EVALUATION OF SELECTED MRI SEED MAIZE (*ZEA MAYS*. L) INBRED LINES FOR TOLERANCE TO SEEDLING DROUGHT STRESS.

By

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A dissertation submitted to the Graduate School of Agricultural Sciences of the University of Zambia in partial fulfilment of the requirements for the Masters of Science (Agronomy) Degree.

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DECLARATION

I, Milupi Milupi, declare that this thesis represents my own work and that it has not previously been submitted for a degree at this or any other University.

Signature ……………………………

Date……………………………………………

APPROVAL

This dissertation of Milupi Milupi is approved as fulfilling the requirements for the award of the degree of Masters of Science (Agronomy) by the University of Zambia.

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ABSTRACT

Zambia's many seed companies are involved in maize cultivar development with the primary focus of breeding for yield and disease tolerance. Despite having high yielding potential, most available maize cultivars are greatly affected by drought and heat stress. The parental line stock used in the development of most cultivars have not been evaluated for resilience to drought and climate shocks. Therefore, the aim of this study was to evaluate 18 selected MRI-Seed maize (*Zea mays* L.) inbred lines for tolerance to drought stress imposed at seedling growth stage. The study was conducted at Chakana Farm where twenty seedlings for each of the 18 MRI seed maize inbred lines were raised in a Randomized Complete Block Design (RCBD) with three replicates. The plants were observed for 42 days for their phenotypic expression and growth performance under water stress conditions and data on vegetative seedling traits were recorded. Data collected was subjected to Analysis of Variance (ANOVA), Principal Component Analysis (PCA) and correlation analysis. Results of the study showed significant differences at $p \leq 0.05$ and $p \leq 0.01$ among the parental lines on moisture content of root, fresh shoot weight, total fresh biomass and seedling aspect, indicating that there was wide variability in the response of the genotypes for tolerance to drought at seedling stage. Principal Component Analysis also confirmed these traits as the primary ones contributing to diversity among the parental lines under induced drought stress at seedling growth stage, in that they were loaded under PC1 with Eigen values greater than 0.3 and contributing 27% of total effect observed while other traits collected were loaded in PC2 and PC3 with total effect contribution of 22% and 19% respectively. Correlation analysis showed differential association among traits of the parental lines, indicating that mechanism of tolerance to drought among the lines were different and that seedling aspect, moisture content of root , fresh shoot biomass, and total fresh biomass were important drought adaptive traits that could be considered for base indexing when selecting drought tolerant maize genotypes at seedling stage. The correlation coefficients of root moisture content to seedling aspect and total biomass was $r = 0.56^{*}$ and $r = 0.99^{**}$ (at $p \leq 0.05$ and $p \leq 0.01$) respectively. Overall, the study identified five MRI inbred lines, coded as 15ZMB990298, 15ZMB990309, 16ZM901059, 16ZM902623 and 16ZM920511 to be well tolerant to water stress at seedling growth stage. These inbred lines could be recommended for inclusion in crossing blocks to generate F1 drought tolerant hybrids at seedling stage depending on their General Combining Ability (GCA) and also Specific Combining Ability (SCA).

Key words: Maize, principal component, biomass, seedling aspect, root moisture

DEDICATION

I dedicate this work to my family of five. My wife Charity Chiguma Milupi, my three daughters (Namaya, Namukoma and Tebuho) and my son Micheal for all the support rendered and the endurance they underwent staying without a close relationship of the husband and father during my study period.

ACKNOWLEDGEMENTS

I would like to acknowledge the great support received from my supervisors, Dr. T. T Kambikambi and Dr. D.M. Lungu for their untiring guidance in this research both on paper and in the field.

My humble appreciation also goes to MRI seed (Z) limited management for supporting the study by provision of inputs and approval of a third-party collaborator at which the research was conducted.

Many thanks also go to the MRI seed Zambia, Research and Development team, especially Mr. Friday Mulopa for his role in helping on technical trial management.

To many more whom I may not have mentioned, please let the Almighty God kindly bless you for your kindness and love and help rendered to my work.

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ABA	Abscisic Acid
ANOVA	Analysis of Variance
CDKs	Cyclin-Dependent Kinases
CIMMYT	International maize and wheat improvement centre
CO_2	Carbon Dioxide
CSO	Central Statistics Office
CT	Canopy Temperature
GCA	General combining ability
GM	Geometric Mean
IAPRI	Indaba Agricultural Policy Institute
IPCC	Intergovernmental Panel on Climate Change
MRI	Maize Research Institute
PCA	Principal Component analysis
RCB	Randomised Complete Block Design
ROS	Reactive oxygen species
RSR	Root-Shoot ratio
SCA	Specific combining ability
SPSS	Statistical Package for the Social Science
WEMA	Water Efficient Maize for Africa

CHAPTER 1

1.0 INTRODUCTION

The most important downside risk farmers face in sub-Saharan Africa (SSA) is crop production risk that is manifested through unpredictably variable agricultural yield. This risk is enormously enhanced in SSA due to the uncertainty surrounding the occurrence, spatial distribution, and intensity of drought. Drought is generally considered as a normal part of climate for virtually every country. It is a slow-onset, creeping phenomenon with serious economic, environmental, and social impacts. It affects more people than any other natural hazard (Glantz and Katz, 1977; ISDR., 2003). It might be considered in general terms a consequence of a reduction over an extended period of time in the amount of precipitation that is received, usually over a season or more in length.

Across large areas of SSA, drought is a widespread phenomenon, with an estimated 22% of mid-altitude/subtropical and 25% of lowland tropical maize growing regions affected annually due to inadequate water supply during the growing season (Heisey and Edmeades, 1999). Climate change is likely to lead to increased temperature by an average of 2.1 °C in SSA and water scarcity, particularly in southern Africa, in the coming decades (Hendrix and Glaser, 2007; Lobell *et al.*, 2011). The immediate impacts of drought risk in southern Africa are manifested through the low and declining performance of the agricultural sector in general and that of maize production in particular. Maize (*Zea mays. L*) and its production define livelihoods of millions of people in southern Africa, serving as the most important source of calories for the poor African small holder farming communities (Lobell *et al.*, 2008).

In Zambia, maize stands out as the primary crop both in terms of acreage and absolute yield levels however, production has of late been constrained by natural forces. Amongst the natural forces, drought has repeatedly been reported to be the most important challenge of maize production in SSA, Zambia inclusive (Kassie *et al.*, 2012). Climate is a dynamic natural phenomenon, and hence exposure to drought varies over time. Global warming and the probability that drought and other extreme climatic events may become more frequent in the future may translate into increased exposure to drought (Wilhite *et al.*, 2000; ISDR., 2003; World Bank, 2006) and puts the poor rural small holder farmers, entirely dependent on maize production at an extreme risk of hunger. The need to mitigate this risk through breeding cultivars that are drought resilient and climate smart cannot be over emphasized, especially that maize is the staple food crop for Zambia and many countries in SSA.

1.1 Importance of maize

Worldwide, maize (*Zea mays* L.) is a major cereal crop, with a record production of 1134.75 million tones and corresponding yield of 5.75 tons/ha in 2017 (FAO, 2018). During the same year, Africa's total production was 73.5 million tonnes with an average yield of 2.1 tons/ha. In Zambia, production for 2017 was 3.61million tons with an average yield of 2.19 tons/ha while 2018's production was 2.3 million tons and corresponding yield of 1.72 tons /ha (CFS, 2018) as illustrated on (figure1) below.

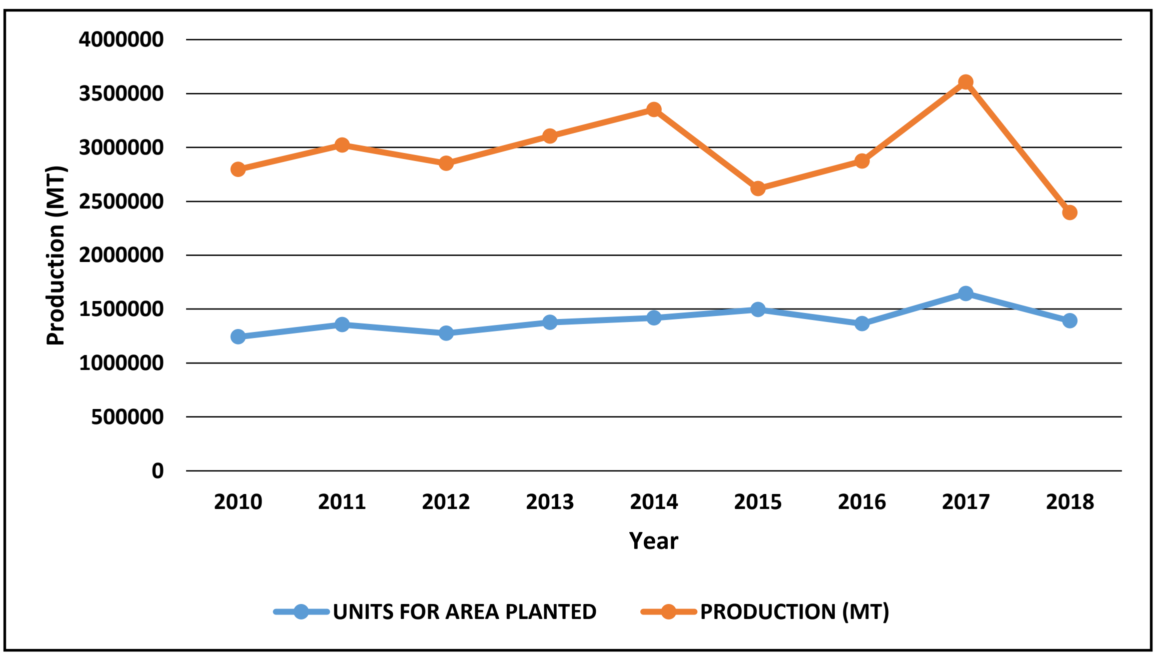

Figure 1 Maize production (MT) in Zambia from 2010 to 2018

Source: CSO/MOA (2018)

Globally, millions of people rely on maize as a staple food and also as source of income. It is also used as feed for livestock as well as a raw material for industrial purposes. In Africa, maize contributes at least one fifth of the total daily calories and accounts for 17 to 60% of the total daily protein supply of individuals in 12 countries as estimated by FAO food balance sheets (Krivanek *et al.*, 2007; FAO, 2012; FAO, 2014). Maize (*Zea mays* L.) is the most important food crop in Zambia and is produced in all the ten provinces of the country. It is used primarily for human consumption as porridge, nshima (local name) or fresh green maize (Mungoma, 1997) and is eaten every day in most households.

According to CSO (2006a) and Indaba Agricultural Policy Institute (IAPRI), rural livelihood report of 2015, about 65% of the households in Zambia are agricultural; of these about 84% are located in rural areas. Over 90% of agricultural households are small-scale farmers; 69% cultivate only up to 2 ha (CSO, 2006b). About 86% of the agricultural households grow maize while only 9% grow millet, the second most widely cultivated cereal as observed on (Table 1) below.

Table 1 Percentage (%) of households growing major cereal crop in zambia

Crop grown	National	Central	Copper belt	Eastern	Luapula	Lusaka	Muchinga	Northern	North western	Southern
Maize	89.4	95.0	100.0	99.5	67.5	98.9	94.4	71.7	91.3	94.7
Millet	10.1	7.4	1.0	0.9	3.4	1.3	27.4	30.6	3.6	4.5
Rice	4.9	0.0	0.0	2.3	3.4	0.0	11.6	11.1	1.9	0.0
Sorghum	3.7	2.2	1.8	0.4	0.3	0.5	8.1	1.0	4.5	10.2

Source: Rural livelihood survey report (IAPRI, 2015)

1.2 Maize Production Constraints in Zambia

The major constraints to maize production include both biotic and abiotic factors. The main biotic factors are pests and diseases. Examples of pests include fall armyworms, stock borers and root worms. Some of the important diseases are Maize streak virus (MSV), Leaf blights, Grey leaf spot and leaf rust. The most common abiotic factors are drought, extreme temperatures, low soil fertility (especially low nitrogen), high soil Aluminium (soil acidity), flooding and salinity (Mweshi, 2009). Drought is a common phenomenon in tropical environments, and it is one of the major factors contributing to yield losses in maize production. It may cause yield losses of up to 60% (Edmeades *et al*., 1999) in southern Africa.

1.3 Problem statement and justification

Zambia has abundant surface and underground water resources. It has numerous rivers, lakes and dams. The total country's ground water is estimated at 1,740,380 million cubic metres while the ground water recharge is estimated at 160,080 million cubic meters per annum. While the potential irrigable land is estimated at 2.75 million hectares, less than 155,000 hectares or six percent is actually irrigated. This is concentrated mostly on commercial farms for the production of sugarcane, wheat and plantation crops (Hamududu *et al*., 2018). Despite the abundant water resource in Zambia, drought still remains a major threat to Zambia's food security. This is because mostly, maize is produced as a rain fed crop and also that small scale farmers who are significant contributors to maize production lack capital to invest in irrigation equipment. This problem is aggravated by limited availability of drought tolerant food crop cultivars to the farming community.

A number of seed companies in Zambia are involved in maize cultivar development with the primary focus of breeding for yield and disease tolerance. Despite having high yielding potential, most available maize cultivars are greatly affected by drought and heat stress. The parental line stock used in the development of most cultivars have also not been evaluated for resilience to drought and climate shock. Drought tolerant lines could be available but such lines have not yet been identified.

Prevailing climatic change, with metrological forecasting of increase in temperature of 0.6^{o}c per decade and more likelihood of drought than ever before (MTENR, 2007), agitates a crucial requirement for breeding for drought tolerant and climate smart maize genotypes. Breeding for drought tolerance starts with understanding of how available parental line stocks can perform in drought environments and possibility of introgression of the parental drought resilience traits on to the progenies. Therefore, there is need to evaluate inbred lines for tolerance to drought stress and identify those with potential to be used as testers in drought tolerance hybridization programmes.

1.4 Main objectives

The main objective of the study was to evaluate selected MRI-Seed maize (*Zea mays* L.) inbred lines for tolerance to drought stress imposed at seedling growth stage

1.4.1 Specific Objectives

The specific objectives were;

i. To identify in bred lines tolerant to seedling drought stress.
ii. To identify adaptive phenotypic traits to be used as selection criteria for drought tolerant maize genotypes.

1.5 Research Hypotheses

i. There are no differences among inbred lines on their tolerance to drought stress.
ii. There is no relationship between adaptive phenotypic traits and drought tolerance among maize genotypes.

CHAPTER 2

2.0 LITEREATURE REVIEW

2.1 Maize origin, domestication and germplasm diversity

Maize belongs to the tribe Maydeae of the grass family *Poaceae*. “*Zea*” (zela) was derived from an old Greek name for a food grass. The genus *Zea* consists of four species of which *Zea mays* L. is economically important. The centre of origin for *Zea mays* has been established as the Mesoamerican region, now Mexico *and Central America* (Chaudhary *at al*., 2014; Watson and Dallwitz, 1992). Archaeological records suggest that domestication of maize began at least 6000 years ago, occurring independently in regions of the southwestern United States, Mexico, and Central America (Mangelsdorf, 1974).

Maize germplasm resources are preserved *ex-situ* in many parts of the world. However, only in the Meso-American region there still exists, *in-situ*, the original ancient maize that gave rise to improved varieties that are grown in all regions of the world. Most of the maize variation can be found in the Meso-American region and the northern part of South America. The great diversity of environments and conditions have created the basis for the development of maize varieties well adapted to harsh conditions of soil and climate as well as to biotic stresses. There is a growing trend in developing countries to adopt improved maize varieties, primarily to meet market demand. The narrowing of genetic diversity in modern maize varieties emphasizes the importance of conserving genetic traits for future plant breeding. International Maize and Wheat Improvement Centre (CIMMYT) has taken the lead in preserving maize germplasm. It has the world’s largest collection of maize accessions, with over 17,000 lines (CIMMYT, 2000).

2.2 Cultivation Requirements of Maize

Maize is grown all over the world from about latitudes 55° North to 40° South and from sea level to 3 800m altitude. It has adapted to a wide range of environments with its growing period ranging from 65 days in the lowland tropics, to approximately 12 months in the tropical highlands (Fischer and Palmer, 1984). It performs well on well-drained fertile soils in areas with moderately high temperatures and adequate, but not excessive rainfall (Jugenheimer, 1976; Mungoma, 1997). It requires about 450-600 mm of water during its growing cycle and yields about 20kg ha-1 of grain for each mm of water, giving an average potential yield of 9-12-ton ha^{-1} (Pendleton, 1979). With minimum average rainfall of about 600 mm season^{-1}, Zambia receives enough rain to support maize production and achieve high yields. For normal growth, maize requires essential elements, of which nitrogen (N), phosphorous (P) and potassium (K) are the most important. The minimum levels of these three elements required in dry soil to support maize production are 3.0% N, 0.25% P and 1.9% K (Mohr and Dickson, 1979). Much of the soil in Zambia is of savanna type and contains very small amounts of N because much of the nutrient is lost through leaching and/or de-nitrification (Vanlauwe *et al*., 2001). In Zambia about 112kg ha^{-1} of N is recommended for application to maize and this could enable farmers to realize grain yields of about 6-8t ha^{-1} (Wellving, 1984). Some N is also naturally made available to plants through decomposition of organic matter in the soil. However, the general N recommendation may not be appropriate for semi-arid areas in Region I where soils generally lack moisture. Region I receives less rain and experiences higher temperatures than the other regions, hence the low soil moisture. Therefore, the limited available soil moisture is inadequate to dissolve the applied inorganic fertilizers which remain unavailable to plants. Shamudzarira and Robertson (2002) found that moderate rates of about 30kg N ha^{-1} gave greater N response than lower rates (15kg N ha^{-1}) in semi-arid areas in Zimbabwe. The recommended rates may be too high in the dry Region I of Zambia. A maize plant optimizes its growth at 24-30°C (Pendleton, 1979). According to Muchinda (1985), average temperatures range from 20-26°C, during summer when much of the maize is grown in Zambia. This is close to optimal temperature for maize growth and development and confirms the suitability of maize cultivation in Zambia.

2.3 Drought and its effects on maize production

Drought is the main abiotic factor most responsible for limiting maize production and productivity in the developing world (Edmeades *et al*., 1992). Three types of drought exist, meteorological drought, hydrological drought and agricultural drought. Meteorological drought is defined as an interval of time, generally in the order of months or years during which the actual moisture supply at a given place consistently falls below the climatically appropriate moisture supply (Passioura, 1996). On the other hand, hydrological drought refers to deficiencies in surface and subsurface water supplies. Blum (1996) defined agricultural drought as the relative ability to maintain plant function under a dehydrated state. The effect of drought on maize has been extensively studied, and plants are affected at various levels of their growth (Edmeades *et al*., 2006). Maize has been shown to be very sensitive to drought during seedling establishment and flowering. A deficiency of water during early stages of crop growth was shown to result in low grain yields. Drought tolerance is a polygenic trait, that is, its expression is controlled by several genes. As a result, genotypes vary in their ability to tolerate drought stress (Ribaut *et al*., 2006).

Water is vitally needed for every organism in specified amount and any deficiency in that particular amount imposes the stressful conditions. Water requirement is variable across the tissues and across the growth stages of same species of crop plant and maize crop has no exception so, far. Assessment of optimum plant water requirements is prerequisite to determine the water deficiency in plants. Water requirement of maize crop is low at early growth stages then reaches on peak at reproductive growth stages and during terminal growth stages requirement of water again lowers down. During reproductive growth stage, 8–9 mm water is needed per day to a single plant. Four weeks are most crucial regarding water requirement which includes two weeks before and two weeks after pollination. Pollination is most critical growth stage for water requirement and all leaves are kept unfolded and grain yield is also decided at this stage. Grain filling and soft dough formation are most sensitive to water deficiency, whereas, pre-tasseling and physiological maturity are relatively insensitive to water deficiency.

Drought stress during vegetative growth stages especially during V1–V5, reduces growth rate, prolong vegetative growth stage and conversely, duration of reproductive growth stage is reduced (Pannar 2012). Each millimeter of water produces 15.00 kg of kernels and total 450–600 mm is needed across the whole season (Du Plessis 2003). On average, a total of 250litres water is consumed by the maize plant till maturity (Du Plessis 2003). Relative water contents, water potential, stomatal resistance, leaf temperature and rate transpiration are responsible for maintaining the plant water relation and any imbalance in these or any one of these traits disturb the plant water relation (Anjum *et al*. 2011).

Relative water contents determine the status of metabolic activities of the cell or tissue. During early leaf development, relative water contents of the leaves are higher and tend to decline towards maturity. Strong correlation is reported between relative water contents, water uptake and transpiration rate. Under drought stress, relative water contents and water potential is reduced, resultantly, leaf temperature is increased due to reduced transpirational cooling (Siddique *et al*.

2001). This phenomenon, easily reviews that plant water status is dependent on stomatal activity (Anjum *et al*. 2011). Transpiration ratio is described as number of water molecules lost in order to fix one molecule of carbon. Soybean, wheat and maize have 704, 613 and 388 transpiration ratio respectively which shows that maize is relatively efficient water user crop (Aslam *et al*., (2015): Jensen (1973). Despite of being efficient water user, maize is badly affected by drought stress due to hypersensitivity against water deficiency. In maize, developmental stages starting from germination to harvest maturity including seedling establishment, vegetative growth and development and reproductive growth stages are very much prone to drought stress. Effects of drought on maize at different growth stages and organizational levels have been presented on the (figure 2) below and described in subsequent sessions.

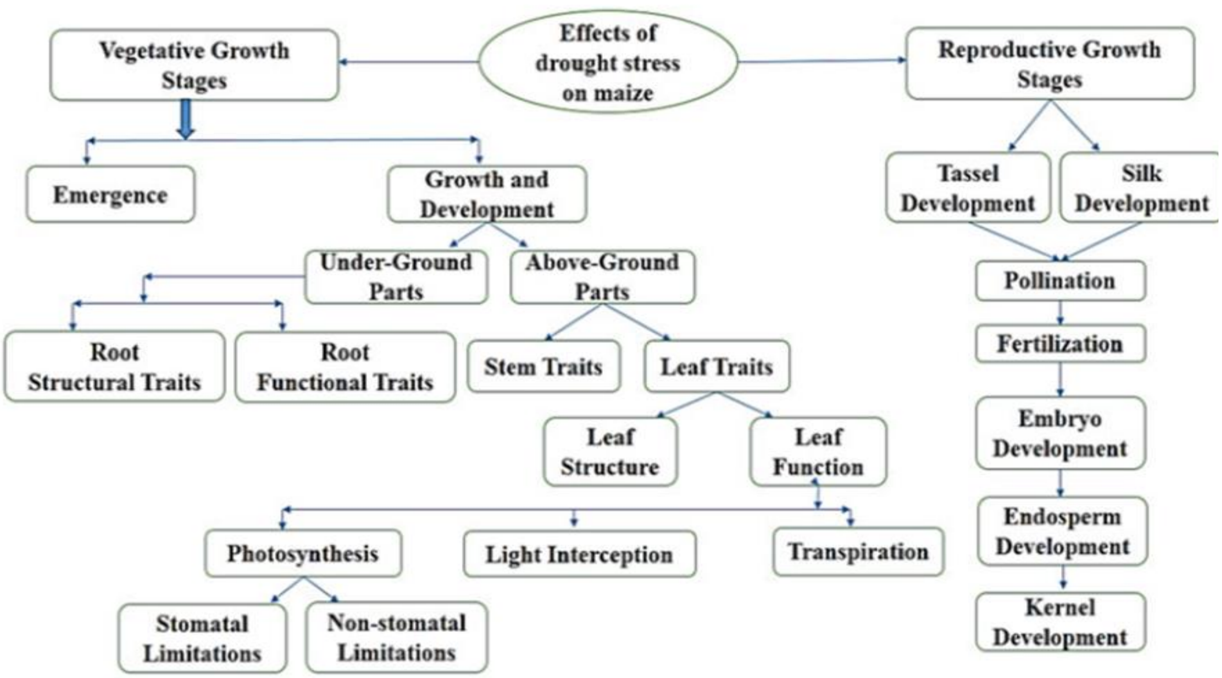

Figure 2: Drought effect on plants- diagramical presentation

Source: Aslam *et al*., (2015)

2.4 Drought effects on Growth and development

Development and growth of crop plants is dependent on establishment of normal plant structures that carry out all physiological and metabolic processes and give potential yield. Drought stress has been observed to seriously hinder growth and development in maize production. Growth and development comprises of numerous component parameters which are estimated by different traits like, plant height, leaf area, structural and functional characters of root, plant biomass, plant fresh weight, plant dry weight and stem diameter. Plant height, stem diameter, plant biomass and leaf area are reduced under drought stress (Khan *et al*. 2001; Zhao *et al*. 2006). Growth is described as increase in size of plant which is directly associated with increase in number of cells and cell size. Meristematic tissues are involved in active elongation of plant by active cell division. Cell division and cell size are reduced by reduction in water potential of cells which causes the reduction in plant growth (Nonami 1998). Leaves in maize ranges from 8 to 20 and these are present alternatively on nodes. Leaf is comprised of structural and functional components. Leaf growth consists of leaf size and number of leaves which are structural components. Photosynthesis, transpiration and light interception are the functional elements of leaves. Leaf size and number of leaves are reduced in maize by drought stress. Turgor pressure, light interception and flux assimilation are determinant of leaf elongation (Rucker *et al*. 1995). Wedge shaped motor cells are present on the upper leaf surface and these keep the leaves unfolded whereas, under drought stress turgor of leaves is reduced and leaves are curled or folded (Du Plessis 2003). Leaf folding reduces the leaf area and resultantly light interception is reduced which decreases the photosynthetic activity. Leaf area and photosynthesis are directly proportional to each other (Stoskopf, 1981). Cell division and cell elongation are reduced under drought stress which reduces the leaf area. Reduction in leaf area under drought stress conditions is taken as adaptive strategy by maize plants. Leaf area index is considered as an important parameter for maize breeding against drought stress (Hajibabaee *et al*., 2012). Plant water requirement is reduced by reducing the leaf area and probability of plant survival is increased under limited water availability (Belaygue *et al.*, 1996) but chlorophyll contents, chloroplast contents and photosynthetic activity are reduced which reduced the grain yield (Flagella *et al*. 2002; Goksoy *et al*., 2004).

Kinases protein family and cyclin-dependent kinases (CDKs) are involved in the active progression of cell cycle. CDK activity is reduced under water deficit conditions which increased the duration of cell division and decrease the number of cell divisions per unit time that ultimately reduces the growth of leaves and plant (Granier *et al*., 2000). Cell elongation is found to be reduced across all points on leaf. Common regulatory pathway is involved in cell division and cell elongation (Tardieu *et al*. 2000). Drought stress increases the leaf to stem ratio which is indication of high level of growth retardation in stems than leaves (Hajibabaee *et al*., 2012). Reduced water potential in roots interrupts the optimal water supply to the elongating cells and resultantly cell elongation is reduced. Water potential less than−10.0 Bars causes the reduction in leaf growth (Tanguilig *et al*., 1987).

Light interception is reduced after reduction of leaf area. *Less interception of solar radiations* causes the reduction in biomass production (Delfine *et al*., 2001). Besides light interception, stomatal activity is also responsible for lower biomass production (Delfine *et al*., 2001; Medrano *et al*., 2002). Rise in leaf temperature under drought stress, inhibits the enzymatic activity and reduces photosynthesis (Chaves *et al*., 2002). Photosynthetic machinery is inactivated by increase in leaf temperature above threshold temperature which is 30 °C (Crafts-Brandner and Salvucci, 2002).Stomatal closure, reduced transpiration and its homeostatic effects are the cause of rise in leaf temperature under limited water availability (Jones 1992). The photosynthetic activity in maize plant is reduced by stomatal and non-stomatal limiting factors. Reduced turgor pressure of the leaf and root originated signals along with lower plant water status trigger the closure of the stomata. Reduction of water potential in the roots transduces the signals for stomatal closure. CO_2 diffusion in the leaves is reduced by stomatal closure and supply of CO_2 to the RUBISCO is hampered (Flexas *et al*., 2007). Reduced CO_2 diffusion is considered as main reason for decline of photosynthesis. Abscisic acid (ABA) accumulation is increased in the leaves in response to drought induced signals which triggers the stomatal closure (Wilkinson and Davies 2010). Cellular environment becomes alkaline under drought stress. Rise in cellular pH increases ABA accumulation in the leaves (ABA trapping) which induced the stomatal closure (*Jia* and Davies 2007).

Stomatal closure has protective role in saving the water loss and increasing water use efficiency under mild drought stress but under severe drought stress stomatal closure becomes inevitable evil (Chaves *et al*. 2009). Stomatal conductance and transpiration rate modulate the CO_2 diffusion in leaves which are directly linked with stomatal opening. CO_2 fixation rate, intercellular CO_2

concentration and net photosynthetic rate are the parameters used for assessment of stomatal conductance and photosynthetic activity under drought stress (Sage and Zhu, 2011). Passive and active stomata closures occur under normal conditions and stress prevalence respectively. Different genes are regulated to maintain the production and consumption equilibrium by alteration of redox state in leaves under drought stress. Reactive oxygen species (ROS), electron acceptors and electron carriers have potential role in regulation of stomatal conductance (Chaves *et al*., 2009).

2.5 Mechanisms of drought tolerance

Plants are able to resist drought due to expression of different mechanisms. According to Blum (1996), drought resistance mechanisms include drought escape, drought avoidance and drought tolerance. Drought escape is a mechanism by which plants grow and complete their life cycle before severe drought occurs (Ribaut *et al.*, 2006). Drought escape also involves developmental plasticity, which is the ability of plants to stop growth during water deficit. A cultural practice that can be used to escape drought stress is the use of early maturing cultivars. As reported by Edmeades *et al*. (1999), the benefits of early maturing cultivars are only realized when water deficit is severe. However, yields will be less when there is adequate water since yield is highly correlated with maturity dates (Edmeades *et al*., 1999). Cultivars that can escape drought stress still experience yield losses during water deficits. Under such conditions, drought avoidance mechanisms will be required to survive drought (Blum, 2005). Drought avoidance is a mechanism by which plants are able to maintain high tissue water potential despite soil water deficit. This is achieved through xeromorphic characters such as leaf rolling, stomatal closure and leaf senescence (Chaves *et al*., 2003).

The third mechanism through which plants cope with drought is drought tolerance. It is defined as the ability of plants to withstand water deficits and maintain physiological processes although low tissue water potential develops. Drought tolerance at the cellular level is achieved by accumulation of solutes (osmotic adjustment), an increase in cellular elasticity and a decrease in cell size. Solutes that accumulate and are synthesized as a result of water deficit include amino acids, organic acids and sugars (Blum, 2005). Blum (2005) reported that genetic variations in osmotic adjustments exist among cultivars which determine their ability to tolerate drought.

2.6 Screening for tolerance to seedling drought stress

Plant breeders are continuously seeking new traits and breeding methods to improve drought tolerance (Ribaut *et al*., 2006). This is because there are several difficulties in directly selecting for grain yield under drought stress, since grain yield is a polygenically controlled complex trait. Breeders therefore have been using less complex traits called secondary traits as selection criteria (Bruce *et al*., 2002). A suitable secondary trait is genetically associated with grain yield under drought, is highly heritable, stable, easy to measure, and is also not associated with yield loss under ideal growing conditions (Banziger *et al*., 2000).

Several morphological and physiological traits can be used as selection indices for drought tolerance in maize (Lafitte *et al.,* 2004). Identification and measurement of secondary traits associated with grain yield provides a guide to specific mechanisms that contribute to grain yield under drought stress. Thus, water depletion patterns, canopy temperature and leaf rolling are indicative of water extraction capacity, while chlorophyll concentration is a measure of the functional stay green character (Bazinger *et al*., 2001). While some secondary traits are associated with specific development stages such as vegetative or flowering, others such as photosynthetic rate are indicative of plant growth throughout the life cycle of the crop.

2.7 Secondary traits associated with maize yield under drought stress

2.7.1 Root traits

Root traits are important for improving maize growth under drought stress. Significant genetic varietal differences in root growth and development under drought and optimum conditions have been observed among maize seedlings (Camacho and Caraballo, 1994; Hund *et al*., 2009). Root length could be used as a selection criterion for improved drought tolerance in maize seedlings. Maize plants with more roots (higher dry and fresh weight) at seedling stage subsequently develop stronger root systems, produce more green matter and have higher values for most characters determining development and yield (Blum, 1998).

2.7.2 Nutrients and water uptake by the root structures

Root traits affect the amount of water and nutrient absorption, and are important for survivability and maintaining crop yield under drought stress conditions in plants (Narayanan *et al.,* 2014).

Plants with higher main root diameter have more growth potential as it has direct relation with water absorption (Richards *et al.,* 2001), and have more ability to explore for moisture in compact soils (Bao *et al.,* 2014). Fine roots are most permeable and thought to have greater ability to absorb water, especially in herbaceous plants. Root architecture also has a significant impact on nitrogen use efficiency (Cosmas *et al.,* 2012). Increased early vigour results in deeper and faster root growth, forming more adventitious roots in the upper soil layer, which increases nutrient and water uptake and reduces surface soil evaporative losses (Sinclair and Paulsen, 1994). In addition to these traits, several morphological root traits such as root tissue density, specific surface area and specific root length are correlated with increased moisture preservation, crop resilience and high productivity under drought conditions (Vadez *et al.,* 2013). Root diameter and root tissue density control the root surface area and length; and hence, encapsulate the overall effect in terms of root length per dry biomass allocated to root system of a plant (Turner *et al.,* 2001).

2.7.3 Leaf rolling

Leaf rolling is a common response to drought in maize (Banziger *et al*., 2000; Edmeades, 2008). Many maize genotypes can reduce the amount of radiation that they intercept by rolling their leaves when exposed to drought. Leaf rolling is both a dehydration avoidance and protective mechanism. The reduction of radiation interception due to changes in leaf orientation is significant as it allows the radiation load on the canopy to be reduced. Firstly, the damage caused by increased leaf temperature resulting from high levels of solar radiation incident on the leaf surfaces is minimized by reducing the effective leaf area presented to the sun's rays (Kadioglu and Terzi, 2007). This results in less radiation being intercepted by the leaf. Secondly, through leaf rolling, the transpiration rate can be reduced through the creation of a microclimate that has both high humidity and boundary layer resistance near the leaf surfaces, thereby conserving the scarce water resources (Kadioglu and Terzi, 2007).

The degree of leaf rolling is calculated as the percentage reduction in leaf width resulting from rolling. The degree of rolling may be closely linked to the water potential. For instance, in maize, it was found that leaf rolling scores were linearly correlated with leaf water potential (O'Toole and Cruz, 1980). Rolling which increases drought avoidance in maize is an adaptive trait and controls plant water metabolism by relieving water stress.

2.7.4 Leaf senescence

When plants are exposed to drought, the leaves begin to lose proteins and chlorophyll. Gradually, the leaves dry. Drying starts from the tip and edges of the leaf until eventual death of leaf tissue occurs (Blum, 2005). Under drought stress, breeders select for delayed leaf senescence, a trait that has moderate heritability (Banziger, 2000). Delayed leaf senescence, also known as the stay green trait, is essentially the capacity of a plant to postpone or prevent leaf senescence during pre-anthes or post anthesis drought stress. However, some evidence suggests that maize leaves exhibiting the stay green trait may not always remain metabolically active under drought stress (Li *et al.*, 2006). Leaf senescence is measured by scoring plant leaves on a scale from 1 to 10 and multiplying the score by 10 as illustrated by Banziger *et al.* (2000).

2.7.5 Canopy size

Decreasing the distance between neighbour rows at any particular plant population has several potential advantages. First, it reduces competition among plants within rows for light, water and nutrients due to a more equidistant plant arrangement (Olson & Sander, 1988; Porter *et al*., 1997). The more favourable planting pattern provided by closer rows enhances maize growth rate early in the season (Bullock *et al*., 1988), leading to a better interception of sun light, a higher radiation use efficiency and a greater grain yield (Westgate *et al*., 1997). The maximization of light interception derived from early canopy closure also is a factor to consider as it reduces light transmittance through the canopy (McLachlan *et al*., 1993). The smaller amount of sun light striking the ground decreases the potential for weed interference, especially for shade intolerant species *(Gunsolus, 1990;* Teasdale, 1995; Johnson *et al*., 1998).

2.7.6 Canopy temperature

Canopy temperature (CT) refers to the temperature of plant canopies and indicates the ability of transpiration to cool the leaves under a demanding environmental load such as drought stress (Blum, 2005). Canopy temperature can be measured using an infrared thermometry, which is a quick and relatively accurate means of detecting differences in water transpired by the crop. Since the major role of transpiration is to cool the leaf, reduction of CT, relative to ambient air temperature, is an indication of how capable transpiration is in cooling the leaves during drought stress.

When selecting for drought tolerance, the interest is in finding genotypes that maintain lower CT compared to other genotypes under the same drought conditions. Relatively lower CT in drought stressed crop plants indicates a relatively better capacity for taking up soil moisture and for maintaining better plant water status (Zharfa *et al*., 2010). This capacity, as expressed in relatively lower CTs, has been shown to be positively correlated with final yield under drought and heat stress (Zaidi *et al*., 2007). Canopy temperature is also affected by the relative amount of desiccated and dead leaves in the canopy, and thus it was found to be positively correlated with 'leaf death scores' (Araus *et al*., 2008).

2.7.7 Drought tolerance indices

The response of a plant to drought stress depends on the genotype, intensity and duration of drought, plant age and soil characteristics. Genotypes evaluated under drought and non- drought stress can be ranked according to their drought tolerance using different indices. This facilitates identification of the best performing drought tolerant genotypes. Rosielle and Hamblin, (1981) proposed use of the stress tolerance index (TOL) for evaluating the differences in yield under stress and optimum environments. Fernandez (1992) suggested the use of a stress tolerance index (STI), which can be used to identify genotypes that produce high yield under drought and non-drought stress. Another yield-based estimate of drought tolerance is the use of the geometric mean (GM) (Schneider *et al.,* 1997). Fischer and Maurer, (1978) proposed the use of a drought susceptibility index (DSI) for each genotype. Drought susceptibility index has been used by several researchers to identify drought tolerant genotypes in different crops (Grezsiak *et al.,* 2007; Anwar *et al*., 2011; Grezsiak *et al*., 2012).

CHAPTER 3

3.0 MATERIALS AND METHODS

3.1 Site description

The study was conducted at Chakana farm in Chiawa area during the 2018 winter season. Chiawa is located south –east of Lusaka, about 120km away in the lower Zambezi valley. This area was chosen for the study due to its favourable maize production weather conditions in winter unlike the plateau area. In the absence of rainfall in winter, all other climatological requirements for maize production are suitable for maize growing in Chiawa- valley area, making the area suitable for the study. The climate of this area is primarily under Region I classification of the Agro-ecological Zones of Zambia, generally hot and dry with average temperature of 34^{o}c and annual rainfall of less than 500mm (Table 2 below). The soils are predominantly very shallow gravelly and rocky (Skeletal). Areas close to the river bank consists of colluvial soils and are deeper, reaching to the alluvial deposits that get deposited out of the Zambezi river, making such portions of the land very suitable for crop production .

Table 2 Geographical description of Chiawa

Site Name	Longitude (^{0}E)	Latitude (^{0}S)	Altitude (Masl)	Temperature ^{0} C			Average annual precipitation (mm)
				Min	Max	Av	
Chiawa	29.0500	-15.8833	337	28	37	34	331.5

Source: World weather online; https://www.worldweatheronline.com/chiawa-weather averages/lusaka/zm.aspx

3.2 Genotypes used in the study

Eighteen inbred lines were used in the study as listed on (table 3). Out of these, sixteen (16) were the elite inbred lines sourced from Maize Research Institute (MRI) seed while the other two (2) were the WEMA lines sourced from CIMMYT. The WEMA lines were developed for water stress tolerance and it is for this reason that they were used as checks in the experiment.

Table 2 List of genotypes evaluated for drought tolearance induced at seedling growth stage at chakana farm in chiawa during the winter season of 2017.

ENTRY NO.	LINE CODE	SOURCE
1	14ZMB990024	Maize Research Institute
2	14ZMB990027	Maize Research Institute
3	14ZMB990019	Maize Research Institute
4	15ZMB990298	Maize Research Institute
5	15ZMB990309	Maize Research Institute
6	16ZM901013	Maize Research Institute
7	16ZM901059	Maize Research Institute
8	16ZM920715	Maize Research Institute
9	16ZM920664	Maize Research Institute
10	16ZM920205	Maize Research Institute
11	16ZM902347	Maize Research Institute
12	16ZM904371	Maize Research Institute
13	16ZM902623	Maize Research Institute
14	16ZM902669	Maize Research Institute
15	16ZM920511	Maize Research Institute
16	16ZM903798	Maize Research Institute
17	**CML442 (Check)**	WEMA Project
18	**CML444 (Check)**	WEMA project

3.3 Field Trial Design and Management

The experiment was conducted in the winter season of 2018 in an open field, during which no rainfall was recorded. The eighteen maize inbred lines (16 MRI and 2 WEMA lines) were laid out in a Randomised Complete Block design with three replicates. The two WEMA lines were used as checks. Each entry was planted in two-row plots that were 2.5m long and plants were 0.25 m apart within the row and 0.75 m between rows. Two border rows on each side of the experiment were planted to reduce border effects. The seedlings were adequately irrigated for 7days, to allow for germination and thereafter water was withdrawn and the lines were observed on their phenotypic expression for 42 days under water stress conditions.

3.4 Data Collection

During data collection, traits were measured following standard procedures as recommended by CIMMYT according to Magorokosho *et al*., (2009). In every plot, 10 plants were sampled for measurements and then the average was calculated and recorded.

i. Seedling emergence; recorded when 50% of seedlings had emerged.
ii. Leaf number; count of total number of leaves on the sampled plants
iii. Seedling aspect: a visual score of how tolerance to water stress the seedlings in a plot looked. A score of 1 to 5 was used where 1= tolerant and 5= susceptible. The lower the value, the better tolerant the genotype is and vice versa.
iv. Seedling height; height from base ground to the tip of the plant, measured in centimetres.
v. Average fresh shoot weight; the above ground fresh biomass in each plot was weighed using a digital scale, and the weight recorded in grams.
vi. Average fresh root weight; the fresh root biomass in each plot was weighed using the digital scale and the weight was recorded in grams.
vii. Shoot dry weight; the above ground dry biomass was the weight of oven dry fresh shoot weight after at 80 degrees until constant weight was achieved and recorded in grams.
viii. Root dry weight: the dry root biomass was recorded after oven drying the fresh root biomass at 80 degrees until constant weight was achieved and recorded in grams.
ix. Shoot Moisture: was recorded as the difference between the fresh shoot biomass and dry shoot biomass, and the difference was expressed as a percentage on dry biomass basis.
x. Shoot: root ratio (Dry) was recorded as the ratio of dry shoot value to that of dry root value.
xi. Shoot: root ratio (Fresh) was recorded as the ratio of fresh shoot value to that of fresh root value.
xii. Total fresh biomass; was recorded as the sum of fresh shoot biomass and fresh root biomass.
xiii. Total dry biomass; was recorded as the sum of dry shoot biomass and dry root biomass.
xiv. Leaf area (LA) was measured by keeping the leaves flat, while using the ruler , to measure the length of each sampled leaf from the pointy part at one end of the leaf to the point where the leaf joins the stalk at the other end.

3.5 Data Analysis.

3.5.1 Single Site Analysis of variance

Analysis of variance (ANOVA) for all the collected traits was done using GenStat software 18th edition (Payne et al. 2014). The below statistical model was used.

$$Y_{ijk} = \mu + H_i + r_j + B_{K(j)} + \varepsilon_{ijk}$$

Where, Y_{ijkl} = main effect; μ = overall mean or grand mean; H_i = the effect of the i th inbred line and l=1,2,3…50; r_j = number of replications and j=1,2; $B_{k(j)}$= estimate of the complete block within replication and k=1,2; and ε_{ijk} = overall random error.

3.5.2 Correlations analysis

Simple Pearson Phenotypic correlation using genotype means of traits data calculated using the IBM SPSS version 25 software (IBM, 2017).

The phenotypic correlation was calculated as follows:

$$r_p = \frac{\text{cov}(x, y)}{\sqrt{(\text{var}(x) \times \text{var}(y))}}$$

Where, r_p =phenotypic correlation; $\text{cov}(x, y)$ =phenotypic variance of x and y characters; and $\sqrt{(\text{var}(x) \times \text{var}(y))}$ = square root of the phenotypic variance of x and y of character

3.5.3 Principal Component Analysis (PCA)

To identify traits that made important contribution to each PC axis, a restriction that stipulates eigenvectors greater than or equal to 0.3 as the logical cut-off points was applied as proposed by Badu-Apraku *et al.*, (2006) and IBM SPSS version 25 software (IBM, 2017) was used for Principal component Analysis based on the correlation matrix. The number of variables was reduced into fewer uncorrelated principal components and the contribution of each trait to the variation was estimated.

CHAPTER 4

4.0 RESULTS

4.1 Analysis of Variance

Results of the analysis of variance of seedling traits showed significant differences among the eighteen inbred lines for seedling height, average root fresh weight and seedling aspect, root moisture content and total fresh weight (Tables 4 and 5). Other traits such as, number of dead leaves, total number of leaves and root length were not significantly different among the genotypes.

Table 3 Analysis of Variance for selected shoot characteristics measured for the 18 maize inbredlines evaluated for seedling drought stress induced at seedling growth stage

Source	df	Seedling height (cm)	Leaf area	Number of leaves	Number of dead leaves	Shoot fresh weight (g)	Shoot dry weight (g)	Shoot moisture	Seedling aspect
Rep	2	8.095	84.64*	208.1*	3.5*	104.1	49.7	220.2*	2.9
Genotypes	17	52.372*	52.53	46.1	1.6961	145.4**	73.2	110.1	10.71 **
Error	34	2.243	72.12	35.63	0.3877	10.9	73.1	32.3	0.9
R square		67	79	63	90	73	76	60	93
CV%		12.1	28	18	24	22	30	30	11.1

*, ** Significant at 0.05 and 0.01 probability, respectively

Table 4 Analysis of Various (ANOVA) for some root and biomass characteristics

Source	DF	Root length	Fresh root weight (g)	Dry root weight (g)	Root moisture content	Root-shoot Ratio (fresh weight)	Root-shoot Ratio (dry weight)	Total Fresh biomass (g)	Total dry biomass (g)
Replication	2	30.2	1.94	2.67	6.42	34.9*	109.36	190	101.1
Genotypes	17	29.7	255.11	23.42	218.1**	21.2	97.81	394.17**	100.9
Error	34	16.4	25.4	9	1.93	2.8	31.2	99.8	100
R square (%)		60	54	40	70	46	43	72	55
CV (%)		24	36	44	19	18	22	23	36

*, ** Significant at 0.05 and 0.01 probability, respectively

The two tables above (Table 4 and 5) show differences in response to drought stress amongst the genotypes after analysis of variance on traits collected. For both the shoot (above ground structures) and root traits (Underground structures), the trend observed on the data was that fresh biomass resulted in significant differences compared to dry biomass traits. Significant variations were also observed (Table 5) on seedling height.

4.2 Genotype means for all seedling parameters measured

The mean values (Table 6 after next page) of genotypes based on seedling height ranged from 12.6 cm for the line 16ZM904371 to 27.7cm for the line 16ZM920664. Root moisture content, a trait which show the ability to store moisture within the plant ranged from 11.4% for the line 14ZMB990019 to 30.8% for the line 15ZMB990298. Genotypes with low seedling height means were also observed to have low fresh biomass that ranged from 54.3g to 206.3g. Seedling aspect, which is a visual score ranged from 1.1 for both checks lines CML442 and CML444 to 4.1 for the line 14ZMB990019. The line 15ZMB990309 had the lowest root length of 21.6 cm while the line 16ZM903798 recorded the highest root length of 36.2cm. In general, mean values of MRI inbred lines were significantly different (at $p \leq 0.05$ and $p \leq 0.01$) among themselves as well as when compared to the checks (WEMA lines) in all the traits measured. Notably from the results was that the five MRI seed inbred lines performed the same as the checks in terms of root moisture content and seedling aspect scores. Values of 29.1 and 29.3 for Root moisture content and corresponding seedling aspect scores of 1.1 and 1.1 were observed for the check (WEMA) inbred lines respectively, while root moisture content values for the five MRI lines ranged from 25.5 to 30.8 with seedling aspect scores of 1.1 to 2.1 respectively. The 11 other inbred lines were observed to be significantly different (at $p \leq 0.05$ and $p \leq 0.01$) when compared to the check in all traits measured and had lower mean values. A corresponding trend between root moisture content and seedling aspect scores was observed from the results. Genotypes that had high root moisture content mean values, had also low seedling aspect score values .Lower seeding aspect values , meant high ability to tolerate drought stress while high values meant more susceptibility to drought stress.

4.3 Relationship among seedling traits

4.3.1 Correlation analysis

Results of the correlation analysis of all parameters for the 18 maize inbred genotypes (Table 7) revealed significant relationship of root length with average fresh root weight (r = 0.51*), average dry root weight (r = 0.59*) and also total dry biomass (r = 0.49*). Seedling aspect had significant correlation with average fresh shoot weight (r = -0.85**), shoot moisture content (r = -0.86**), root-shoot ratio based on dry weight (r = -0.77**) and total fresh biomass (r = -0.80).

Table 5 Means of selected seedling traits for the 18 maize inbred lines evaluated for drought tolerance at seedling stage

Entry No	Inbred line code	Seedling height (cm)	Number of leaves	Number of shed leaves	Fresh shoot biomass (g)	Average dry shoot weight (g)	Moisture content of shoot %	Average Fresh root weight (g)	Average dry root weight (g)	Moisture content of root	Average root length (cm)	Seedling aspect	Total Fresh biomass (g)
1	14ZMB990024	23.7	8	1.7	124.7	24.1	100.5	19.7	6.0	24.3	14.4	2.3	144.3
2	14ZMB990027	20.1	7	0.7	69.7	14.1	55.6	17.0	5.1	15.1	12.8	3.2	86.7
3	14ZMB990019	22.1	10	1.7	53.7	10.4	43.2	14.0	3.8	11.4	13.4	4.1	67.7
4	15ZMB990298	26.9*	9	0.3	178.0**	40.8*	137.2*	28.3*	6.8	30.8*	19.4*	1.2*	206.3**
5	15ZMB990309	25.9	9	0.3	136.1*	37.9	178.8**	34.3	9.6	28.1*	11.6	1.2*	170.4**
6	16ZM901013	22.5*	9	0.7	129.0*	25.4*	103.6**	33.4*	9.3	22.9*	14.2*	2.3	162.4**
7	16ZM901059	24.7*	8	1.7	132.9*	14.5*	60.5*	30.5*	9.7	30.7*	13.1*	1.3*	163.4**
8	16ZM920715	18.0	8	1.3	77.7	15.6	62.1	14.0	4.8	16.2	10.3	3.4	91.7
9	16ZM920664	27.7	7	1.0	71.7	13.0	58.7	15.7	7.4	14.9	14.0	3.2	87.3
10	16ZM920205	22.6	9	2.0	115.0	38.0	54.1	21.7	7.4	23.2	17.2	2.5	136.7
11	16ZM902347	20.4	9	1.7	51.0	17.8	23.6	12.0	4.7	15.8	14.5	3.3	63.0
12	16ZM904371	12.6	7	3.0	43.9	6.2	17.5	10.4	3.0	12.2	10.9	4.2	54.3
13	16ZM902623	27.0*	8	0.7	123.7*	5.4	18.7	10.0	3.7	25.5*	17.0*	2.1*	133.7
14	16ZM902669	24.9	7	0.7	119.9	5.9	21.1	10.7	3.5	24.2	14.1	2.4	130.6
15	16ZM920511	24.7*	7	0.3	125.6*	3.4*	23.3*	27.8*	2.9	31.9*	15.1*	1.1*	153.4*
16	16ZM903798	15.3	8	1.0	78.3	3.1	5.9	13.6	2.4	14.8	26.2	3.2	91.9
17	CML442(Check)	23.8*	8	2.0	134.3*	27.07*	107*	33.9*	4.78	29.1*	13.6*	1.1*	168.2*
18	CML444(Check)	27.1*	9	0.3	132.3*	42.2*	170.4*	29.9*	8.9	29.3*	14.8*	1.1*	162.2*
	Grand mean	22.8	8.2	1.17	105.4	18.6	68.2	19.85	5.76	22.24	8.9	2.4	98.3
	LSD	4.6	1.0	0.79	14.7	18.6	2.4	1.62	0.3	4.3	0.9559	1.1	14.9

Values with *, ** are significant at 0.05 and 0.01 probability, respectively

Table 6 Correlation of 18 maize inbreed lines evaluated for drought stress tolerance induced at seedling growth stage

Trait	Root length (cm)	Seedling aspect	Total fresh biomass (g)	Total dry biomass(g)
Seedling height (cm)	0.04	0.05	0.00	0.30
Root length (cm)	-	0.02	0.21	0.47*
Number of leaves	0.14	-0.20	0.10	0.01
Number of leaves shed	-0.33	0.02	-0.18	-0.07
Average Fresh shoot weight (g)	0.18	-0.85**	1.00**	0.37
Average Fresh root weight (g)	0.51*	0.27	0.04	0.79**
Average dry shoot weight (g)	0.43	-0.29	0.49*	0.96**
Average dry root weight (g)	0.59*	0.19	0.18	0.68**
Moisture content of shoot	0.27	-0.86**	-0.09	0.56*
Moisture content of root%	0.18	0.56*	0.99**	0.26
Root: Shoot ratio (fresh)	-0.22	-0.47*	0.38	0.19
Root: shoot ratio (Dry)	-0.17	-0.77**	0.66**	-0.18
Total fresh biomass	0.22	-0.80**	-	0.43*
Total dry biomass	0.49*	-0.17	0.43*	-
Seedling aspect	0.02	-	-0.86**	-0.19

*, ** Significant at 0.05 and 0.01 probability, respectively

4.3.2 Principal component analysis (PCA)

Results of the principal component analysis (Table 8) showed that the first 3 principal component axes contributed significantly to variation among genotypes. The first three principle components accounted for 68% of the total variation among the genotypes. PC1 contributed 27% while PC2 and PC3 accounted for 22% and19%, respectively

Based on the restriction recommendation by Badu Apraku et al., (2006) , seedling aspect, average fresh shoot weight (g), moisture content of the root (%), root: shoot ratio (dry) and total fresh biomass had higher loadings in PC 1. The higher loadings in PC 2 were observed on leaf number, average fresh shoot weight (g) average dry root weight (g), and total dry biomass. In PC 3 seedling emergence (%) seedling height (cm) and moisture content of the shoot had the highest loadings.

Table 7 . Eigen vectors of the first three Component Analysis (PC1,PC2,PC3) for 18 maize inbred lines evaluated for drught stress

TRAIT	PC1	PC2	PC3	P4
Seedling Emergence (%)	-0.28	-0.07	**-0.37**	
Seedling height (cm)	0.02	0.25	**0.39**	
Root length (cm)	0.06	0.26	-0.21	
Number of leaf	0.05	-0.21	-0.12	
Number of dead leaves	-0.19	0.19	0.22	
Seedling Aspect	**-0.43**	0.07	-0.08	
Average Fresh root weight (g)	-0.05	**0.41**	-0.15	
Average Fresh shoot weight (g)	**0.43**	0.02	-0.03	
Average dry root weight (g)	0.00	**0.33**	-0.04	
Average dry shoot weight (g)	0.18	**0.31**	0.02	
Moisture content of shoot %	-0.07	**0.33**	**0.34**	
Moisture content of root%	**0.42**	-0.02	-0.08	
Root: Shoot ratio (fresh)	0.22	0.02	-0.07	
Root: shoot ratio (Dry)	**0.32**	-0.23	-0.31	
Total fresh biomass	**0.43**	0.05	-0.08	
Total dry biomass	0.15	**0.37**	-0.15	
Proportion (%)	27	22	19	
Cumulative (%)	27	49	68	

Values in bold indicates Eigen vectors higher or equal to 0.3

CHAPTER 5

5.0 DISCUSSION

Analysis of variance (ANOVA) revealed significant differences among the genotypes for seedling height, seedling aspect, fresh shoot weight, root moisture content, and total fresh biomass. This indicated that these are reliable traits that could be used as indicators of drought tolerance at seedling stage and it is suggestive that a base index that incorporate these traits could be computed for selecting maize genotypes for drought tolerance at seedling and early vegetative stages. The identification of total fresh biomass is consistent with findings of Farooq *et al*, (2008). Root-shoot ratio has also been identified as important trait indicator of drought tolerance in maize. Li *et al.,* (2014) and Naveed *et al.,* (2014) explained that although both shoot and root growth were inhibited by drought stress, shoot growth was more sensitive than root growth, thus shoot-root ratio was typically reduced, implying that lower shoot –root ratio values is an indication of drought stress tolerance ability . The sensitivity to water stress by the above ground structures (shoot) makes visual score method (seedling aspect visual ratings) an important way of screening drought tolerance among genotypes at seedling growth stage. This further implied that under drought stress, plants allocate more resources to the root than the shoot growth in order to enhance water acquisition and limit evaporation (Lynch and Ho, 2005). Other traits such as number of leaves, shoot moisture content, root length, fresh root weight, and total dry biomass could only be useful when doing morpho-phenotypic characterization of genotypes and not good indicators of drought tolerance at seedling stage as reviewed by the results of this study. Contrary to this study findings though, some earlier studies identified root length and density as good indicators of drought tolerance in cultivated crops (Ober *et al,* 2005; Gowda *et al,* 2011; Iqbal *et al,* 2011). However, the findings of this study is in agreement with the reports of other studies who reported no relationships between length and density of root with drought tolerance (Kashiwagi *et al.*, 2006; Jongrungklang *et al,* 2012). Avramova *et al.*, (2016) identified total root length and shoot dry weight as reliable measurements of drought tolerance at the seedling stage under field conditions in maize. Vadez (2014) observed that although roots have long been thought of as a major avenue to improve crop adaptation to water limitations based on the assumption that deeper and more robust root structures could access extra moisture from the soil profile and alleviate drought effects, success in breeding cultivars with improved root systems is limited. He reported that the role that the roots play in drought adaptation may not necessarily be associated with their density or depth, but rather from their hydraulic ability characteristics.

Based on analysis of data from a lysimetric system that allows monitoring and comparing plant water use over the entire crop life cycle and yield, Vadez (2014) reported that the role of roots as adaptive features under drought may not be on the basis of their length, density or depth, but rather on their hydraulic characteristics (ability to effectively access and utilize available water into their tissues). In this study, moisture content of the root, which is an indication of water holding capacity as well as measure of the effectiveness of the roots to draw moisture into its tissues, was found to be more important rather than root length under drought conditions at seedling growth stage. A positive relationship between root moisture content and seedling aspect (Table 7) was observed, revealing a trend of moisture content values of >25.5 and corresponding seedling aspect scores of < 2.1 for tolerant genotypes, implying that though the shoot is very sensitive to water stress, as long as the roots held sufficient moisture levels underground, damage or morphological change on the shoot will not be visibly seen , conversely, if less moisture is available in the root structures, it will be easily noticed on the shoot. Cantao *et al*., (2008) also reported significant differences for root morphological attributes and root and canopy growth. They observed that drought tolerant inbred lines showed distinct root system than susceptible lines, by presenting longer root, surface area, and volume under drought stress at seedling stage.

Plant productivity under drought stress is strongly related to the process of dry matter partitioning and temporal biomass distribution (Kage *et al*., 2004). Farooq *et al.,* (2008) reported that water stress on crop plants generally cause significant reduction in fresh and dry biomass production. In general, the result of this study identified more above the ground biomass (seedling shoot) characteristics as indicators of drought tolerance than the underground biomass (seedling root) characteristics. The results further reviewed significant variability among the genotypes based on the fresh weights than the dry weights data assays, implying therefore, that fresh weight biomass parameters are better distinguishing traits under drought conditions than the dry weights.

Based on root moisture content results, supported by seedling aspect scores, there were 5 MRI inbred lines with moisture content values of >25.5 and seedling aspect score values of < 2.1 with non-significant differences in comparison to the two WEMA line checks, implying that the five MRI inbred lines had root structures well adapted to access and also preserve sufficient moisture levels during drought conditions, thereby keeping the above ground structures (shoot) succulent. The five genotypes are coded as (15ZMB990298, 15ZMB990309, 16ZM901059, 16ZM902623, 16ZM920511.). These inbred lines could be advanced for use as testers among themselves to generate F1 hybrids, depending on their general and specific combining ability (GCA and SCA) that will be more drought tolerant at seedling stage.

Another important objective of this study was to reveal relationships among seedling traits with a view to identify novel traits for measuring drought tolerance at seedling traits among the genotypes. Correlation analysis showed that root length had non-significant relationship with other traits except other root attributes such as both fresh and dry root weights. This implied that root length is not an important adaptive trait for drought tolerance at seedling stage in maize genotypes. Seedling aspect on the other hand had significant negative correlation with average fresh shoot weight (r = -0.85**), shoot moisture content (r = -0.86**), root moisture content (r = 0.57**) root-shoot ratio based on dry weight (r = -0.77**), root-shoot ratio based on fresh weight (r = -0.47*), and total fresh biomass (r = -0.80**). Many of these traits with significant correlation with seedling aspect had been identified as important traits in earlier studies (Moser, 2004; Vadex, 2014). This indicates that seedling aspect could serve singly as a selection criterion for drought tolerance at seedling stage or in combination with other traits in a selection index. A careful look into the relationship among traits reviewed that root length had significant positive relationship with average fresh and dry root weights as well as total dry biomass. This implied that root structures contributed significantly to dry matter accumulation among the genotypes. It has been reported earlier that under drought stress, roots elongate in search of water for survival and that deeper and more robust root systems could tap extra water from the soil profile and alleviate drought effects while shoots are reduced to conserve moisture (Vadez, 2014). Premised on these earlier findings, the root has been considered as a major avenue to improve crop adaptation to water limitations at seedling growth stage.

Principal Component Analysis results (PCA) reviewed that seedling aspect, average fresh shoot weight, moisture content of the root, root: shoot ratio (dry) and total fresh biomass were the traits that contributed to variation, and had higher loadings in PC1. The traits with high loading on the PC1 should be assigned a greater weight than those loaded on other PCs axes (PC2 and PC3). The identification of seedling aspect, average fresh shoot weight, moisture content of root, Root: Shoot ratio (Dry), and total fresh biomass as traits with high loading under the first principal component axis revealed that the traits are of primary importance in determining maize genotypes with tolerance to drought at seedling stage. These traits should be included and considered important phenotypic traits for drought tolerant genotypes selection at seedling growth stage. Unlike root attributes, selecting drought tolerant genotypes based on seedling aspect is faster because seedling aspect is scored between 16 and 20 days after planting or between 9 and 13 days after water supply is withdrawn; scoring is easier and does not involve destructive sampling. The fact that it was significantly correlated with some of the adaptive traits reported with other studies such as Root-Shoot ratio (Fresh), Root-Shoot ratio (Dry) and total fresh biomass indicated that it is a reliable trait that can represent these other traits in a base index for seedling drought tolerance. Base index is a novel approach to develop cultivars that combine many good and important traits (Yan and Kang, 2003). Other traits with high loadings on PC2 such as average fresh root weight, average dry shoot weight, moisture content of shoot and total dry biomass could also be considered as adaptive traits for drought tolerance selection at seedling stage and less weights should be attached to them.

CHAPTER 6

6.0 CONCLUSION

The MRI inbred lines responded differently to drought conditions imposed at seedling growth stage in terms of interrelationship among phenotypic adaptive seedling traits. Based on Analysis of Variance (ANOVA) of traits collected during the study, five (5) MRI maize inbred lines coded as ((15ZMB990298, 15ZMB990309, 16ZM901059, 16ZM902623, 16ZM920511.) were discovered to be well tolerant to water stress at seedling stage and these could be recommended for inclusion in crossing blocks to generate F1 drought tolerant hybrids at seedling stage, depending on successful assessment of their General Combining Ability (GCA) and Specific Combining Ability (SCA). They can as well be used for starting new populations meant to generate drought tolerant hybrids at seedling growth stage.

It was also concluded from the study that there is a positive relationship between drought tolerance at seedling stage and seedling phenotypic trait expression. Six (6) phenotypic seedling traits (seedling aspect, average fresh shoot weight, shoot-root ratio of dry matter, moisture content of the root, and total fresh biomass) were identified as differential traits that could be used to select for drought tolerance at seedling growth stage in maize genotypes.

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