stages has several benefits, such as low cost, ease of handling, less laborious and getting rid of susceptible genotypes at the earliest. Furthermore seedling traits have also shown moderate to high heritability with additive type of genetic variance within and over environments (Rauf *et al.,* 2008). An alternative approach to field experiments related to moisture stress is to induce stress using PEG in an *in vitro* condition. Water, the most important component of life, is rapidly becoming a critically short commodity for humans and their crops. Shortage of water limits plant growth and crop productivity in arid regions more than any other single environmental factor (Oraki *et al.*, 2012). Arid and semi arid environment besides other factors may induce water stress during crop growth and development, resulting in a reduction in crop yield (Raza *et al*., 2012).

Turgor maintenance plays an important role in drought tolerance of plants which may be due to its involvement in stomatal regulation and hence photosynthesis

(Osakabe *et al*., 2014). Water stress reduces crop yield regardless of the growth stage at which it occurs. However, the adverse effect of water stress on crop yield may be more pronounced at some particular growth stage (Cakir, 2004) depending upon the nature of crop species and even genotypes within the species. Several physiological characteristics have been reported as being reliable indicators for the selection of germplasm possessing drought tolerance. These characteristics include seed germination and seedling growth in nutrient solutions with low osmotic potential the degree of electrolyte leakage (cell membrane stability, CMS) from drought-damaged leaf cells and the water relations of plants (Chai, *et al*., 2010).

Screening of different crop plants to abiotic stresses is used to find out most resistant variety (Zafar-ul-Hye *et al.,* 2007). While screening the wheat cultivars for most drought sensitive and most drought tolerant genotype, it was considered that the success of these approaches under green house and lab conditions depends on their same behavior under field condition also (Zafar-ul-Hye *et al.,* 2007).

Wheat production in ASALs is hampered by low moisture levels during the growing season. Decreased plant water potential leads to reduced photosynthesis, increased stomatal diffusion resistance (Blum, 2011) and subsequently lowers yields. Drought stress from heading to maturity gives greater reduction in grain yield than from emergence to tillering (Mushatq *et al*., 2015). However, post anthesis grain yield loss is associated with kernel abortion or reduction in kernel growth leading to fewer grains per head and low kernel weights (Kimurto *et al*., 2003). Drought stress at seedling and flowering stages of maize is estimated to cause annual yield losses of about 17% in the tropics (Sande *et al.*, 2014). Severe water stress from seedling stage to maturity reportedly reduced all grain yield components, particularly the number of fertile ears per unit area by 60%, grain number per head by 48%, dry matter and harvest index (Kiliç and Yağbasanlar, 2010).

Breeding for drought tolerance is complicated by the fact that several types of abiotic stress can challenge crop plants simultaneously. High temperatures, high irradiance, scarcity of water, and nutrient deficiencies are commonly encountered under normal growing conditions but may not be amenable to management through traditional farm practices. Certain soil properties such as composition and structure can also affect the balance of these different stresses (Whitmore and Whalley, 2009).

Some biochemical mechanisms may have opposing effects under different stresses; therefore tackling tolerance to one stress may lead to sensitivity to another. For example, some plants avoid heat stress by increasing stomatal conductance and, consequently, evaporative cooling. However, closing the stomata helps to decrease the loss of water and maintain turgor under conditions of low soil water potential. The two mechanisms will conflict when high temperature and drought occur simultaneously, which is frequently the case in a Mediterranean climate. Moreover, the osmo-protectant amino acid proline has a toxic effect under heat stress and its accumulation may not be an appropriate tolerance mechanism in field conditions when heat and drought stresses are combined (Salekdeh *et al.*, 2009).

Although the reductionist approach of studying isolated stress has considerably increased our knowledge of tolerance mechanisms, interaction between multiple stresses and stress combinations should be studied so as to make progress relevant to field conditions. In addition to these confounding environmental factors, a drought research programme should also consider plant phenology. By completing its life cycle before the onset of severe water deficit, plants are often able to escape drought (Chaves *et al*., 2003). This mechanism of avoidance is deployed by rapid phenological development, developmental plasticity, and remobilization of pre-anthesis assimilates to grain. A short life cycle is particularly advantageous in environments with terminal drought stress or where physical or chemical barriers inhibit root growth (Fleury *et al*., 2010).

Plant maturity strongly influences grain yield under dry conditions (Ouk *et al*., 2006). A further confounding factor is plant morphology, particularly plant height and tillering. Small plants with few tillers can show higher WUE than tall multi-tillered plants. Since the genotypic variation of WUE is mainly driven by variations in water use rather than by variations in plant assimilation, the selection for high WUE may result in smaller plants, instead of high yield under drought (Blum, 2005). Some QTLs for carbon isotope discrimination (a measurement of WUE) in wheat were actually associated with variation in heading date and plant height (Rebetzke *et al.*, 2008). Breeding for a shortened crop life cycle has been a very successful strategy in Mediterranean conditions (Araus *et al*., 2002).

However, in well developed agricultural regions, crop flowering time has already been optimized by breeders so that the plant’s phenology matches its environment (Passioura, 2007). Therefore, research should now focus on optimizing vegetative development to manage biomass and ensure effective assimilates remobilization to grain when water supply becomes limiting.

Rain, especially in East Africa is erratic and favourable seasons are often interspersed with unfavourable ones (Kimurto *et al*., 2003). Kenya´s dry lands have long been unfit for agriculture, being at best merely grazing areas for wild animals and livestock. Today, the landscape is more picturesque and productive, lined with golden stalks of wheat-yielding precious grain for Kenya´s farms and families. This has been due to development of wheat varieties that are drought tolerant (Kinyua, *et al*., 2010).

Kenya agriculture, livestock research organization, which is the country's premier institution for agricultural research and technology transfer, has now released a new wheat variety (Kenya IBIS), one that is much higher yielding than other varieties grown in these regions. As a result, small farming families will realize harvests on farmlands once considered too poor to cultivate, to the country's social and economic benefit (Kinyua, *et al*., 2010). Arid and Semi- Arid Lands have low and erratic amounts of rainfall ranging between 500mm and 1000mm annually (Njuguna et al 2010). These rains result in short growing seasons which are unsuitable for production of conventional wheat varieties. Several drought tolerant wheat varieties such as Ngamia, Duma, Njoro Bread wheat 1, and Chozi are suited for these marginal areas.

2.5 Components and Mechanisms of Drought Resistance

There is no unified abiotic stress resistance mechanism for drought at the level of the whole plant or the single gene (Blum, 2004). The traits associated with avoidance and tolerance can be constitutive (differing between genotypes) or adaptive (vary with the stage of the life cycle). Drought avoidance and drought tolerance involve different mechanisms and processes, and phenology is the single most important factor influencing whether a plant avoids drought. Drought stress is highly variable in its timing, duration and severity, and this result in high environmental variation and $G{\times}E$ variation (Bänziger *et al*. 2006). The whole-plant response to stress is complex because it is determined by component traits that interact and differ in their individual responses to the intensity and duration of water deficits and temperature.

The use of managed stress environments can be very effective in breeding for drought tolerance. However, it is important to apply sufficient drought stress intensity to maximize *G*×*E* (Bänziger *et al*. 2006).

2.6 Morphological Derivations of Drought Tolerance in Wheat

According to a study by Deňcić *et al*. (2000), wheat is paid special attention due to its morphological traits during drought stress including leaf (shape, expansion, area, size, senescence, pubescence, waxiness, and cuticle tolerance) and root (dry weight, density, and length). Shi *et al.* (2010) stated that drought can affect vegetative and reproductive stages. Therefore, understanding plants' responses to drought at every life stage is crucial to progress in genetic engineering and breeding. Rizza *et al*. (2004) observed that early maturity, small plant size and reduced leaf area can be related to drought tolerance. Lonbani and Arzani (2011) claimed that the length and area of flag leaf in wheat increased while the width of the flag leaf did not significantly change under drought stress. Leaf extension can also be limited under water stress in order to get a balance between the water absorbed by roots and the water status of plant tissues (Nezhadahmadi *et al.,* 2013). According to the study of Rucker *et al*. (1995), drought can reduce leaf area which can consequently lessen photosynthesis. Moreover, the number of leaves per plant, leaf size, and leaf longevity can be shrunk by water stress (Shao *et al.,* 2008). It was observed that leaf development was more susceptible to water stress in wheat (Nezhadahmadi *et al.,* 2013).

Root is an important organ as it has the capability to move in order to find water (Hawes, 2000). It is the first organ to be induced by drought stress (Shimazaki *et al.,* 2005). In drought stress condition, roots continue to grow to find water, but the hairy organs are limited to development. This different growth response of shoots and roots to drought is an adaptation to arid conditions (Niu *et al.*, 2008). To facilitate water absorption, root-to-shoot ratio rises under drought conditions (Nezhadahmadi *et al.*, 2013) which are linked to the Abscisic Acid (ABA) content of roots and shoots (Rane and Maheshwari, 2001). The growth rate of wheat roots is diminished under moderate and high drought conditions. In wheat, the root growth was not markedly decreased under drought (Nezhadahmadi *et al.*, 2013).

2.7 Drought Management

2.7.1 Drought-Tolerant Varieties

In the past decade, there have been several efforts to generate drought-tolerant wheat through breeding. Cross-breeding among wild wheat species at the International Centre for Agricultural Research in the Dry Areas (ICARDA) created germplasm that have higher yields under drought. In wheat breeding programs, seeking for increased yield has been a priority to improve drought tolerance of plants. However, before successful genetic manipulation can be made, it is important to characterize the physiological parameters of drought-tolerant or sensitive cultivars (Veesar *et al.*, 2007). Analysing physiological determinants for yield which responds to water stress may also be helpful in breeding for higher yields and stability of genotypes under drought conditions. Traits to select either for stress escape, avoidance or tolerance, and the framework where breeding for drought stress is addressed will depend on the level and timing of stress in the targeted areas.

However, selecting for yield itself under stress-alleviated conditions appears to produce superior cultivars, not only for optimum environments, but also for those characterized by frequent mild and moderate stress conditions (Veesar, *et al.*, 2007). This implies that broad avoidance/tolerance to mild/moderate stresses is given by constitutive traits also expressed under stress-free conditions (Araus *et al.*, 2002). Keeping in view the importance of identifying water-stress tolerant wheat genotypes, water stress conditions can be imposed to wheat at various stages of crop growth and development. The stresses can be given at tillering, booting, and grain forming stages. Root system size (RSS) of wheat can be a selection target for drought tolerance. During dry periods, crops expand their roots to deeper soil regions and they are able to alter their morphology. For instance, the hairy organ mass is decreased but the mass of roots is increased. Wheat genotypes with good water management are able to bear high yields in drought conditions (Manschadi *et al*., 2006). Genotypes with proper water management could be used to create new breeding lines and cultivars with developed drought tolerance.

2.7.2 Agronomic Practices in Drought Management

Drought stress includes different agronomic, soil, and climatic factors which vary in the time of occurrence, duration, and intensity. It has effect on yield and can also diminish benefits of crop handling performances including management of fertilizer or pest and disease (Schneekloth *et al*., 2012). Drought management strategies are very important and have to concentrate on extraction of available soil moisture, crop establishment, growth, biomass, and grain yield. There are many agronomical ways to manage drought stress such as control of field irrigation methods (surface or furrow, sprinkled, and drip) and identification of drought tolerant sources through developing screening methods under environmental conditions. So, for drought screening, not only analysing sources of replications, variation among plots, and repeated experiments are needed, but also sprinkler irrigation, rainout shelters, and evaluation of drought susceptibility index (DSI) are important (Schneekloth *et al.*, 2012).

In drought management strategies, increasing biomass and seed yield, crop establishment, and maximum crop growth have to be considered. For example, to improve yield in drought-prone area, these steps are essential: frequency of drought stress occurrence in the target environment, matching phenology of crop (sowing, growth period, flowering, and seed filling) with period of soil moisture and climatic regimes, developing a way for the better use of irrigation, and increasing soil water to crop through agronomic management practices. Furthermore, good knowledge of what type of stress is more frequent in target environment is essential in drought breeding. Yield stability under water shortage condition and crop water productivity should be the goal (Schneekloth *et al*., 2012).

In drought stress condition, the aim is to preserve the source of water. These sources include snow, rain, and irrigation water. Water conservation can be achieved by surface residue during the growing season. Wheat residue diminished the evaporation rate during the season (Nezhadahmadi *et al.*, 2013). Residue also slows movement of water and allows much time for the water to penetrate into the soil. Rotation of crop can preserve the total water needs by irrigation. In winter wheat, it can decline requirements for irrigation. Rotation of crops also makes the irrigation season to have much time frame in comparison with a single crop. In breeding for drought tolerance, productions of biomass and WUE are imperative elements of agronomy (Pierre *et al*., 2012). There is a risen interest in improving WUE of plant genotypes so that plants can develop and bear better under drought condition (Nezhadahmadi *et al.,* 2013).

Current climate change is projected to have a significant impact on temperature and precipitation profiles, increasing the incidence and severity of drought. Drought is the single largest abiotic stress factor leading to reduced crop yields, so high-yielding crops even in environmentally stressful conditions are essential (Fleury *et al*., 2010). This is not the first time we face this situation, in which increasing demands on existing resources are not feasible, and higher-yielding crops are required to balance crop production with increasing human food consumption. A similar scenario occurred 50 years ago due to the high rate of population growth, and it was overcome by selective breeding of high grain yielding semi dwarf Mutants of wheat, a process coined Green Revolution (Tester and Langridge, 2010). In relation to current development of cultivars, which are higher yielding even in water-limited environments, one of the major targets is *Triticum* species, being one of the leading human food sources, accounting for more than half of total human consumption (Fleury *et al*., 2010).

The increasing incidence and importance of drought in relation to crop production has rendered it as a major focus of research for several decades. However, studying drought response is challenged by the complex and quantitative nature of the trait. Drought tolerance is complicated with environmental interactions. In the analysis of a plant’s drought response, the mode, timing, and severity of the dehydration stress and its occurrence with other abiotic and biotic stress factors are significant (Reynolds, 2006). Furthermore different species, subspecies, and cultivars of crops show variation in their drought tolerance under same conditions, emphasizing the importance of genetic diversity as an underlying factor of drought and its significance in drought-related research. Plants exhibiting high drought tolerance are the most suitable targets of drought-related research and are the most promising sources of drought-related gene and gene regions to be used in the improvement of modern crop varieties. These include the natural progenitors of cultivated crops, and for wheat improvement, *Ae tauschii*, which is more drought tolerant than *Triticum* and wild emmer wheat (*T. dicoccoides*), which harbors drought tolerance characteristics, lost during cultivation of modern lines, is of great importance (Ashraf *et al.*, 2009).

Although development of higher-yielding crops under water-limited environments is the most viable solution to stabilizing and increasing wheat production under current climatic conditions, it is challenged by the nature of drought response as a trait and the complex genomic constitution of wheat (Farooq, 2009).

2.8 Use of Poly-Ethylene Glycol (PEG) in Screening for Drought Stress Tolerance

The high-molecular-weight Poly-Ethylene Glycol is the most selective agent used to induce water stress *in vitro* (Rao and Jabeen, 2013). Poly-Ethylene Glycol which is a water-soluble polymer, nontoxic and non-metabolized by the cells, is available in a wide range of molecular weights (e.g., PEG-4000, PEG-4500, PEG-6000, PEG-8000 and PEG-10000) (Lawlor, 1970). Because of its high molecular weight, PEG cannot cross membranes and cannot get into the cell to change its osmotic potential (Yang *et al.*, 2010).

It simulates the water deficit *in vitro* in a manner similar to that observed *in vivo* in cells of intact plants subjected to drought conditions. Due to many reasons, PEG is considered superior to other solutes to induce water stress (Bouiamrine and Diouri, 2012). This selective agent was first used to select drought tolerant genotypes in sorghum (Duncan *et al*., 1995), durum (Hsissou and Bouharmont, 1994) and soft wheat (El-Haris and Barakat, 1998). The insertion of *in vitro* techniques in a breeding program offers considerable opportunities for genetic improvement of plants by saving space and time required by conventional methods (Bouiamrine and Diouri, 2012).

Polyethylene glycol with a Mw P 6000 (PEG 6000) is an osmotic solutions that has frequently been used to induce water stress and maintain a uniform water potential through-out an experimental period (El Siddig *et al*,. 2013). Thus, cell cultures surviving under water stress can be selected and raised as drought resistant cell lines. Dragiiska *et al*., (1996) developed a system for *in vitro* selection during somatic embryogenesis in alfalfa using PEG as a selective agent for osmo-tolerance. Hady *et al.,* (2001) subjected embryogenic calli of five wheat cultivars to *in vitro* selection for drought tolerance using 5, 10 and 20% PEG. One way of increasing productivity in stressful environments is to breed crops more tolerant to stress.

Poly ethylene glycol provides a means of quantifying the water stress that roots experience. The use of PEG-6000 for the experimental control of moisture stress in Petri dish and pot culture studies has been considered very useful tool for the studying the effect of water stress for seed germination and seedling growth and consequently for evaluating the drought tolerant character of plants (Datta *et al*., 2011). It has been suggested to use PEG for imposing and studying the experimentally determinable moisture stress in crop plants under laboratory as well as under field conditions.

Controlled and uniformly repeated simulation of drought in the field cannot be easily achieved (Shaheen and Hood-Nowotny 2005). The slow progress in developing drought-resistant cultivars also reflects the lack of a specific method for screening the large numbers of genotypes required in breeding for drought (Tuberosa, 2012). Using natural field conditions is difficult because rainfall can eliminate water deficits. However, *in vitro* drought-screening methods are facilitating progress in our understanding of drought-tolerant traits and in our selection of drought-resistant genotypes. Germination is a useful criterion in screening for water stress tolerance (Sammar Saleem and Khan, 2012). Khakwani, *et al*., (2011) demonstrated that among the six varieties of wheat tested; those which were tolerant to drought during *in vitro* germination tests were similarly tolerant in field conditions. In addition, Agili *et al*., (2012) confirmed this finding with experiments on sweet potato.

Water stress is one of the limiting factors for plant growth and crop production. Upon exposure to water deficit, plants react by complex mechanisms involving morphological, physiological, biochemical and molecular factors, both at cellular and whole-plant levels (Hasegawa *et al.*, 2000). At the cellular level, the effect of water stress on the slowdown of cell divisions and elongation by the loss of turgor has been widely reported (Kakaei *et al.*, 2013). The addition of PEG in the medium causes cell dehydration by reducing water availability to cells, which leads to a loss of cell turgor and hence a loss of growth (Soliman and Hendawy, 2013). However, the concentration of PEG inhibiting growth depends on the genotype of the species studied (Bouiamrine and Diouri, 2012).

2.9 Drought Resistance at Seedling Stage

Water stress is one of the most important abiotic stresses, mainly combined with rise in temperature (Siddiqui *et al.*, 2008). Breeding programs of bread wheat seeking increased yield have usually attempt to improve water stress tolerance of plants

(Kumari *et al*., 2000). Successful genetic manipulation is the only way to characterize the physiological traits to overcome water stress tolerance or sensitiveness (Pessarakli, 2014). Several methods based on physiological and agronomical traits as selection criteria, such as WUE, early seedling vigor, shoot and root length, dry root /shoot ratio and seed reserve mobilization ((Dhanda *et al*., 2004; Noorka, 2013) have suggested to screen the germplasm for drought tolerance.

A near perfect crop stand can be obtained by using high quality seed that ensure high germination percentage under favorable conditions. Among seedling traits, emergence percentage has been extensively used as an indication for seedling vigor (Noorka *et al*., 2007). Therefore, high emergence percentage followed by high seedling vigor is necessary for attaining good crop stand. A reliable and efficient screening technique may facilitate isolation of drought tolerant genotypes. Good survival of genotypes after desiccation will be useful in developing a stable variety (Noorka *et al.,* 2007). Poor germination and uneven crop stand are main constraints of low yield in food crops (Du and Tuong, 2002). The seedling survival was considered a next important trait by Farooq *et al*., (2006). Studies have been done on the impact of drought during the reproductive phase of wheat and it was found out that high yields under early-season drought were attributed to high leaf area index under stress and, upon recovery, and to high tiller survival rate (Nezhadahmadi *et al*., 2013). Similarly, Johari-Pireivatlou (2010) investigated the effect of water stress on growth of *Triticum* durum in relation to sugar accumulation and water status of wheat plant.

Water stress or water deficit is inevitable in the future of global agriculture. About one-third of the world’s potentially arable land suffers due to water shortage, and most of the crops production is often reduced by drought and diseases (Ahmad, *et al*., 2010). Being an integral part of plants water plays a pivotal role in the initiation of growth, subsequent maintenance of developmental process throughout the plant’s life and ultimately economy of a country (Shafi, *et al*., 2012). Seedling trait is an important aspect of any crop breeding programme, since the final stand of a crop mostly depends on seedling characteristics. Various factors like seed germination, growth rate, mean emergence time and desiccation tolerance affect the yield of a crop (Noorka and Khaliq, 2007).

Seedling survivability is a time tested, simple and an efficient screening method for screening germ plasm under an artificially induced water stress environment. The screening has been used as a selection criterion (Chang and Leresto, 1986) in cowpea (Singh, *et al*., 1999) and wheat (Tomar and Kumar, 2004). Simple and valuable traits may be measured during the screening process according to the work of Noorka and Khaliq (2007).

2.9.1 Emergence Percentage

The appearance of the radicle marks the end of germination and the beginning of "establishment", a period that utilizes the food reserves stored in the seed (Raven *et al*., 2005). It is the hidden ability and power of the crop seed to emerge from itself. It is considered that good emergence can give a good crop stand (Subrahmanyam, *et al*., 2006). Emergence percentage is the ability of a plant to emerge its aerial parts from the soil (Heydecker, 1960) and has been considered a very important component of seedling vigour (Basra *et al*., 2003). Poor germination and uneven crop stand are the main constraints of a good crop (Noorka *et al*., 2009). Emergence percentage is calculated according to the formula derived by Smith and Millet, (1964).

$$E\% = \frac{\text{Total number of seedlings emerged 18 DAS}}{\text{Total number of seedlings grown}} \times 100$$

2.9.2 Emergence Index

Germination and establishment as an independent process are critical phases in the life of a plant when they are the most vulnerable to injury, disease, and water stress (Raven *et al*., 2005). Emergence index (EI) is an estimate of the speed of emerging seedlings and can be calculated according to the method described in the Association of official seed analysis (1983). EI = number of seeds emerged at first count +……..+ number of seeds emerged at final count divided by Days of first count + ……..+ days of final count

2.9.3 Emergence Rate Index

Emergence rate index describes how many seeds of a particular plant species, variety or seedlot are likely to germinate over a given period. It is a measure of germination time (Raven *et al*., 2005). Emergence rate index is arrived at by dividing EI with the emergence percentage and can be calculated by the method proposed by Noorka *et al*., (2013) as follows

ERI = emergence index /emergence percentage

2.9.4 Energy of Emergence

Seed germination and seedling establishment are the most sensitive processes to water stress (Zheng, 2006). The processes and traits of germination and field emergence of a plant are very important for subsequent growth and development and form the basis for future plant growth and development in standard and stressed conditions such as drought (Bláha and Středa, 2016). Energy of emergence (EE) is the power of a seed to emerge from the seed testa and soil. The EE is calculated with the formula given by Ruan *et al*. (2002). That is counting the number of seedling that emerged three days after sowing, and then divided by the total number of seeds planted for each variety calculated as a percentage.

2.9.5 Mean Emergence Time

It is common for seedlings emerging early in the season to do better than those emerging later, as they have a higher rate of survival, improved growth or even fitness (Verdu´ and Traveset, 2005). The relative importance that the timing of emergence exerts in determining seedling success may, however, be mediated by the environmental conditions encountered by the seedlings (Quintana et al., 2004). Mean emergence time (MET) is the average time by the seed to emergence from the soil and is dependent on genetic potential as shown in the equation of Ellis and Roberts (1981)

$$\mathrm{MET} = \sum \frac{\text{number of emerged seedlngs}}{\text{day of counting}}$$

2.9.6 Desiccation Tolerance

Vegetative desiccation tolerance is broadly distributed among modern day plant taxa (Alpert, 2000). Desiccation tolerance is one of the most outstanding features in the plant kingdom. It can be defined as the ability of the cell to rehydrate successfully after the removal of 80-90 % of protoplasmic water, reaching moisture content below 0.3 g H_2O /g dry matter (Hoekstra *et al.*, 2001). Seeds that possess such an attribute, the so-called orthodox seeds, can be dried and stored for many years without significant loss of viability. Most crop species such as wheat, rice and maize produce orthodox seeds. In contrast, seeds that lack this characteristic, the so-called recalcitrant seeds, shed at high water content, metabolically active and have short life spans (Faria *et al*., 2005). Orthodox seeds acquire desiccation tolerance during their development and dry mature seeds are extremely tolerant to desiccation (Pammenter and Berjak, 2000). During germination; however, desiccation tolerance is rapidly lost depending upon the species. For example, the seedlings of wheat up to fourth day following inhibition are able to survive severe dehydration. This desiccation tolerance period for other species is either shorter as in soybean (Senaratna and McKersie, 1983), or longer as in pea (Corbineau *et al*., 2000).

Desiccation tolerance can be constitutive as in many seeds, or inducible as in whole plants, e.g. those from the genus Craterostigma. Different mechanisms of desiccation tolerance have been proposed (Buitink, Hoekstra and Leprince 2002). As membranes are considered a primary target of desiccation injury, membrane stabilization is thought to be a key mechanism of desiccation tolerance (Oliver *et al*., 2005).

2.10. Mutation breeding in crops

Mutation is a permanent change in the Deoxyribonucleic Acid (DNA) sequence of a gene which can alter the amino acid sequence of the protein encoded by the gene. The DNA sequence is interpreted in groups of three nucleotide bases, called codons. Each codon specifies a single amino acid in a protein (IAEA, 2013). Application of biotechnology and mutation techniques for the improvement of Crops, improve local varieties of basic food crops for yield and quality, early maturity and tolerance to biotic and abiotic stresses (IAEA, 2013). Mutation breeding is often used to correct a specific deficiency in an adopted and high yielding genotype. This otherwise desirable genotype may be susceptible to a disease or may need modification in plant breeding (Acquaah, 2012).

Wheat mutation breeding is the use of a variety of physical factors and chemical mutagens to induce mutation or mutation of genetic material, in a short time to be of value in the Mutant, breeding new varieties or create new germplasm resources. Generally, seeds are exposed to the mutagen at an appropriate dosage. Dosages too high can cause excessive genome disruption decreasing the total Mutant yield (Zhaochun *et al*., 2010).

2.11. Morphological Characterization of Crop Plants

Traditionally, genetic diversity was evaluated in crop species based on differences in morphological characters and qualitative traits (Malek *et al*., 2014). Morphological characterization is the first step in genetic relationship studies in most breeding programmes (Chipojola *et al*., 2009). Phenotypic identification of plants has been used as a powerful tool in the classification of genotypes and to study taxonomic status, based on morphological traits recorded in the field. Most important agronomic characteristics are controlled by multiple genes and are subjected to varying degrees of environmental modifications and interactions. Plant morphological traits are grouped as either polygenic (variable) or monogenic (constant). Polygenic characteristics are associated with large genotype by environment interaction. Monogenic characteristics are salient, thus identifying the species or genotype, for example, petiole color, root skin and pulp color, and stem color (Elias, *et al*., 2001). Morphological characterization has been used for various purposes including; studies of genetic variation patterns, identification of duplicates and correlation with characteristics of agronomic importance (Fekadu *et al*., 2013).

In the past, plant breeders made selection of breeding material on the basis of morphological characteristics that were readily observable and that were co-inherited with desired traits. Although this method remains effective, morphological comparisons have limitations including the influence of environmental practices (Gepts and Hancock, 2006). However, the use of morphological traits depends on biochemical traits and most of them are ambiguous descriptors. They have limited use for cultivar identification such characteristics are often controlled by multiple genes and are subject to varying degrees of environmental modification and interaction with qualitative traits (Magloire, 2005). Yield performance and quality characters which are of major importance in breeding are usually focused on during evaluation of accessions. Some morphological characterization variables that will be scored using the agronomic wheat descriptors as given by the International Board for Plant Genetic Resources (IBPGR, 1985) in this study include:

2.11.1 Plant Height

Plant height is the distance between the upper boundary of the main photosynthetic tissues (excluding inflorescences) on a plant and the ground level, expressed in metres or centimetres. Plant height is the maximum stature a typical mature individual of a species attains in a given habitat. Plant height is associated with growth form, position of the species in the vertical light gradient of the vegetation, competitive vigour, reproductive size, whole-plant fecundity, potential lifespan, and whether a species is able to establish and attain reproductive size after disturbance events such as e.g. fire, storm, ploughing, grazing (Wright *et al*., 2007). In wheat it is measured from ground to top of spike, excluding awns

2.11.2 Spike Length

Wheat grains are borne on a spike or ear. In the case of wheat, the major axis, called the rachis, bears two rows of spikelets in alternating order. The mature wheat spike may contain 15-18 spikelets or more attached on alternating sides of the rachis (Edwards, 2010). The length of a spike therefore determines the number of spikelets and in turn the yield of a wheat variety. Spike length is quantitative trait and it is in relation with other yield components (Madić *et al.*, 2010). Measurement of spike length in centimetre that is distance from the base to the end of spike

2.11.3 Number of Spikelets Per Spike

The head of the wheat plant has a rachis (stem) made up of nodes and short, flattened internodes. At the nodes are the floral structures, called spikelets, that hold up to 10 florets containing the flower of the wheat plant, where grain is formed. Each floret is enclosed within two protective bracts called the lemma and palea. These structures wrap around the carpel. The carpel contains the ovary with the feathery stigmas, three stamens holding the anthers (pollen sacs), and the ovule. Once fertilized, the ovule forms the grain (White and Edwards, 2007).

2.11.4 Number of Seeds per Spikelet

The spike (head) of a plant may contain 14–17 spikelets, each spike containing about 25–30 grains. Large spikes may contain between 50 and 75 grains. The grain size varies within the spikelet, the largest being the second grain from the bottom and decreasing in size progressively towards the tip of the spike (Acquaah, 2012). It is the average number of seeds from a spikelet - obtained from the central portion of the spike.

2.11.5 Days to 50% Heading

The heading stage extends from the time of emergence of the tip of the head from the flag leaf sheath to when the head has completely emerged but has not yet started to flower. This is the number of days required for 50% of the wheat plants being studied for the inflorescence (panicle) to emerge from the flag leaf of a plant or a group of plants in a study.

2.11.6 Number of Tillers at Booting

Tillers, which have the same basic structure as the main shoot, arise from the axils of the basal leaves. At anthesis, only some of the tillers that have developed survive to produce an ear. Others die and may be difficult to find in the mature plant (Kirby, 2002).

This study, therefore, sought to determine drought tolerance in mutant wheat using seedling traits and *in vitro* techniques which are a fast and effective method for screening plants. Morphological characterization of the mutant wheat was done to determine the different agronomic traits.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Site

University of Eldoret is located on geographical coordinates 0° 30′ 0″ north, 35º 15′ 0″ east. The experimental site is located 10 km of Eldoret town, in Uasin Gishu county of Kenya. The University is located at an altitude of 2180m above sea level. It consists primarily of an agro-ecological zone LH3. The site is among major wheat growing region in Kenya. University of Eldoret receives unimodal rainfall which begins in March. The average annual rainfall range is between 900mm and 1100mm and mean annual temperature of 16.6ºC.The soils are shallow, ferralsol, well drained, non humic cambisols with high nutrient availability and moisture storage (Jaetzold, *et al.,* 1983)

Mimea international, Kitengela. Kitengela is located in Kajiado County which borders Nairobi County. It is situated between Longitudes 360 5’ and 370 5’ East and between Latitudes 10 0’ and 30 0’ South. The county has a bi-modal rainfall pattern. The short rains fall between October and December while the long rains fall between March and May with rainfall amount ranging from 300mm to 1250mm. Temperatures vary from about 34^0C are recorded around Lake Magadi and 10^0C in the eastern slopes of Mt. Kilimanjaro. The coolest period is between July and August, while the hottest months are from November to April (RoK, 2013).

3.2 Generation of Mutant Wheat Varieties

Several Kenyan wheat varieties were sent to the International Atomic Energy Agency (IAEA) laboratory in Vienna, Austria and subjected to gamma radiation at an irradiation dose of 300 gy (gray) to obtain M1 (mutated seed that gives rise to the first generation of Mutants). The M1 seed was planted at University of Eldoret in an experimental field for advancement to the next several generations and preliminary evaluation for positive effects of radiation. After several generations of screening for stem rust resistance, two Mutant lines were selected having shown resistance to stem rust. These two Mutant lines were used in the subsequent experiments to screen for their drought tolerance *in vitro*, at seedling stage and effects of induced mutagenesis on their morphological traits.

3.3. Screening for Drought Tolerance in the Mutant Selection in PEG 6000

The experiment was conducted at Mimea International, Kitengela, under laboratory conditions. Two Mutant wheat lines (M1 and M2) that showed resistance to stem rust disease in the field were used in the present study and were obtained from University of Eldoret. The other seeds were two known drought resistant commercial varieties of wheat (Duma and Chozi) which were obtained from Kenya Agriculture and Livestock Research Organization (KALRO-Njoro). Both Duma and Chozi wheat varieties have been developed for the dry areas of Kenya. They can be grown in dry areas with low rainfall and areas with short growing periods. In total four wheat varieties were tested.

3.3.1 *In vitro* Osmotic Stress Experiment

Seeds were initially surface sterilized with 70% ethanol for 15 min. Residual ethanol was removed by thorough washing with sterilized distilled water. Twenty-five randomly selected seeds of each wheat variety, that is, Chozi, Duma, M1 and M2, were placed in Petri dishes on moistened filter paper to provide appropriate moisture stress for seed germination as suggested by Bayoumi *et al*., (2008). Water stress was exerted by preparing different water potential values, -3.0, -9.0 and -15.0 bars, produced by dissolving 138, 222 and 270 grams of PEG in 1000 ml of distilled water, respectively following the method of Hadas (1976). A control set was also included using distilled water (zero bars). All the Petri dishes were placed at random in a growth chamber for 10 days, at average temperature of day and night of 22±2^{o} C and at 50% relative humidity. Five ml of distilled water was added to each Petri dish every 2 days to compensate for losses through evaporation.

At the same time, 5 ml of PEG solution was added to each Petri dish under osmotic stress conditions of -3.0, -9.0 and -15.0 bars. When seedlings were at the stage of first true leaf initiation (10 days after treatment) data was taken at four different treatments (0, -3.0, -9.0 and -15.0 bars). These included root length, shoot length and root length to shoot length ratio all measured in centimetres. The experiment was laid out in a completely randomized design with two factors: wheat variety types and water stress and three replicates. Data was subjected to Analysis of Variance, and means were separated by Duncans multiple range test at $P < 0.05$ where the differences were significant. All the analyses were done using Genstat Statistical software version 12.

3.4 Screening for Drought Tolerance at Seedling Stage

The experiment was conducted in a green house at Mimea International, Kitengela. The experiment was laid out in CRD with three replications. The seeds were sown in 18x9 cm polythene bags filled with measured quantity of normal field soil, that was approximately 450 grams per bag, as described previously by Noorka and Khaliq, (2007). The bags were arranged in iron trays, each genotype comprising three bags per replication. Two seeds of each variety were sown in the three bags at uniform depth of 3 cm to ensure full crop stand. The parameters used to determine drought tolerance at seedling stage were emergence percentage, emergence index, emergence rate index, energy of emergence, mean emergence time, desiccation tolerance index and percentage seedling recovery.

3.4.1 Emergence Percentage (E%)

Data collection began instantly after the emergence of first seedling in any bag and onwards measurements were made on daily basis at 1600 h. Eighteen days after sowing (DAS), the number of visible seedlings was recorded. Data collection continued until there was no further emergence. Emergence percentage was then calculated according to the formula derived by Smith and Millet, (1964).

$$E\% = \frac{\text{Total number of seedlings emerged 18 DAS}}{\text{Total number of seedlings grown}} \times 100$$

3.4.2 Emergence Index (EI)

Emegence index is the estimate of emergence rate of seedlings and was calculated by the formula as delineated in Association of Official Seed Analysis (AOSA) (1983). Each day, seedling at least 1.5 cm long from the different wheat varieties were counted and removed until no further seeds germinated. This total was then divided by the sum of the number of days after sowing for each day. This was then calculated as a percentage.

$$EI\% = \frac{\text{No. of seeds emerged at first count} + \ldots\ldots + \text{No of seeds emerged at final count}}{\text{Days of first count} + \ldots\ldots\ldots\ldots\ldots + \text{days of final count}}$$

3.4.3 Emergence Rate Index (ERI)

Emergence rate index for each treatment and replication was calculated as follows:

ERI = Emergence index/Emergence percentage

3.4.4 Energy of Emergence (EE)

Energy of emergence was computed according to the method as outlined by Ruan *et al*. (2002). It is the percentage of emerged seedlings three days after sowing. This was calculated by counting the number of seedling that emerged three days after sowing for the different wheat varieties. This was then divided by the total number of seeds planted for each variety and then calculated as a percentage.

3.4.5 Mean Emergence Time (MET)

Mean emergence time was calculated in accordance with the equation of Ellis and Roberts, (1981) as under:

$$MET = \Sigma Dn / \Sigma n$$

where n is the number of seeds germinated on day D and D is the number of days counted from the beginning of emergence.

3.4.6 Desiccation Tolerance Index (DTI)

To check the potential of the genotype to bear the water stress condition, the plants were watered until they sprouted two to three leaves. This is considered the best stage to evaluate the genotype for their water stress tolerance and susceptibility as suggested by ISTA (Anonymous, 1997). To induce artificial water stress condition, the water provision was withheld for two weeks a result of which most of seedlings died. Irrigation was started again and survival was noted on re-growth of plants in each replication. The number of live as well as dead seedlings was counted daily following the procedure described by Noorka and Khaliq, (2007). This was done by counting the number of dead plants after the resumption of irrigation and dividing it by the number of live seedlings in all the wheat varieties.

Desiccation tolerance index = Final number of dead seedlings/Final seedlings emergence number

3.4.7 Percent Seedling Recovery (PSR)

After resumption of the irrigation water, drought tolerant genotypes will recover. The recovery percentage of the seedlings after desiccation was calculated with the formula (Noorka *et al.*, 2013). Hameed *et al.* (2010) suggested survivability (late and early dying) and seedling growth behavior under drought indicated that root/shoot length ratio was the most important trait for screening drought tolerance at the seedling stage.

It is the measure of percent recovery or re-growth of seedlings after desiccation and is calculated by the formula as used by Noorka and Khaliq (2007).

$$\text{Percent seedling recovery} = \frac{\text{Number of plants resuming growth}}{\text{Total number of seedlings}} \times 100$$

3.5 Screening for the Effects of Induced Mutagenesis on Wheat Lines Morphological Traits

The morphological study was conducted at the University of Eldoret. Morphological features were used to characterise the two Mutant wheat varieties (M1 and M2) and the two drought tolerant wheat varieties (Chozi and Duma). Seeds from each entry were sown in 1M rows. The experimental units were separated by 0.3m and 0.5m wide alleyways within and between the blocks, respectively with three replicates.

Sowing was done at an equivalent seeding rate of 125kg/ha. At planting time, di-ammonium phosphate (DAP) fertilizer was applied at an equivalent rate of 125kg/ha. Weeds growths were managed by applying both pre - and post-emergent herbicides. Stomp® 500 EC (pendimethalin) a broad spectrum, pre-emergent herbicide was applied at an equivalent rate of 2.5 l/ha. At tillering stage, the plots were sprayed with Buctril MC (bromoxynil +MCPA) at an equivalent rate of 1.5l/ha to control broad-leaved weeds. The trial was top dressed with Calcium Ammonium Nitrate (CAN) at stem elongation stage (Carisse, 2010)

At physiological maturity 10 plants/plot were selected randomly and used to measure plant height (from plant base to the tip of spike excluding awns), spike length, number of tillers and days to 50% heading. Spike length was measured from the base of the ear to the tip of the spike (excluding the awns) based on an evaluation of all the spikes from the ten plants. Spikelet's per spikes and number of tillers at booting was done by actual count of individual spikeletes and tillers respectively. Days to 50% heading was the number of days counted from planting up to 50% heading. Yield was determined by counting the number of grains per spike. Spikes were thrashed manually with a wooden hand thresher and the number of grains was counted. The data was then averaged over the spikes to determine the number of grains per spike for the given varieties.

3.5 Data analysis.

Data on *in vitro* test using PEG, seedling test and morphological traits were subjected to analysis of variance at p=0.05 using GENSTAT 12th edition and means separated using Duncan Multiple range test. Correlation was done by Pearson Correlation

Coefficients to determine significant associations among the morphological variables. Statistical model used in RCBD was: $X_{ij}=\mu+t_i+\beta_j+e_{ij}$

Where, X_{ij}= observation, μ=overall mean, t_i=treatment effect, β_j=block effect and e_{ij}=experimental error

Statistical model used in CRD was: $Y_{ij}=\mu+\tau_i+\varepsilon_{ij}$

where: Yij is the jth observation of the ith treatment, μ is the population mean, i τ is the treatment effect of the ith treatment, and E is the random error.

CHAPTER FOUR

RESULTS

4.1 Effects of Polyethylene Glycol Concentration on Germination of Wheat

In the control, Duma (23.0) had the highest mean of germinated seeds, followed by Mutant 1 (22.33), Mutant 2 (22.0) and lastly Chozi (14.0). At -3 PEG concentrations, Mutant 1 (21.67) had the highest mean germination, followed by Mutant 2 (21.0), Duma (10.33) and lastly Chozi (6.0). At -9 PEG concentrations, Mutant 2 (20.33) had the highest mean germination, followed by Mutant 1 (19.33), Duma (5) and lastly Chozi (2.33). At -15 PEG concentration, Mutant 1 (18.33) had the highest mean germination followed by Mutant 2 (12.0). Chozi and Duma did not germinate at this concentration (Table 4.1). At -9 PEG concentrations, Duma had a higher mean of germinated plants compared to Chozi. At this same concentration, Mutant line 2 and Mutant 1 had similar means after mean separation with Duncans multiple range test.

Table 4.1: Means of plants germinated 3 days after planting in different PEG concentrations (P=0.05)

PEG conc	**Variety**			
	Chozi	**Duma**	**Mutant 1**	**Mutant 2**
0	14.00a	23.00b	22.33b	22.00b
3	6.00a	10.33b	21.67c	21.00c
9	2.33a	5.00b	19.33c	20.33c
15	0.00a	0.00a	18.33b	12.00b

Means separated by Duncans multiple range test ($p \leq 0.05$). Means which are homogeneous have the same letter

4.1.1 Effects of polyethylene glycol on Energy of Emergence, Emergence Index and Mean Emergence Time

The four wheat varieties revealed significant differences ($p \leq 0.05$) under different PEG concentrations for Energy of Emergence, Emergence Index and Mean Emergence Time. There was a decreasing trend in all the parameters calculated as the PEG concentration increased as shown in Table 4.2. In the control, Duma had the highest energy of emergence of 93, followed by Mutant 2 (88.3), Mutant 1 (85.3) and lastly Chozi (56). On the emergence index, Mutant 1 had the highest index of 9.3, followed by Mutant 2 (8), Duma (6.2) and lastly Chozi (2.8) in the control. On the mean emergence time, Mutant 1 had the highest of 7.9, followed by Mutant 2 (7), Duma (4.8) and lastly Chozi (3.8) in the control.

At PEG concentration of 3, in the energy of emergence, Mutant 1 (83.7) was the highest followed by Mutant 2 (86), Duma (41.3) and lastly Chozi (24). In the emergence index, Mutant 1 (8.9) had the highest followed by Mutant 2 (6), Duma (1.7) and lastly Chozi (1). In the mean emergence time, Mutant 1 (7) had the highest followed by Mutant 2 (5) and Duma (3) and Chozi (3) having the same mean emergence time (Table 4.2).

At PEG concentration of 9, in the energy of emergence, Mutant 1 (77) was the highest followed by Mutant 2 (81), Duma (20) and lastly Chozi (9.3). In the emergence index, Mutant 1 (6.1) had the highest followed by Mutant 2 (3.3), Duma (0.8) and lastly Chozi (0.4). In the mean emergence time, Mutant 1 (5) had the highest followed by Mutant 2 (3), Duma (3) and Chozi (3) all having the same mean emergence time (Table 4.2).

At PEG conc of 15, there was significant difference in the energy of emergence among the varieties with M1 recording the highest energy of emergence (EE) of 70 followed by M2 (48). Chozi and Duma had no significant difference and both an EE of zero in the emergence index, Mutant 1 (3.2) had the highest followed by Mutant 2 (2), Duma and Chozi both had 0. In the mean emergence time, Mutant 1 and Mutant 2 both had 3 while Duma and Chozi both had 0 (Table 4.2).

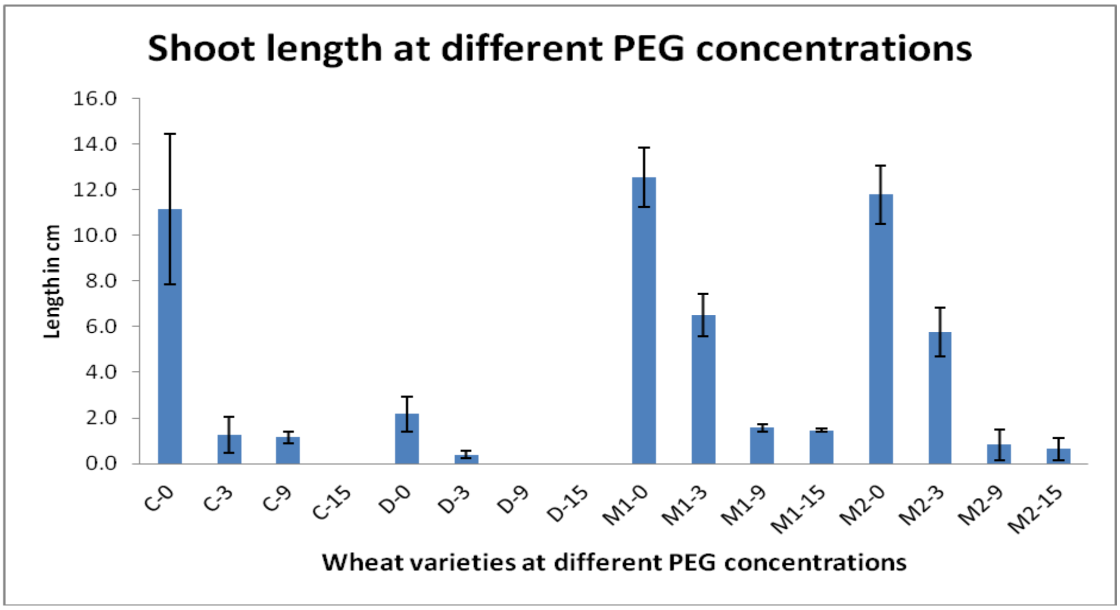
Table 4.2: Table on Energy of Emergence, Emergence Index and Mean Emergence Time

PEG Conc	Wheat varieties	Energy of Emergence	Emergence Index	Mean Emergence Time
0	Mutant 1	85.3 b	9.3d	7.9c
	Mutant 2	88.3c	8.0c	7.0c
	Duma	93.0d	6.2b	4.8b
	Chozi	56.0a	2.8a	3.8a
3	Mutant 1	83.7c	8.9c	7.0c
	Mutant 2	86.0d	6.0b	5.0b
	Duma	41.3b	1.7a	3.0a
	Chozi	24.0a	1.0a	3.0a
9	Mutant 1	77.0c	6.1c	5.0a
	Mutant 2	81.0d	3.3b	3.0a
	Duma	20.0b	0.8a	3.0a
	Chozi	9.3a	0.4a	3.0a
15	Mutant 1	70.0c	3.2c	3.0b
	Mutant 2	48.0b	2.0b	3.0b
	Duma	0.0a	0.0a	0.0a
	Chozi	0.0a	0.0a	0.0a

Means separated by Duncans multiple range test (p=0.05). Means which are homogeneous have the same letter

4.1.2 Effects of different concentrations of PEG on Shoot Length

The shoot length was measured in terms of centimetres. There was a significant difference between the different wheat varieties in terms of shoot length in the different PEG concentrations. Figure 4.1 below shows that in all the wheat varieties there was a decrease in shoot length as the PEG concentration increased. In the control, Mutant 1 had the highest shoot length (12.5 cm) followed by Mutant 2 (11.8 cm), Chozi (11.2 cm) and lastly Duma (2.2 cm). At -3 PEG concentration, Mutant 1 had (6.5 cm), Mutant 2 (5.8 cm), Chozi (1.3 cm) and lastly Duma (2.2 cm). At -9 PEG concentration, Mutant 1 had (1.6 cm), Mutant 2 (0.8 cm), Chozi (1.2 cm) and lastly Duma (0 cm). At -15 PEG concentration, Mutant 1 had (1.5 cm), Mutant 2 (0.6 cm), Chozi (0 cm) and lastly Duma (0 cm).

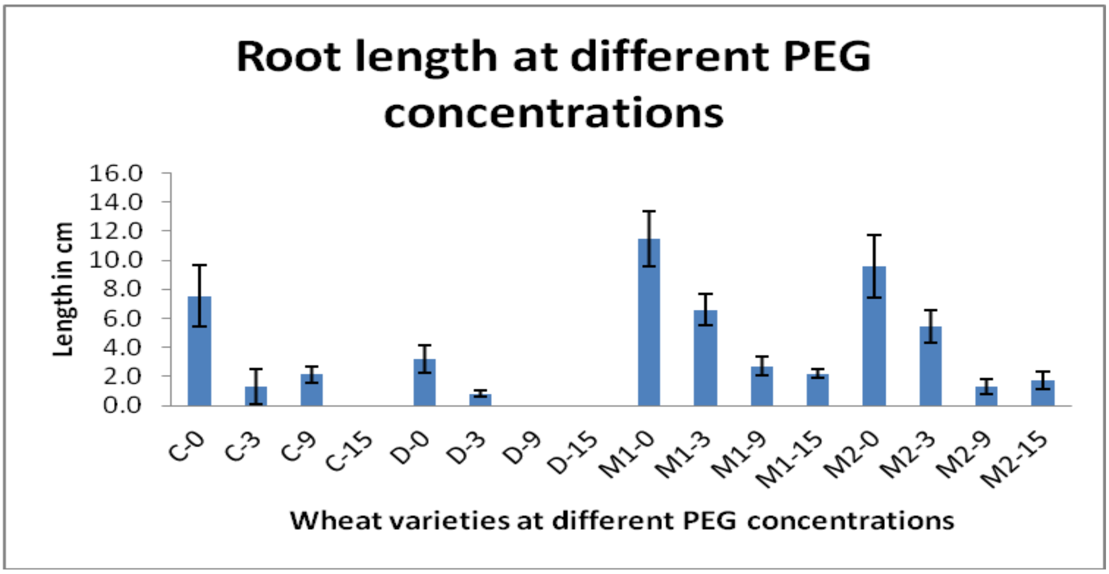


Key: C = Chozi, D = Duma, M1 = Mutant 1 M2 = Mutant 2

Figure 4.1: Shoot length at different PEG concentration against for the different wheat varieties.

4.1.3 Effects of Different Concentrations of PEG on Root Length

The root length was measured in terms of centimetres. There was a significant difference between the different wheat varieties in terms of root length in the different PEG concentrations. Figure 4.2 below shows that in all the wheat varieties there was a decrease in root length as the PEG concentration increased. In the control, Mutant 1 had the highest root length (11.5 cm) followed by Mutant 2 (9.5 cm), Chozi (7.5 cm) and lastly Duma (3.2 cm). At -3 PEG concentration, Mutant 1 had (6.6 cm), Mutant 2 (5.4 cm), Chozi (1.3 cm) and lastly Duma (0.8 cm). At -9 PEG concentration, Mutant 1 had (2.7 cm), Mutant 2 (1.3 cm), Chozi (2.1 cm) and lastly Duma (0 cm). At -15 PEG concentration, Mutant 1 had (2.2 cm), Mutant 2 (1.76 cm), Chozi (0 cm) and lastly Duma (0 cm).



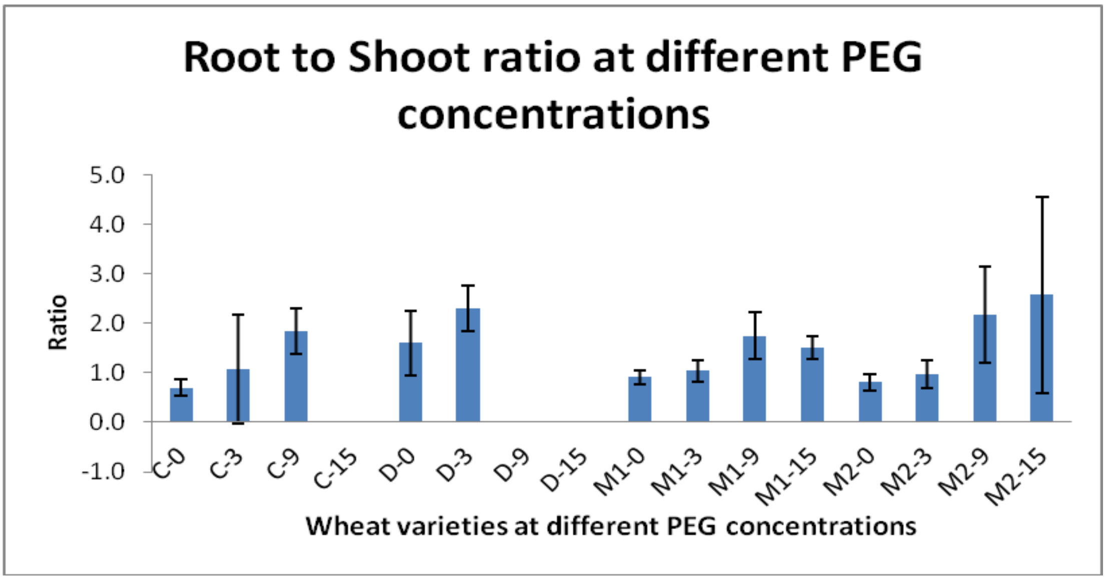
Key: C = Chozi, D = Duma, M1 = Mutant 1 M2 = Mutant 2

Figure 4.2: Root length at different PEG concentration for the different wheat varieties.

4.1.4 Effects of Different Concentrations of PEG on Root to Shoot Ratio

There was a significant difference between the different wheat varieties in the root shoot ratio at differing PEG concentrations. Figure 4.3 below shows that in all the wheat varieties there was an increase in the root to shoot ratio as the PEG concentration increased. In the control, Duma had the highest ratio (1.60) followed by Mutant 1 (0.92), Mutant 2 (0.81) and lastly Chozi (0.69). At -3 PEG concentration, Duma had (2.30), Chozi had (1.08), Mutant 1 (1.03) and lastly Mutant 2 (0.97). At -9

PEG concentration, Mutant 2 had (2.16), Chozi (1.85) Mutant 1 (1.75) and lastly Duma (0). At -15 PEG concentration, Mutant 2 had (2.57), Mutant 1 (1.5) and lastly Chozi and Duma 0 each.



Key: C = Chozi, D = Duma, M1 = Mutant line 1, M2 = Mutant line 2

Figure 4.3: Root to shoot ratio at different PEG concentration for the different wheat varieties.

4.1.5 Correlation Coefficient Between the Root, Shoot and Shoot to Root Ratio

Correlation of data using Pearson correlation ($p \leq 0.01$) shows that there was a negative correlation between the PEG concentration, shoot length and root length. However there was a positive correlation between PEG concentration and shoot to root ratio. There was a positive correlation between root length and shoot length. The wheat varieties used had a negative correlation with the root length.

Table 4.3: Correlation coefficient between the root, shoot and shoot to root ratio

	Variety	PEG Conc	Shoot	Root	Shoot _Root
Variety	1	-0.139	-0.148	-0.256**	-0.060
PEG conc		1	-0.698**	-0.649**	0.407**
Shoot			1	0.913**	-0.530**
Root				1	-0.364**
Shoot _Root					1

** Correlation is significant at the 0.01 level

4.2: Screening Drought Tolerance at Seedling Stage

4.2.1 Emergence Percentage

Mutant 2 had the highest emergence percentage of 77.8% followed by Mutant 1 (72.2%) then Duma (66.7%). Chozi had the least emergence percentage of 38.9% (Table 4.4).

4.2.2 Emergence Index

Mutant 2 had the highest emergence index (91.7) followed by Mutant 1 (77.4) then Duma (72.0). Chozi had the least emergence index of 49.4 (Table 4.4).

4.2.3 Emergence Rate Index

Chozi had the highest Emergence Rate Index (1.3) followed by Mutant 2 (1.2). Mutant 1 and Duma had an Emergence Rate Index of 1.1 (Table 4.4).

4.2.4 Energy of Emergence

Mutant 2 had the highest Energy of Emergence of 27.8 followed by Mutant 1 (22.2) and Duma (16.7). Chozi had EE of 0 (Table 4.4).

4.2.5 Mean Emergence Time

Chozi had the highest Mean Emergence Time of 13.4 followed by Duma (12.3). Mutant 1 had a Mean Emergence Time of 12.0 and lastly Mutant 2 had a Mean Emergence Time of 11.9 (Table 4.4).

4.2.6 Desiccation Tolerance Index,

Chozi had the highest Desiccation Tolerance Index of 0.7 followed by Duma (0.6). Mutant 1 had had a Desiccation Tolerance Index of 0.5 and lastly Mutant 2 with 0.4 (Table 4.4).

4.2.7 Percent Seedling Recovery

Mutant 2 had the highest Percent Seedling Recovery (57.1%) followed by Mutant 1 (46.2%). Duma had a Percent Seedling Recovery of 41.7% and lastly Chozi had a Percent Seedling Recovery of 28.6%. (Table 4.4)

Table 4.4: Table on emergence percentage, emergence index, emergence rate index, energy of emergence, mean emergence time, desiccation tolerance index and percent seedling recovery

Variety	EP	EI	ERI	EE	MET	DTI	PSR
M2	77.8 d	91.7 d	1.2 a	27.8 d	11.9 a	0.4 a	57.1 d
M1	72.2 c	77.4 c	1.1 a	22.2 c	12.0 a	0.5 b	46.2 c
C	38.9 a	49.4 a	1.3 b	0 a	13.4 b	0.7 d	28.6 a
D	66.7 b	72.0 b	1.1 a	16.7 b	12.3 a	0.6 c	41.7 b

Key: M1 = Mutant line 1; C = Chozi ; M2 = Mutant line 2; D = Duma

Key: EP – emergence percentage, EI – emergence index, ERI – emergence rate index, EE – energy of emergence, MET – mean emergence time, DTI – desiccation tolerance index, PSR - percent seedling recovery

Mean separation using Duncan Multiple Range test at $p \leq 0.05$; means followed by the same letter are not significantly different from each other.

4.2.8 Correlation coefficient between the different parameters used to evaluate Drought Tolerance at Seedling Stage

The correlation coefficient table 4.5 shows that there was a positive correlation between emergence percentage (EP), emergence index (EI) and percent seedling recovery (PSR). However, EP had a negative correlation with emergence rate index (ERI), mean emergence time (MET) and drought tolerance index (DTI).

Table 4.5 Correlation coefficient between the different parameters used to evaluate Drought Tolerance at Seedling Stage

	variety	EP	EI	ERI	EE	MET	DTI	PSR
variety	1	$-.495^{**}$	$-.638^{**}$	-.039	$-.596^{**}$	$.343^{*}$	$.633^{**}$	$-.699^{**}$
EP		1	$.965^{**}$	$-.465^{**}$	$.990^{**}$	$-.694^{**}$	$-.862^{**}$	$.941^{**}$
EI			1	$-.365^{*}$	$.985^{**}$	$-.680^{**}$	$-.903^{**}$	$.995^{**}$
ERI				1	$-.406^{*}$	.259	.276	-.311
EE					1	$-.701^{**}$	$-.895^{**}$	$.972^{**}$
MET						1	$.644^{**}$	$-.663^{**}$
DTI							1	$-.906^{**}$
PSR								1

**. Correlation is significant at the 0.01 level.

*Correlation is significant at the 0.05 level.

4.3 Morphological Characterization

Morphological characterization was used to compare the two Mutant wheat varieties with the two drought tolerant wheat varieties.

4.2.1 Plant height

Mutagenesis had a significant effect on the plant height in the various Mutant lines as observed $p \leq 05$ (Table 4.6). There was a significant difference between the different wheat varieties. Chozi was the tallest with a mean height of 110.73cm followed by Mutant 1 90.3 cm, Mutant 2 89.23 cm and lastly Duma with 85.03 cm (Table 4.6).

4.2.2. Spike length

There was a significant difference between the different wheat varieties with Chozi having the longest average spike length of 11.83 cm followed by Duma 10.43 cm, Mutant 2 10.13 cm and lastly Mutant 1 9.70 cm (Table 4.6).

4.2.3. Spikelets per spike

There was a significant difference between the different wheat varieties. Chozi had the highest average spikelets per spike 32.1 followed by Mutant 2 31.67. Mutant 1 had 28.03 and lastly Duma 26.03 (Table 4.6).

4.2.4 Days to heading

There was a significant difference between the different wheat varieties. Duma had the longest average days to 50% heading (59) followed by Mutant 2 (58). Mutant 1 and Chozi had both an average days to 50% heading of 57 (Table 4.6).

4.2.5 Grain per spike

There was a significant difference between the different wheat varieties. Mutant 2 had the highest average grain per spike of 65.70 followed by Chozi 62.27 then Mutant 1 57.7 and lastly Duma (55.45) (Table 4.6).

4.2.6 Number of tillers

There was a significant difference between the different wheat varieties. Mutant 1 had the highest average number of tillers (7.17) followed by Mutant 1 (6.17). However, no significant difference between Chozi and Duma varieties that had an average of 4.83 and 4.67 respectively (Table 4.6).

Table 4.6: Mean separation of the different wheat variety traits ($p \leq 0.05$)

Wheat variety	**Plant height (cm)**	**Spike length (cm)**	**Spikelets per spikes**	**Grains per spike**	**No. of tillers at booting**	**Days to heading**
M1	90.30 b	9.70 a	28.03 b	57.70 a	7.17 c	57 a
M2	89.23 b	10.13 ab	31.67 c	65.70 c	6.17 b	58 b
C	110.73 c	11.83 c	32.1 c	62.27 b	4.83 a	57 a
D	85.03 a	10.43 b	26.03 a	55.40 a	4.67 a	59 c

Key: M1 = Mutant line 1; C = Chozi ; M2 = Mutant line 2; D = Duma. Mean separation using Duncan's multiple range test at $\alpha \leq 0.05$; means followed by the same letter are not significantly different from each other

There was a positive correlation between plant height and spikelets per spike, grains per spike and spike length. However, there was a negative between plant height and days to heading. There was a positive correlation between spikelets per spike and grains per spike and spike length (Table 4.7)

Table 4.7: Correlations coefficient between the different morphological traits

	Height	Spikelets per spike	Days to heading	Grains	Spike length	Tillers
Height	-					
Spikelets per spike	0.5161*	-				
Days to heading	-0.5265	-0.3994	-			
Grains	0.2829	0.5046*	-0.2392	-		
Spike length	0.5422	0.2383	-0.0709	0.3080*	-	
Tillers	0.264	0.354	0.453	0.2323*	0.3324	-

*Correlation is significant at the $p \leq 0.05$ level

CHAPTER FIVE

DISCUSSION

5.1 Effects of different PEG concentration

5.1.1 Effects of different concentrations of PEG on germination

The four wheat varieties revealed significant differences ($p \leq 0.05$) under different PEG concentrations for all growth characteristics indicating a level of genetic variability (Table 4.1). In this study it was found out that PEG decreased the available water required for seed germination and plant growth. The inhibitory effect of PEG on seed germination of different plants was recorded by many investigators on cotton, pea, wheat and on *Pennisetum americannum* (Heikal and Shaddad, 1982; Kuhad *et al*., 1987). Polyethylene glycol causes osmotic stress and could be used as a drought stimulator (Turhan, 1997). In the present investigation PEG-6000 was used to create the osmotic stress, as most of the researchers (Hu and Jones, 2004) utilized it for the development of water deficit environment in growth chamber studies.

Seed germination is first critical and the most sensitive stage in the life cycle of plants (Ashraf and Mehmood, 1990) and the seeds exposed to unfavorable environmental conditions like water stress may have to compromise the seedlings establishment (Albuquerque and Carvalho, 2003). Therefore, it seems that water potential exerts major influence on seed germination (Larson and Kiemnec, 1997) and osmotic stress delayed the emergence of the radicle and further development of the seedling. This was observed in table 4.2 where energy of emergence, emergence index and mean emergence time of all the varieties tested decreased as the concentration of PEG increased from 0 to concentration of 15. This was mostly observed in Chozi and Duma varieties compared to M1 and M2 lines. Mutation breeding is used to improve varieties that are susceptible to certain biotic and abiotic factors (Acquaah, 2012). It is possible that the effects of mutation positively influenced M1 and M2 lines

This is similar to the report of Maliwal and Paliwal (1970) who studied 15 Bajra and 11 Maize varieties for their relative salt and alkali tolerance at germination stage and found that the percentage germination decreased as the salinity increased. The retardation of germination by PEG may be due to their osmotic or ionic effects or a combination of both (Greenway and Munns, 1980).

Polyethylene glycol shows that it moderates intensities delayed germination and elevated doses reduced final germination (Almansouri *et al.,* 2001). In this study, there was no germination observed in Chozi and Duma at PEG concentration of 15. Mutant 1 and Mutant 2 lines however were able to germinate at PEG concentration 15. This could be due to the positive effects of mutation on the Mutant wheat genome (Acquaah, 2012). Kaur et al. (1998) also found decrease in percent germination in chickpea with increasing concentrations of exogenous PEG 6000. The results of this study are consistent with those of other studies that have reported that high concentrations of PEG reduce the final germination percentages of lentil (Somarin and Mahmoodabad, 2011).

Inhibition of seed germination is directly related to reserve mobilization, energy production through respiration, enzyme and hormonal activity, and dilution of the protoplasm to increase metabolism for successful embryonic growth (McDonald, 2007; Haouari *et al*., 2013). Since PEG does not enter to seeds (Mehra *et* *al*., 2003), these differences can be specifically associated to mechanisms that control ion homeostasis and toxicity (Bohnert *et al*., 1999).

Water deficit affects the germination of seed and the growth of seedlings negatively (Khayatnezhad *et al*., 2010). Germination is one of the most important traits in early stage of growth in most plants, Mutant line 1 (M1) followed by Mutant line 2 (M2) in drought stress condition had more resistance than other two cultivars (Chozi and Duma).

Water availability is usually the limiting factor for the germination of non-dormant seeds, affecting the percentage, speed and uniformity of emergence (Kaydan and Yagmur 2008). The use of PEG for the experimental control of external water potential has proved to be a very effective method for studying the effect of water stress on seed germination and seedling growth characters (Radhouane, 2007) and is a simple, accurate cost effective method to screen a large set of germplasm within a short period (Kulkarni and Deshpande, 2007). This was demonstrated in this study with the use of M1 and M2 lines being screened for drought tolerance.

5.1.2 Effects of different concentrations of PEG on shoot length

There was a significant difference on the shoot length in the different PEG concentrations (Figure 4.1). Reduction in shoot length in cereal crops is mostly linked to drought tolerance (Bibi *et al.*, 2012). The decrease in shoot length in this study in the Mutant genotypes may be due to osmotic regulation, which enables them to maintain cell turgor to assist growth under severe stress conditions Mutant 1 and Mutant 2 had shoot growth even at higher PEG concentration of -15 even when Chozi and Duma varieties were not able to grow. This could be due to alteration in the genetic makeup due to mutation (Acquaah, 2012) enabling the two Mutant lines withstand drought simulation situation in the laboratory. They could therefore be additional candidates for drought tolerant varieties in Kenya.

The variability in the decreasing trend of osmotic regulation of the genotypes indicates the genotypic variability in response to water deficit stress. Similar findings were reported by Raziuddin *et al*. (2010) in wheat, Takele, (2000), Ambika *et al*. (2011) and Khodarahumpour (2011) in sorghum in relation to the reduction in coleoptiles elongation.

5.1.3 Effects of different concentrations of PEG on root length

There was a significant difference on the root length in the different PEG concentrations. The response of root growth to drought can be variable; under moderate moisture stress, root growth is favored whereas, severe drought often limits root growth (Prasad *et al.*, 2008). The extent of root development is closely related to the ability of the plant to absorb water and the tolerant genotypes have higher capacity of these character. M1 and M2 had longer root lengths in all the different PEG concentrations tested compared to Chozi and Duma varieties and could thus be considered as drought tolerant varieties.

Generally in the genotypes scrutinized, reduction in root length across the four PEG stress levels was found. The findings of this study are in line with earlier studies where severe water stress reduced root length in cereals (Kamran *et al.,* 2009). Generally plants accumulate some kinds of organic and inorganic solutes in the cytosol to raise osmotic pressure and thereby maintain both turgor and the driving gradient for water uptake. Under mild drought stress, pattern of resource allocation shifts to the roots rather than to the shoot. Water deficit favors the growth of seminal and lateral roots in seedlings (Abdi *et al.*, 2010). Such an increase in root length in response to PEG induced water stress might be due to limited water up take by the amount of roots in a particular volume of growth media. In this study, Mutant 1 and Mutant 2 lines had longer roots at higher PEG concentrations of 9 and 15 as compared to Chozi and Duma varieties.

Matsuura et al. (1996) reported a positive correlation between drought tolerance traits and root length in sorghum and millet. Similarly, a better root development under drought stress enables plant to reach deeper available water in the soil and hence survive to maturity (Radhouane, 2007). Researchers indicated that the addition of PEG in the medium causes cell dehydration by reducing water availability to cells, which leads to a loss of cell turgor and hence a loss of growth.

The ability to develop extensive root systems contributes to differences among cultivars for drought tolerance and root length is considered an important trait in selection of drought resistant cultivars (Abdi *et al.,* 2010). Thus, root morphology and/or growth rate may be instrumental to select drought tolerant varieties (Malik *et al*., 2002).

In this study total root length decreased in all the varieties studied with increasing PEG concentrations, however, M2 and M1 (Figure 4.3) were able to grow even in higher PEG concentration compared to Chozi and Duma. This indicates that M1 and M2 had a higher sensitivity to osmotic stress. The root length reduction in M1 and M2 under drought stress may be associated to a reduced cellular division and elongation during germination (Frazer *et al.* 1990).

The results of this study indicate that the M1 variety which had longer roots than other wheat varieties at zero and 3 PEG concentration (Figure 4.3). M1 plant line could therefore be used as a drought tolerant variety. Many plants successful in dry habitats have no specific adaptation for controlling water loss but rely on the development of very extensive and deep root systems that can obtain water from a large volume of soil deep in the water table (Ridge, 1991).

Roots of drought tolerant genotypes elongate at a higher rate per week than the roots of susceptible genotypes (Kinyua, 1991; Gesimba, 2000). With more roots all of them elongating at a higher rate, it is imperative that drought tolerant genotypes should have higher total root lengths. In this study M1 had the longest roots at zero PEG concentration followed by M2 (Figure 4.3). This could infer that M1 could have drought tolerance properties.

As indicated earlier many plants successful in dry habitats rely on the development of very extensive and deep root systems that can obtain water from a large volume of soil deep in the water table (Gesimba, 2000). In this study, root elongation was more than 7 cm long (M1, M2 and Chozi) (figure 4.3) at the zero PEG concentration. Duma had the least root length (3 cm) at zero PEG concentration.

Root traits are critical for soil exploration and water and nutrient uptake, and are important for crop improvement under drought conditions (Gupta *et al.,* 2010). The effectiveness of a deep root system in maintaining yield under drought conditions has been confirmed by simulation studies across several years and environments in the USA (Sinclair, 1994). A deep root system helps the plant to avoid drought stress by extracting water stored in deep soil layers (Boyer, 1996). Total root length was associated with drought tolerance in wheat because it affects the distribution of roots in the soil and influences the amount of water uptake (Manschadi, 2006).

5.1.4 Effect of Osmotic Stress on Root/shoot Ratio

Apart from the root and shoot lengths, root/shoot ratio also plays a major role in selecting the line for drought tolerance as balanced root and shoot growth was observed in drought resistant genotypes (Gesimba, 2000). The results of this study revealed significant variations for the root/shoot ratio among the cultivars (figure 4.4). Dhanda *et al*., (2004) also reported positive association of root length with coleoptile length which is in agreement with the results of this study.

Root and shoot lengths are envisaged as prominent characters for screening for drought resistant in wheat (Bayoumi *et al.*, 2008). According to Fraser *et al*., (1990), reduction in root and shoot lengths may be due to an impediment of cell division and elongation leading to a kind of tuberization. This tuberization and lignification of the root system allow the stressed plant to enter a slowing growth state, while waiting for the conditions to become favorable. The results reported in this study are similar to earlier studies of Dhanda et al. (2004) in wheat; Radhouane (2007) and Kulkarni and Desphpande (2007) in tomato (*Lycopersicon esculentum* L.) and Govindaraj et al. (2010) in pearl millet (*Pennisetum glaucum* L.). The authors have reported the effect of drought stress induced by PEG on the plants roots and shoots. It could be concluded from the results of this study that variety M2 was the most sensitive compared to the other cultivars, since it had the highest shoot to root ratio.

5.2 Drought Tolerance at the Seedling Stage

The reproducibility and consistency of the genotypic difference determined at the seedling growth stage and correlation information suggests seedling growth test could be reliable and efficient technique for screening moisture stress tolerance in wheat germplasm. A large range of variability was observed in emergence percentage, emergence index, emergence rate index, energy of emergence, mean emergence time, desiccation tolerance index and percent seedling recovery (Table 4.4).

The different parameter studied under present study can be beneficial in screening wheat genotypes at seedling stage. High positive correlation coefficient between energy of emergence and emergence index and between energy of emergence and emergence rate index and between emergence percentage and emergence index (Table 4.5) which predicts an importance in breeding program (Jaffri *et al.* 1991).

It is evident that good emergence percentages as well as maximum energy of emergence and less mean emergence time having maximum present seedling recovery favored a variety to escape through the hazards of the stress conditions. The information on correlation permits the feasibility of indirect selection for various parameters such relationship provides useful information to the plant breeder in identifying traits that have little or no importance in selection programs (Table 4.5).

A great magnitude of variability was observed in emergence percentage, emergence index and energy of emergence (Table 4.4). Earlier and rapid emergence was observed in genotypes which have maximum energy of emergence and emergence rate index ranging from 91.7 to 49.4 and 27.8 to 16.7 respectively. Desiccation tolerance index showed that only those genotypes which survived had good emergence and low Desiccation tolerance index (Table 4.4).

There is an inverse relation between emergence percentage, emergence index, energy of emergence and mean emergence time. High the emergence percentage, emergence index and energy of emergence and lower mean emergence time indicated earlier and rapid germination (Table 4.6). These findings support the earlier work on Canola (Brassica compestris) by Zheng *et al*., (1994), wheat (*Triticum aestivum* L.) by (Hameed *et al*., 2010), and on rice (Oryza sativa) by Basra *et al*., (2003).

Variety M2 showed lower desiccation tolerance index and higher percent seedling recovery (Table 4.4). These results are supported by the early findings of Milthorpe (1950) who showed that such varieties could be potential for drought tolerance studies. Survival after desiccation proved useful indices for rapid evaluation of water stress response in wheat breeding. Similar findings had been reported by Muhammad and Hussain (2012).

Higher values of emergence percentage, emergence index and energy of emergence and lower mean emergence time indicated earlier and rapid germination. These finding support the earlier work on Canola (*Brassica compestris*) by Zheng et al., (1994), wheat (*Triticum aestivum* L.) by Nayyar et al., (1995) and rice (*Oryza sativa*) by Basra et al., (2003).

5.3 Morphological Characterization

Analysis of variance shows that there was significant difference ($p \leq 0.05$) between the different wheat varieties in terms of plant height, spike length, spikelets per spike, days to 50% heading and grains per spike (appendix 1).

5.3.1 Plant Height

Results of this study showed that there was a significant difference in the plant height with Chozi having the highest mean height (110.77cm) followed by Mutant 1 (90.3cm). The lowest was Duma with a mean of 85.03 cm (table 4.6). These results are similar to those found by Rafiullah *et al.*, (2007) who found that different genotypes showed highly significant difference ($p \leq 0.01$) for plant height. This was also supported by Mohammad *et al.*, (2004).

Results also showed there was a positive correlation between the plant height and the number of spikelets per spike with the taller a plant was, the more the number of spikelets it had (table 4.6). According to Zaheer (1991), yield could be increased through selection of plants with taller plant height and more spikelets per spike.

5.3.2 Spike Length

Results indicate a significant difference ($p \leq 0.05$) in the means of the spike lengths of the different wheat varieties. These results are similar to those of Mohammad *et al.*, (2004) who also reported highly significant differences for spike length. There was a positive correlation between the plant height and spike length with the taller a plant was the longer the spike length was. Spike length can therefore be used as effective selection criteria for yield (table 4.6).

Khan *et al*. (2005), however, reported plant height and spike length had negative direct effect on grain yield. Haq *et al.* (2010) observed that spike length, spikelets per spike, grains per spike, tillers per m2, 1000-grain weight had positive correlation with grain yield.

5.3.3 Spikelets per spike

There was a significant difference in the number of spikelets per spike (table 4.6). A study by Zaheer (1991) showed that yield could be increased through selection of plants with taller plant height and more spikelets per spike. The results from this study are in line with those of Kirby and Appleyard (1984) who found the number of spikelet per spike varying from 20 to 30. The results also concur with those of Degewione and Alamerew (2013) who studied genetic diversity in bread wheat genotypes and found that there was a significant difference between genotypes.

5.3.4 Number of tillers

Tillers are an important component of wheat yield because they have the potential to develop grain-bearing heads. The results from this study (table 4.6) show that there was a significant difference in the number of tillers in the two mutant genotypes. These results are similar to those of Singh et al (2014) who studied Indian wheat genotypes. The number of productive tillers depends on genotype and environment and is strongly influenced by planting density (FAO, 2002). Tillers per plant may be used as effective selection criteria for yield as Khan *et al*. (2005) reported that tillers per plant had the highest positive direct effect on grain yield. In this study, M1 and M2 had the highest mean number of tillers with 7.17 and 6.17 tillers respectively.

5.3.5 Days to 50% heading

Analysis of variance (ANOVA) showed that there was a significant difference ($p \leq 0.05$) in the days to heading. This could be attributed to the fact that normal agronomic practices that were undertaken to all wheat varieties that were grown. However, when the means were separated by Duncan's multiple range test ($p \leq 0.05$) (Table 4.6), Mutant 1 and Chozi variety were similar (57 days). Mutant 2 had an average of 58 days while Duma had the longest days to 50% heading with 59 Days (table 4.6).

These results are different from those of Mollasadeghi *et al* (2010) who compared 12 bread wheat genotypes based on number of phenological and morphological traits and found that there was a significant difference ($p \leq 0.05$). However, in my study, only four wheat varieties were studied.

5.3.6 Number of grains per spike

The number of grains in each spike were counted and the results showed that there was significant difference ($p= 0.05$). Mutant 2 had the highest average number of grains per spike (65.7) (Table 4.6) followed by Chozi (62.27) and Mutant 2 (57.7) and lastly Duma variety (55.4). These results collaborate with those of Ahmad *et al*., (2003) who found a highly significant difference ($p\leq0.01$) in mixed genotypes showing diverse types of wheat and triticale genotypes. This could be attributed to the different genetic makeup of the four wheat varieties.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

1. Mutant lines had a higher number of tillers and grains per spike and shorter days to heading compare to Chozi and Duma. Chozi was however, taller and had longer spike length and more spikeletes per spike compared to the two mutant lines.

2. Compared to Chozi and Duma, mutant lines M1 and M2 exhibited higher drought tolerance characterized by root elongation, increased root/shoot ratio in response to increased PEG treatment.

3. Mutant lines 1 and 2 had higher emergence percentage, emergence index, energy of emergence and percentage seed recovery and lower mean emergence time compared to Chozi and Duma. These parameters are indicative of drought tolerance.

6.2 Recommendations

1. The two mutant lines be further screened for other biotic and abiotic stresses that affect wheat production in Kenya, for purposes of determining their suitability as potential varieties.

2. Other morphological traits such as leaf size, waxiness, stomata size could be used to characterise the difference between the Mutant wheat and other wheat varieties.

3. Molecular work should be done to identify the resistant gene that was created during the mutation process.

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APPENDICES

Appendix 1: ANOVA tables

Analysis of variance (ANOVA) of PEG and number of days

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
PEG conc	Between Groups	.000	3	.000	.000	1.000
	Within Groups	57.000	44	1.295		
	Total	57.000	47			
replicate	Between Groups	.000	3	.000	.000	1.000
	Within Groups	32.000	44	.727		
	Total	32.000	47			
DAY 1	Between Groups	486.729	3	162.243	10.175	.000
	Within Groups	701.583	44	15.945		
	Total	1188.313	47			
DAY 2	Between Groups	1088.917	3	362.972	7.858	.000
	Within Groups	2032.333	44	46.189		
	Total	3121.250	47			
DAY 3	Between Groups	1943.063	3	647.688	18.756	.000
	Within Groups	1519.417	44	34.532		
	Total	3462.479	47			

Analysis of variance (ANOVA) of PEG, shoot length, root length and shoot to root ratio

ANOVA						
		Sum of Squares	Df	Mean Square	F	Sig.
peg_conc	Between Groups	9.028	3	3.009	3.607	.015
	Within Groups	141.845	170	.834		
	Total	150.874	173			
shoot	Between Groups	757.438	3	252.479	12.107	.000
	Within Groups	3545.180	170	20.854		
	Total	4302.618	173			
root	Between Groups	426.564	3	142.188	11.843	.000
	Within Groups	2041.029	170	12.006		
	Total	2467.593	173			
shoot_root	Between Groups	7.632	3	2.544	4.634	.004
	Within Groups	93.334	170	.549		
	Total	100.966	173			

Analysis of variance
Variate: tillers

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.2167	0.1083	0.15	
Wheattype	3	125.6250	41.8750	56.19	<.001
Residual	114	84.9500	0.7452		
Total	119	210.7917			

Analysis of variance
Variate: spikelenght

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.800	0.400	0.24	
Wheattype	3	76.625	25.542	15.13	<.001
Residual	114	192.500	1.689		
Total	119	269.925			

Analysis of variance
Variate: grains

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	7.27	3.63	0.14	
Wheattype	3	1913.80	637.93	23.97	<.001
Residual	114	3034.40	26.62		
Total	119	4955.47			

Analysis of variance
Variate: daystoheading

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.0500	0.0250	0.10	
Wheattype	3	82.5000	27.5000	112.16	<.001
Residual	114	27.9500	0.2452		
Total	119	110.5000			

Analysis of variance
Variate: Spikeletsperspike

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	1.817	0.908	0.39	
Wheattype	3	768.492	256.164	109.18	<.001
Residual	114	267.483	2.346		
Total	119	1037.792			

Analysis of variance
Variate: Height

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	121.85	60.92	1.60	
Wheattype	3	11900.82	3966.94	103.90	<.001
Residual	114	4352.65	38.18		
Total	119	16375.33			

Mutant 1 (P ≤ 0.05)

Wheat	Rep	Plant height	Spike length	Spikelets per spike	Days to heading	Tillers	Grains per spike
M1	1	85	9	27	57	6	51
M1	1	93	9	29	58	5	54
M1	1	92	7	26	57	7	57
M1	1	95	12	28	57	8	60
M1	1	95	10	29	57	6	51
M1	1	94	11	27	57	7	54
M1	1	95	11	26	57	7	51
M1	1	94	8	29	58	7	48
M1	1	88	11	28	57	8	60
M1	1	87	10	29	57	9	66
M1	2	86	8	28	57	7	51
M1	2	96	9	29	57	6	57
M1	2	90	7	28	56	7	60
M1	2	88	10	28	57	7	75
M1	2	86	11	29	57	7	66
M1	2	87	12	27	57	9	54
M1	2	95	11	29	56	8	72
M1	2	85	8	28	57	7	51
M1	2	91	10	27	57	6	51
M1	2	90	11	29	57	6	54
M1	3	95	11	26	57	7	54
M1	3	94	11	29	57	8	51
M1	3	88	8	28	57	9	48
M1	3	87	11	29	58	7	60
M1	3	86	10	28	57	6	66
M1	3	96	8	29	57	7	51
M1	3	90	9	28	57	7	57
M1	3	88	7	28	57	7	60
M1	3	86	10	29	56	9	75
M1	3	87	11	27	57	8	66
Mean		90.3	9.7	28.03	57	7.17	57.7
SE		0.67	0.28	0.18	0.08	0.19	1.41
CV		4.23	15.59	3.57	0.8	14.23	13.56
P		0.05	0.05	0.05	0.05	0.05	0.05
F		1.42	0.56	0.37	0.17	0.38	2.88

Mutant 2 ($P \leq 0.05$)

Wheat	Rep	Plant height	Spike length	Spikelets per spike	Days to heading	Tillers	Grains per spike
M2	1	93	13	32	58	5	72
M2	1	101	12	34	58	6	66
M2	1	95	10	33	58	7	66
M2	1	95	10	31	57	6	65
M2	1	95	11	29	58	6	67
M2	1	95	10	33	58	7	65
M2	1	84	9	32	59	6	64
M2	1	84	10	31	58	7	66
M2	1	84	9	30	57	5	67
M2	2	84	8	31	58	7	66
M2	2	84	10	31	58	6	62
M2	2	84	9	32	59	7	65
M2	2	84	10	33	58	5	67
M2	2	103	13	34	58	6	75
M2	2	99	12	31	58	6	66
M2	2	99	12	32	58	7	66
M2	2	81	11	34	58	6	69
M2	2	81	10	32	58	6	66
M2	2	75	8	31	58	6	45
M2	3	95	10	31	57	6	65
M2	3	95	11	29	58	7	67
M2	3	95	10	33	58	6	65
M2	3	84	9	32	59	7	64
M2	3	84	10	31	58	5	66
M2	3	84	9	30	57	7	67
M2	3	84	8	31	58	6	66
M2	3	84	10	31	58	7	62
M2	3	84	9	32	59	5	65
M2	3	84	10	33	58	6	67
Mean		89.23	10.13	31.67	58	6.17	65.7
SE		1.35	0.25	0.25	0.09	0.13	0.87
CV		0.08	0.13	0.04	0.009	0.12	0.07
P		0.05	0.05	0.05	0.05	0.05	0.05
F		2.77	0.51	0.51	0.2	0.27	1.78

Chozi ($P \leq 0.05$)

Wheat	Rep	Plant height	Spike length	Spikelets per spike	Days to heading	Tillers	Grains per spike
C	1	100	13	35	58	6	63
C	1	103	12	33	57	7	57
C	1	111	10	32	57	4	57
C	1	116	12	32	58	5	64
C	1	115	12	31	57	6	60
C	1	110	12	32	57	6	60
C	1	118	10	33	57	5	65
C	1	126	13	32	56	3	69
C	1	110	12	32	57	5	63
C	2	104	12	31	57	5	66
C	2	107	12	32	57	5	60
C	2	106	12	32	58	4	64
C	2	107	13	32	57	5	63
C	2	112	11	32	57	3	60
C	2	113	11	31	57	5	57
C	2	104	11	32	57	5	57
C	2	115	11	32	56	6	58
C	2	118	12	34	57	5	66
C	2	105	11	32	56	5	63
C	3	116	12	31	57	5	60
C	3	115	12	32	57	3	65
C	3	110	12	33	56	5	69
C	3	118	10	32	57	5	63
C	3	126	13	32	57	5	66
C	3	110	12	31	57	4	60
C	3	104	12	32	58	5	64
C	3	107	12	32	57	3	63
C	3	106	12	32	57	5	60
C	3	107	13	32	57	5	57
Mean		110.77	11.83	32.1	57	4.83	62.27
SE		1.21	0.16	0.16	0.09	0.18	0.66
CV		5.86	7.3	2.68	0.94	20	5.71
P		0.05	0.05	0.05	0.05	0.05	0.05
F		2.48	0.33	0.33	0.20	0.37	1.35

Duma ($P \leq 0.05$)

Wheat	Rep	Plant height	Spike length	Spikelets per spike	Days to heading	Tillers	Grains per spike
D	1	75	10	23	59	4	57
D	1	85	9	26	58	4	53
D	1	90	8	28	59	5	54
D	1	103	12	23	59	5	69
D	1	87	11	28	59	4	56
D	1	96	12	29	59	5	55
D	1	80	10	25	60	6	54
D	1	85	11	22	59	4	58
D	1	77	9	29	58	4	54
D	1	72	7	28	59	5	53
D	2	85	11	24	59	4	54
D	2	86	11	27	59	6	57
D	2	87	10	25	59	5	55
D	2	88	10	22	59	4	56
D	2	88	11	28	59	5	57
D	2	89	12	29	60	4	55
D	2	81	11	26	59	5	54
D	2	86	12	28	59	5	53
D	2	88	10	26	59	4	52
D	2	88	11	26	59	4	53
D	3	86	7	28	59	5	55
D	3	77	11	29	59	4	54
D	3	85	11	25	60	5	58
D	3	80	10	22	59	6	54
D	3	86	10	29	58	4	53
D	3	87	11	28	59	4	54
D	3	88	12	24	59	5	57
D	3	88	11	27	59	4	55
D	3	81	12	25	59	6	56
D	3	77	10	22	59	5	57
MEAN		85.03	10.43	26.03	59	4.67	55.4
SE		1.12	0.25	0.44	0.08	0.13	0.56
CV		7.23	13	9.27	0.77	15.23	5.5
P		0.05	0.05	0.05	0.05	0.05	0.05
F		2.3	0.51	0.9	0.17	0.27	1.14