

**USE OF MORPHO-PHYSIOLOGICAL TRAITS TO ENHANCE
BREEDING FOR DROUGHT TOLERANCE IN TROPICAL MAIZE**

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DECLARATION

The work presented here is my own and has not been submitted to any other university for the award of a PhD degree.

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DEDICATION

To My Parents, My Wife Gloria, and Children, Kudakwashe, Tinotenda, Tawana

And

‘To God Be The Glory’

THESIS OUTLINE AND JOURNAL PUBLICATIONS

This thesis is written in chapter format as per the objectives of the study and following the university thesis guidelines. Chapter 4 is made up of two objectives which are mutually exclusive but interlinked through use of the same materials. The results, discussions and conclusions of these two objectives are however separated. This study resulted in the publication of objective based papers in peer reviewed journals as outlined below:

Objective 1 Publication:

Mhike X, Okori P, Kassie G.T and Chikobvu S (2012) An Appraisal of Farmer variety Selection In Drought Prone Areas and Its implication to Breeding for Drought Tolerance. Canadian Journal of Agricultural Science Volume 4 (6) pp 134 – 145

Objective 2 Publication:

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Objective 3 Publication:

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ABSTRACT

This thesis contains four mutually reinforcing studies conducted with the aim of identifying the most effective secondary morphological traits and approaches that can be used to efficiently select for high yielding and drought tolerant maize genotypes. In objective one, a survey was conducted to gain insights into farmer views on factors that influence choice of varieties in maize dominated farming systems in drought prone areas and their implications to breeding. Objectives two and three aimed at determining the combining ability of the inbred lines, assess efficacy of secondary traits and selection indices during breeding as well as the mode of gene action conditioning drought tolerance. Objective four aimed at mapping quantitative trait loci conditioning secondary traits that are strongly correlated to grain yield under drought conditions. North Carolina Design II was used to develop hybrids which were evaluated under managed drought stress in the dry season by withdrawing irrigation 4 weeks before and after flowering. A 248 F2:3 population derived from a cross CML505 x CZL 00009 elite drought tolerant lines was evaluated under managed drought stress and used to map the QTL using 278 SNP markers. Results showed that farmers were willing to make trait trade-offs when selecting for drought tolerance related traits. Study 2 showed that selection indices STI and GMP were the most suitable to be used in tandem selection with ear per plant, anthesis silking interval and leaf senescence. Study three showed that GCA of secondary traits was predominant over SCA in drought environments while AMMI managed to separate genotype grain yield performance. In study 4, genetic maps covering 1238.8 cM on ten chromosomes were generated and 31 QTL mapped. One QTL position on chromosome seven mapped for both anthesis date and stem lodging. The implications of these finding on maize breeding for drought tolerance are discussed in detail in the thesis with three modified papers from the thesis chapters having been published in peer reviewed journals.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Significance of maize in sub Saharan Africa

Maize (*Zea mays* L.) has accounted for 22 to 25 % of starch consumption in Africa since 1980, representing the largest single source of calories, followed closely by cassava (Smale *et al.*, 2011). It is the most preferred staple crop for 900 million consumers in the world (CIMMYT, 2010). Maize has high nutritive value comprising of 10 % protein, 4.8 % oil, 9.5 % fibre, 3 % sugar, and 1.7 % ash (Chaudhary, 1983; Araus *et al.*, 2003). Maize is used extensively as raw material in industry for various products, including bio-energy, that benefit a large proportion of the world's population.

In Sub-Saharan Africa (SSA), maize currently covers 25 million ha, largely produced by smallholder farmers who cultivate the crop primarily for food with a fluctuation of between 32% and 45% share of total area in primary crops (Jayne *et al.*, 2010). The potential for expanding maize production in SSA is huge with an estimated potential of 88 million ha (Jayne *et al.*, 2010). However, production and productivity of maize in SSA is variable with Southern African countries like Zimbabwe experiencing high variations of up to 41%, Malawi 33% and Zambia 31% (Smale *et al.*, 2011). Climatic factors especially drought are responsible for much of the yield variability, with average yields of less than 1.2 t/ha being realized (Bänziger *et al.*, 2002). Thus successful and continuous production of maize is pivotal to sub Saharan Africa's food security and any disruption in the supply of maize either at the farm level or to the markets has destructive consequences for the most vulnerable smallholder farmers and for industrial raw material supplies globally.

1.2 Drought as a major constraint to maize production

Among the maize production, constraints drought is the single most common cause of severe food shortage in developing countries and climate change is predicted to further exacerbate its impact (Sivakumar *et al.*, 2005; Ribaut *et al.*, 2009). It is also estimated that some 1.8 billion people will suffer from water shortage and two thirds of humanity will be affected by water stress by 2025 compromising cereal production sub Saharan Africa and Latin America (UNESCO, 2007). In maize, moisture stress is most devastating when it occurs at flowering with causing yield losses of 45-60% (Campos *et al.*, 2006). In the tropics, annual maize yield losses due to drought are thought to average about 17% but can reach 80-100% depending on severity and timing of drought (Chapman *et al.*, 1997; Edmeades 2008). In East and Southern Africa the drought prone areas cover an estimated 2 690 000 ha. However ‘drought tolerant’ varieties currently on the market are not fitting into the farmers’ maize ecosystem due to poor drought tolerance and consequently low yields hence their poor adoption by the farmers (Langyintuo *et al.*, 2008). In Southern Africa the 2002-2003 drought left about 14 million people exposed to starvation and food deficit was 3.3 MT (World Food Programme, 2003). East Africa experienced a severe drought in 2011 that left more than 10 million people relying on food aid (World Food Programme, 2011).

1.3 Drought management strategies

1.3.1 Agronomic management options

There are many drought mitigation strategies used which include irrigation, soil management techniques like minimum tillage, cultural practices such as mulching and integrated water management systems that involve soil and water conservation. Addition of organic manure, mulching and minimum tillage are meant to reduce soil moisture loss, control weeds and add organic matter to the soil. However these techniques are cost effective on a small scale such as home gardens as opposed to a large scale maize production as experienced in most of SSA.

Supplementary irrigation is the most effective drought mitigation method but this is a very expensive option for the majority of the resource poor farmers in the drought prone marginal areas where maize is grown. In addition the effective cultural practices of soil and water conservation require good varietal response through increased water uptake and water use efficiency. This leaves development of improved drought tolerant varieties as the most viable options for majority of the resource poor farmers who grow maize in the tropics.

1.3.2 Drought tolerance breeding strategies

Drought tolerance in plants is a complex trait that involves drought escape, plant dehydration avoidance and dehydration tolerance mechanisms (Campos *et al.*, 2004). It can also be defined based on the relative yield or survival of a genotype, compared with other genotypes subjected to the same drought, where drought escape is not a major factor. Understanding the genetic and physiological mechanism of the maize plant is critical for one to develop the best strategy to breed for drought tolerance in maize. There are two approaches to breeding for drought tolerance namely: (a) selecting for yield under non-stressed conditions, and then evaluation at many sites with variable moisture availability or “random stress” (Myers, 1988). (b) Screening germplasm for yield and related secondary traits under drought stress conditions (Blum, 1988). The assumption for the first approach is that genes for drought tolerance are present in elite high yielding material. In the second approach, maize yields are usually very low, with possibility of wide environmental variances affecting performance of test genotypes.

Drought tolerance maize breeding progress has been slow due to genotype x environmental interactions because of year-to-year variations in the timing and intensity of drought stress in field breeding nurseries (Bänziger *et al.*, 2006). Indirect selection through secondary traits associated with grain yield can improve the efficiency of the breeding for drought-tolerant

genotypes. Secondary traits help to overcome the drawbacks of breeding for high grain yield under water-limited conditions, namely the low heritability of yield, which is due to the small genetic variance and the occurrence of poorly understood genotype-by-environment interaction (Messmer, 2006). Maize breeders have resorted to using secondary traits such as anthesis silking interval, leaf senescence and ears per plant that are highly correlated to grain yield when breeding under moisture stress but have not yet fully exploited the selection gains from these traits. In addition the seasonal nature of phenotypic selection can be minimised by use of molecular markers hence breeding materials can be evaluated at least twice a year thereby hastening the breeding process.

1.4 Justification of the study

In Zimbabwe and SSA in general, many maize varieties have been developed and released but there has been low adoption of these varieties in drought prone marginal areas where the majority of the resource poor farmers live. In this thesis effort was made to establish the rationale behind the low adoption of varieties targeted for drought prone marginal areas and to identify factors that farmers consider when choosing cultivars best suited to their agro-ecological needs and conditions. Moreover, breeding progress for drought tolerance in maize is slow because drought tolerance is a complex trait controlled by many genes. Breeders in general improve maize for drought tolerance using secondary traits and selection indices independently to select ideal genotypes. As a result objective two of this thesis explores possibilities of using the two approaches in tandem in-order to improve drought tolerant selection criteria for quicker identification of tolerant inbred lines and hybrids.

Any genetic improvement programme needs to assess the breeding value of its potential parents for developing new varieties. Characterisation of gene action conditioning secondary traits that are positively correlated with grain yield is needed to select lines that can then be

used in developing synthetics or hybrids targeting both drought prone and non stress environments. In the third objective of this thesis combining ability and genetic control of secondary traits for selection of inbred lines and hybrids for grain yield under moisture stress and non stress environments are investigated to gain better understanding of inheritance of various secondary traits. With the advent of genomics and bioinformatics, opportunities for targeted selection of genomes are available. For complex traits, the mapping of gene loci conditioning the trait provides the basis for targeted introgression of gene loci through processes such as marker assisted selection (MAS). In this study quantitative trait loci (QTL) were mapped as an initial step to the development of a marker assisted recurrent selection (MARS) for pyramiding of drought tolerance secondary traits in order to support phenotypic selection (Johnson, 2004). This was done in order to exploit the genetic variability that exist in the lowland tropics and subtropical (inbred lines which were used

1.5 Objectives and hypotheses

1.5.1 General objective

To develop an effective drought tolerance breeding strategy for maize based on secondary morphological traits.

1.5.2 Specific objectives

1. To determine factors that influence choice of varieties in maize dominated farming systems in drought prone areas and their implications to breeding.
2. To validate the use of secondary traits and selection indices in the identification of inbred lines and hybrids that are drought tolerant
3. To assess the combining ability, genetic control and mode of gene action for secondary traits of new drought tolerant inbred lines under stress environments.

4. To map quantitative trait loci of secondary traits that are strongly correlated to grain yield under drought or moisture stress environments

1.5.3. Hypotheses

1. Farmer's perceptions and knowledge influence the success of breeding and adoption of drought tolerant crop varieties.
2. Individual and tandem use of secondary traits and selection indices has different efficacies in improving selection of maize genotypes for drought tolerance.
3. Knowledge of mode of gene action for grain yield and its secondary traits will help in the identification of the most effective breeding strategies for moisture stress tolerance in maize.
4. Mapping of quantitative trait loci of secondary traits will help in the developing drought tolerant genotypes using marker assisted recurrent selection (MARS)

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CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Introduction

Maize is of paramount importance to about 300 million people who depend on it for their livelihoods in Africa (CIMMYT 2010). The biggest threat to maize production is drought, which is likely to increase under climate change. Climate models predict that drought episodes will increase due to the long term effects of global warming and by 2050, many varieties of maize now under cultivation will no longer be viable (Cook and Vیزی, 2007; La Rovere *et al.*, 2010). Development of drought tolerant maize varieties could translate into a 22-25 % yield increase due to increased water use efficiency (CIMMYT 2010). This therefore calls for doubling of efforts in developing drought resilient varieties for sub Saharan Africa. In this thesis effort is made to investigate various conventional, biotechnology and socio economic approaches commonly used in breeding as part of efforts to address the threat posed by drought to maize production under drought stress. The thesis investigated both socio-economic factors influencing adoption as well as genetic tools and the efficiency of genetic tools and approaches commonly used in breeding. The details of these two broad knowledge areas are presented in papers 1 to 4 but in this chapter literature is reviewed to show the scientific knowledge gaps and underpinnings for the studies undertaken.

2.2 Types of drought and their effects on maize production

2.2.1 Agricultural drought

Agricultural drought refers to the shortage of the sufficient water available for a crop at any given stage of its development resulting in impaired growth, wilting and ultimately reduced crop yields (Bänziger *et al.*, 2006). This definition looks at the link between meteorological and/or hydrological droughts and agricultural impacts, and focuses more on the vulnerability of the crops at their different growth stages vis-à-vis plant-water needs. Drought affecting

agriculture is the most prevalent abiotic stress which limits plant growth and productivity worldwide, with devastating socio- economic impacts (Jayne et al., 2010). It is a major cause of food insecurity for many households as it has been estimated to cause annual maize yield loss of 24 million tons in the developing world (FAOSTAT 2010; Edmeades 2008). Breeding for drought tolerance mainly focuses on this type of drought.

2.2.2 Hydrological drought

Hydrological drought refers to the reduction in precipitation on surface and sub-surface water resources which affects water-reliant activities like irrigation, hydro-electricity generation, fishing and recreational activities such as water rafting (Mutasa, 2010). This also affects the availability of groundwater inducing water stress on communities relying on groundwater for their household needs. River basins often face the brunt of hydrological droughts, though such droughts rarely coincide with meteorological and agricultural droughts. Although climate is the primary contributor to hydrological drought, other factors such as land use, land degradation and dam construction also affect hydrological characteristics of the river basin (Moneo and Iglesias, 2004)

2.2.3 Meteorological drought

Meteorological drought occurs when there is a reduction in expected rainfall supply over a particular period. Meteorological drought is based on the degree of dryness and the length of the dry period. The meteorological definitions are site or region-specific, and measure dryness and rainfall received comparing this against a given baseline (Moneo and Iglesias, 2004). This type of drought is often based on an internationally accepted 30-year precipitation period.

2.2.4 Effects of drought on maize plant physiology

Maize is affected by drought during its entire life cycle with varying degrees of damage or loss to the ultimate yield. The impact of restricted water availability on crop performance is dependent on crop growth stage, crop history, leaf area, root volume, atmospheric vapour pressure deficit, temperature and radiation (Bänziger *et al.*, 2000). Maize is comparatively more susceptible to drought than its close relative sorghum, because of its flowering architecture that predisposes developing grain to environmental stresses (Grant *et al.*, 1989). Moisture stress occurring soon after planting results in poor germination, reduction in plant population and consequently yield. Plant growth from emergence to V8 (eighth leaf fully emerged or about 4 weeks after planting) determines the plant and leaf size affecting assimilates supply at grain filling (Monneveux and Ribaut, 2006).

Maize is particularly sensitive to water stress occurring just before and during flowering when the crop's yield potential is defined (Malosetti *et al.*, 2008). The flowering period is the most crucial stage in terms of negative effects of drought on yield. During this stage, one day of water stress can potentially decrease yield by up to 22% (Landi *et al.*, 2007). Silking or the onset of the reproductive stage is the most sensitive stage and water stress during this period coupled with high temperatures can result in 100 percent yield loss since daytime temperatures can kill pollen before it can reach the silks (Banziger *et al.*, 2006). When water stress occurs just before flowering, a delay of silk emergence in relation to male flowering is observed increasing anthesis-silking interval (ASI) which is correlated with lower yield (Bolanos and Edmeades, 1996). When ASI is lengthened the pollen may arrive when silks have withered or senesced or after ovaries have exhausted their starch reserves (Saini and Westgate, 2000). At flowering extreme sensitivity is confined to the period -2 to 22 days after silking with the peak at 7 days. Complete bareness can occur if maize plants are water stressed just before tassel emergence to the beginning of grain filling (Bänziger *et al.*, 2000).

Thus mid season drought spells are critical as they coincide with the flowering period hence the need for genetic improvement of maize to better tolerate moisture stress at this crucial growth stage. Under moisture stress, apical (tassel) dominance is enhanced decreasing allocation of assimilates to cobs, ovules and silks resulting in reduced cob and silk growth rate and increased kernel and ear abortion (Bolanos *et al.*, 1993). Under drought conditions, abscisic acid production increased inhibiting photosynthesis through stomatal closure and further decreasing assimilates supply (Bänziger *et al.*, 2000). Starch metabolism is also inhibited with the enzyme invertase inhibited and reducing starch supply to ovaries or affected pollen (Kulwal *et al.*, 2011). In this study assessment of genotype performance under drought was done using managed water stress during the rain free season by withdrawing water for 4 weeks before and after flowering. This affected the flowering duration and synchronisation of the male and female flowering parts with the focus being assessing the anthesis- silking interval.

Water stress during grain-filling increases leaf senescence, shortens the grain-filling period, increases stem and root lodging and lowers kernel weight. Moisture stress during grain filling, results in incomplete filled kernels and reduced assimilate fluxes to growing organs causing kernel abortion (Messmer, 2006). This bareness may lead to complete loss of grain yield. Grain abortion is highest during the first 2-3 weeks after silking and is as a result of accelerated lower leaf senescence (Schussler and Westgate, 1995). Moisture stress affects photosynthetic pigments damaging photosynthetic apparatus and decreasing the activities of Calvin Cycle enzymes and interception of photosynthetic active radiation (Monakhova and Chernyadev, 2002; Kim *et al.*, 2000). In general, kernels near the ear tip are the last to be fertilised, are less vigorous and most susceptible to abortion under water stress conditions. In addition under water stress lodging occurs because most stalk carbohydrate reserves are

mobilised to the grain (Bänziger *et al.*, 2002). These factors underpin the need to consider the number of ears per plant or bareness trait for measurement as a proxy to grain yield.

2.3 Breeding for drought tolerance

2.3.1 Why breed for drought tolerance and marginal agro-ecologies

Most varieties are specifically adapted to conditions that are at or near the optimum for crop growth. The superiority of these varieties is lost in sub optimal or stress environments. Trials in Zimbabwe between 2000 and 2002 with over one thousand hybrids, under optimum, low N and managed moisture stress generated grain yield that ranged from 1 t/ha to 10 t/ha. Selection differentials were largest between 2-5 t/ha and became less significant at higher yield levels (Bänziger *et al.*, 2006). An Eberhart-Russell, stability analysis, estimated a 40% advantage at 1 t/ha¹ yield level, which decreased to 2.5% at the 10 t/ha yield level (Bänziger *et al.*, 2002). Selection under carefully managed high priority abiotic stresses, including drought, breeding can significantly increase maize yields in highly variable drought prone environments especially at lower yield levels (Ceccarelli *et al.*, 1998 ; Bänziger *et al.*, 2002).

Based on the progress in improving drought tolerance of tropical maize, the ideotype of a drought-tolerant maize plant is characterized by short plants, reduced leaf area especially on the upper part of the stem, small tassels, thick stems, erect leaves, delayed senescence, deeper rooting with less lateral branching, better grain filling capacity, robust growth of spikelets and kernels and good osmotic adjustment at low water potential (Ribaut *et al.*, 2008). These are some of the traits that were being considered in the genotypes under study in this thesis.

2.3.2 Use of secondary traits in drought tolerance breeding

When breeding for moisture stress environments many breeders use secondary agro morphological traits that are highly correlated to grain yield. These secondary traits help to overcome the drawbacks of breeding for high grain yield under water-limited conditions,

namely the low heritability and the occurrence of poorly understood genotype-by-environment interactions (Messmer, 2006). An ideal secondary trait should be observable before flowering or at flowering to avoid undesirable cross-pollinations. Secondary traits are valuable because they demonstrate the degree to which a crop was moisture stressed and when observed before flowering, they can be used for selecting desirable crossing parents thus improving the precision (Edmeades *et al.*, 1999)

Anthesis silking interval is one of the common secondary traits used to select indirectly for moisture stress tolerance. Other secondary traits for selection, which include leaf senescence, leaf rolling, ears per plant (EPP) and of late the normalized difference vegetation index (NDVI). Furthermore, selection indices are used in bringing together the different scores from the different traits and identifying the best materials.

The two main physiological processes affecting leaf senescence in maize are nitrogen remobilization and a disturbed source-sink balance (Bänziger *et al.*, 2000). Tolerance to premature senescence is referred to as stay-green. The “visual stay-green” phenotype is defined as a delay in onset of leaf senescence and is visually characterized by maintenance of green leaf area during the grain filling period (Lee and Tollennar, 2007). There are five types of stay-green, and the stay green inherent in modern maize hybrids can be classified as type C where chlorophyll is retained indefinitely, but photosynthetic capacity of the leaf declines.

Photosynthesis peaks at silking and declines during the grain filling period and this decline in photosynthetic capacity, is what we refer to as “functional stay-green” (Lee and Tollennar 2007). Genotypes with a smaller decline in leaf photosynthesis during the grain filling period are desirable and selected for in maize breeding since plants that stay-green retain green leaves for a longer period of time and produce more grain normally. In general the main secondary traits for drought tolerance in maize are: minimal flowering asynchrony between

male and female flowering structures (i.e., a short ASI), reduced barrenness, stay-green characteristics and epinasty or leaf rolling. Selection of genotypes in this study was advised by these trait considerations.

2.4 Genotype x Environment Interaction (GEI)

The phenotype of an organism is a product of the interaction of its genotype and the environment (Falconer and Mackay, 1996). Environmental factors such as locations and seasons, may have positive or negative impacts on a genotype. This association between the environment and the phenotypic expression of a genotype constitute the genotype by environment interaction (GEI) (Bondari, 2003). The crossover interaction is the most important in crop improvement as it results in change in magnitude and rank (Banziger and Cooper, 2001). Occurrence of GEI complicates selection of superior genotypes for environments. This is as a result of confounding effects of the three components of interaction between genotype and environment, namely genotype x location (G x L), genotype x year (G x Y) and genotype x location x year (G x L x Y). The genotype-environment interaction plays a significant role in relative performance of different varieties in different environments hence stability estimates depend considerably not only on choice of test location but also on choice of genotype (Falconer and MacKay, 1996; Simic *et al.*, 2001).

Plant breeders encounter genotype x environment interactions when testing varieties across environments. Depending upon the magnitude of the interactions or the differential genotypic responses to environments, the varietal rankings can differ greatly across environments. A combined analysis of variance can quantify the interactions, and describe the main effects. However, analysis of variance is uninformative for explaining the genotype x environment interaction. A number of statistical methods have been developed to analyse the GEI data and they include analysis of variance (least squares, restricted maximum likelihood), regression,

variance components, cluster and factor analyses and the additive main effects and multiplicative interaction model (AMMI), (Bondari, 2003). To apply the AMMI model the conventional analysis of variance for additive main effects ($\mu + G_i + E_j$) is combined with the principal component analysis for the multiplicative interaction (non additive residual) effects to analyse the matrix of two way means (Baker 2002). Analysis of GEI in this thesis also used this method in the separation of the variance components.

2.5 Combining Ability

Combining ability estimate is a measure of the value of genotypes based on performance of their offsprings, produced in a definite mating system (Hallauer and Miranda, 1988). It may also be considered as a phenomenon where some parents produce superior F_1 s or progenies from crosses while others do not. Combining ability enables the prediction of performance, but the genotype performance cannot be predicted for traits that are polygenic. It cannot be predicted from the parental phenotype and is assessed by progeny testing from controlled pollinations (Vasal *et al.*, 1992). Combining ability estimates serve as a useful guide during selection of parents for hybridization programmes (Shukla and Pandey, 2008). The selection is based on the performance of hybrids and are related to the general (GCA) and specific (SCA) combining abilities of the inbred lines involved in the cross. Favourable alleles are combined through hybrid combination with high performance with good SCA estimates and having at least one of the parents with high GCA (Marilia *et al.*, 2001). SCA effects should be used in combination with hybrid performance and GCA of particular parents for selection (Shukla and Pandey, 2008).

General combining ability is the mean performance of a line in all its combinations expressed as a deviation from the overall population mean which can either be positive or negative and is trait specific. It is used to identify types of gene interactions governing traits associated

with additive gene effects. Specific combining ability is the deviation of a particular cross performance from that predicted on the GCA basis. It is related to dominance and epistatic effects (non-additive effects) of the genes. It indicates the value of inbred combinations identifies specific inbred line performances. In maize, breeders have used measures of GCA and SCA to establish heterotic patterns (Wray and Visscher, 2008). SCA and GCA estimates are relative to and dependent on a particular set of materials (inbreds or populations) used in the hybrids under evaluation, hence any new germplasm introduced in a breeding programme has to be tested for GCA and SCA (Hallauer *et al.*, 2010). Inbred line superiority on the basis of combining ability estimates is mainly dependent on the breeding programme objectives and outputs with higher GCA effects being preferred in synthetic development while a hybrid programme would desire good SCA effects of parental material selections for hybridisation (Shukla and Pandey 2008). In choosing parents and crosses and to estimate the combining abilities of parents in early generations, plant breeders use the line x tester analysis method. Line x tester analysis provides an efficient approach for identification of suitable parents and crosses exhibiting good performance in traits under consideration (Ahuja and Dhayal, 2007).

2.6 Heritability and Heterosis estimation

Heritability is the proportion of phenotypic variation (V_P) that is due to variation in genetic values (V_G) (Falconer and Mackay, 1996). The combined effect of all loci, including possible allelic interactions within loci (dominance) and between loci (epistasis), is the genotypic value (Wray and Visscher, 2008). The effect of a particular allele on a trait depends on its allele's frequency in a population and the effect of each genotype. This is sometimes termed the average effect of an allele. The additive genetic value of an individual, also called the breeding value, is the sum of the average effects of all the alleles the individual carries (Falconer and Mackay, 1996). Two types of heritability can be estimated namely broad and narrow sense heritability. Broad sense heritability is a ratio of total genetic variance (V_G) to

the phenotypic variance (V_P) and has the formula of $H^2 = V_G/V_P$. The total genetic variance is made up of additive, dominance and epistatic variances, where dominance is the intra-locus value while epistasis is the inter-loci value. On the other hand narrow sense heritability is the ratio of additive genetic variance (V_A) to total phenotypic variance and is calculated as: $h^2 = V_A/V_P$. Additive genetic variance is the variance that causes resemblance between relatives (Hill, *et al.*, 2008).

On the other hand realised heritability is an estimate of what the heritability needs to be, for one to observe the rate of divergence given the selection practiced. This estimate is used by breeders to decide the efficacy of selection, especially phenotypic selection (Thompson, 1986). Direct phenotypic selection for traits with low heritability is not promising. Use of phenotypes from relatives and selection indices can be employed to determine more accurately the underlying genetic merit. Heritability is a property not only of a character but also of the population, of the environmental circumstance to which the individuals are subjected, and of the way in which the phenotype is measured (Falconer and Mackay 1996). In this study, hybrids were used and hence the coefficient of genetic variability was used as an estimate of the actual heritability. In addition selection indices were used in tandem to enhance the selection process. Realised heritability of each trait was also considered before QTL mapping and analysis was done.

Heterosis is defined as the superiority of a hybrid over its two parents. It is the superiority of a hybrid over the mean of its two homozygous parents and is also known as hybrid vigour. Heterosis is then one of several genetic effects that are part of the non additive genetic effects. Breeders have for a long time exploited heterosis, but the causes of this phenomenon are still poorly understood. The effective use of heterosis involves the development of populations or parental lines with high combining ability (Vasal *et al.*, 1992). In maize,

inbreds are low yielding but their hybrids exhibit a high degree of heterosis for yield as well as other traits such as plant height, flowering and maturity (Duvick, 2005)

2.7 Use of molecular markers in cereal breeding

2.7.1 Why use molecular markers

Conventional cereal breeding is time consuming and environment dependent. Using this approach in variety development and evaluation can take 8-12 years with no guarantee of release (Korzun *et al.*, 2001). Genetically complex quantitative traits, involving many genes and environmental effects account for the majority of the economically important agronomic traits in cereals (Semagn *et al.*, 2006a). Cereal breeders are therefore keen on efficient technologies that improve trait selection and reduce the time to variety release and molecular marker technology which adopts a wide range of novel and precise approaches is one option. Marker applications in cereal breeding have been diverse and include identification and finger printing of genotypes, assessing genetic diversity, detection of quantitative trait loci (QTL) and marker assisted selection (MAS) with introgression of QTL into elite germplasm

2.7.2 Types of molecular markers

Plant breeders today have at their disposal a number of genetic tools to use for annotation of candidate genomes as they design MAS programmes. These genetic tools or markers are available mainly as DNA genetic markers generated and analysed using PCR based techniques. The ideal marker system should be highly polymorphic, evenly distributed across the genome and co-dominant. Simple sequence repeats (SSRs) have been a marker of choice for most crops, because of their easy use, relatively low price, and high degree of polymorphism, but have challenges in accurately sizing SSR alleles due to PCR and electrophoresis artifacts (Bernardo, 2008). However increasing amount of sequence information and determination of gene function in cereals has made single nucleotide

polymorphisms (SNPs) more preferred of late due to their abundance in crop species (Collard and Mackill, 2008; Mammadov *et al.*, 2010). A SNP is a single change in the sequence of a section of DNA. SNPs arise from point mutations of the DNA that could be due to substitution, insertion or deletion of one nucleotide for another. SNPs can be di, tri or tetra allelic the vast majority of them are diallelic (Mammadov *et al.*, 2010). SNPs define the individual or variety and when present in genes they determine how the individual interacts with the environment (Henry and Edwards, 2011). Whereas SNP markers are mostly bi-allelic and therefore tend to be less polymorphic than SSRs, the problem is compensated for by their abundance and ability to utilize SNP haplotypes. A very large number of SNP markers are now available for maize, many of which have been developed from the DNA sequence of known genes (Mammadov *et al.*, 2010). It is for this reason that SNP markers are now the assay of choice for a variety of tasks in maize improvement including genetic diversity analysis, linkage map construction, marker trait association or quantitative trait locus (QTL) mapping and MAS (Tang *et al.*, 2010). As such SNPs were selected for use in this study.

2.8 QTL Mapping Strategies and Analysis

2.8.1 QTL mapping strategies

The traditional QTL strategy in breeding mainly involves the use of standard bi-parental mapping F₂ populations or recombinant inbred lines. This approach is used to discover rare alleles that control a phenotype (Zhao *et al.*, 2007). However this strategy has a weakness in that it is difficult to discover the causative gene and that only alleles with major effects are discovered (Tang *et al.*, 2010). Another strategy is association mapping which uses the recombinant events of many lineages. This strategy discovers the causative locus in a gene and it exploits natural variation found in species (Yu *et al.*, 2006). In maize, however, the low level of linkage disequilibrium compared to other species, poses methodological and

technological challenges for association mapping (Zhao *et al.*, 2007). However combining QTL mapping and association mapping has great potential for maize breeding in future.

2.8.2 QTL mapping approaches

Interval mapping is used for estimating the position of a QTL within two markers. Interval mapping is originally based on the maximum likelihood but there are also very good approximations possible with simple regression (Kearsey and Hyne 1994). The major problem with standard interval mapping is that linked and unlinked QTL affect the analysis, causing either modified results or false detection of QTL (Jones *et al.*, 2009). On the other hand composite interval mapping modifies the standard interval mapping approach to include additional markers as cofactors in the analysis (Rafalski, 2002). By utilising the information from these markers the power of QTL detection is greatly improved compared to the standard interval mapping (Jansen, 1993; Jones *et al.*, 2009). Composite interval mapping involves interval analysis, multiple regression and cofactors in the regression. These additional factors produce the following improvements compared to interval mapping (Zeng 1994; Yu *et al.*, 2006):

- i) reduces the need for multiple searches as it confines search to one region at a time
- ii) sensitivity of the test and the precision of QTL mapping is improved
- iii) inclusion of other markers in the analysis increases efficiency of QTL mapping.

The basic principle of marker regression is to utilize the linear functional relationship between the apparent effect of a marker on a quantitative character and the substantial effects of all the related QTL linked to the marker (Whittaker *et al.*, 1996).

2.9 Challenges for QTL mapping in maize for complex traits

Many important agronomic traits such as drought are complex and are in general regulated by many QTL sometimes with minor effects. When working with such complex traits on

biparental crosses the inconsistency of estimated QTL effects has three important implications for plant breeders. First, estimated QTL effects for traits such as grain yield or plant height have limited transferability across populations especially with use of biparental crosses due to the fixed effects model. QTL mapping for such traits will likely have to be repeated for each breeding population unlike when you are using the random effects model from association mapping where QTL mapped can be extended to other populations. This specificity for each population is demonstrated in marker assisted recurrent selection (MARS), where genotyping, phenotyping, and construction of a selection index are repeated for each population (Koebner 2003). Second, because complex traits controlled by many QTL are likely subject to genotype \times environment interaction, QTL mapping for the same population will likely have to be performed for each target set of environments. Third, because the effects of sampling error are large, population sizes of $N = 500$ to 1000 are recommended if the objective is QTL mapping per se for highly complex traits that are likely controlled by many loci (Mammadov *et al.*, 2010). Evaluating $N = 500$ to 1000 progenies for each cross of interest is unfortunately prohibitive in plant breeding programs. However, the majority of QTL in trials conducted under varying water regimes or during different seasons characterized by different rainfall patterns show a high QTL \times environment interaction (Pelleschi *et al.*, 2006). Experimental evidence from trials in which the same maize mapping population was tested under different water regimes indicates that most QTLs for grain yield and drought-related traits are stress adaptive, hence detected mainly under water-limited conditions (Tuberosa *et al.*, 2005) a result that is consistent with the sizeable genotype \times environment interaction usually detected for the same traits when phenotyped across a wide range of water regimes. This therefore implies that in this study the water management of the trials had to be consistent and uniform for the stress effects to be correctly phenotyped thereby improving accuracy and efficiency of the molecular work.

2.10 Introgression of QTL in marker assisted selection

Kulwal *et al.* (2011) summarised the three major steps involved in marker assisted selection (MAS) namely: (i) identification of molecular marker(s) associated with trait(s) of interest to breeders; (ii) validation of identified marker(s) in the genetic background of the targeted genotypes to be improved; and (iii) marker-assisted backcrossing (MABC) to transfer the QTL from the donor genotype into the targeted genotype. Important advantages of MAS are that, it can be effectively utilised for traits with low heritability, for gene pyramiding, selection can be made at seedling stage and above all there are no issues involving genetically modified crops. However despite hundreds of studies having been undertaken, very few have resulted in variety development using marker assisted back crossing. This may be attributed to: i) identification of few markers associated with small-effect QTL; ii) non-validation of markers in elite genotypes; and iii) slow adoption of markers by breeders in their breeding programs (Ribaut and Ragot 2007). First attempts to apply QTL analysis to obtain genetic insights into the drought tolerance response in maize were reported by Lebreton *et al.* (1995).

Drought tolerance is an intricate and multi-genic trait, although single genes, controlling, for example, the time of flowering or osmotic adjustment (OA), may also be involved in the plant response to drought. A number of QTL regulating important morpho-physiological traits and grain yield in drought conditions have been identified in maize (Ribaut *et al.* , 2007). However the limited use of the previous drought QTL studies, has been due to examining of crosses between lines that were not agronomically elite such as landraces or exotic materials. In addition QTL are often germplasm-specific due to the fixed effects model and the costs for applying MAS for many QTL of small effect may be greater than those of conventional cross-breeding (Xu *et al.*, 2009).

Marker assisted selection for drought-related traits based on genetic mapping information should preferably target “major” QTL with a considerable effect, consistent across germplasm. QTL identified under moisture stress tend to cluster in genomic regions related to drought tolerance, whereas those identified under well-watered conditions are related to plant performance. Nevertheless, clusters of QTL are also found in both water regimes and are probably involved in the broad adaptation of plants (Ribaut *et al.*, 2008). Tuberosa *et al.* (2002) also compiled a single genetic map of QTL from various studies and found that drought related QTL were dispersed throughout the maize genome. However since QTL are specific to their original genetic background and the effect of single genes on target traits is usually weak, the number of successful marker-assisted breeding (MAB) experiments for improved drought tolerance is limited.

2.11 Conclusion

Breeding for drought tolerance is a challenging process that needs constant review of approaches and strategies which include consultation with the end user of the maize varieties, improving the phenotypic selection strategies by refining the existing selection approaches such as the use of secondary traits and selection indices and understanding the genetic basis of these traits. In addition in tropical maize, QTL studies in the past have not identified any QTL with sufficiently large effects to be effectively used in MAS programs (Xu, *et al.*, 2009). This justifies the need to continue searching for QTL with major effects and explore other approaches such as MARS for pyramiding moisture stress related traits for development of better drought tolerant inbred lines. Furthermore most of the drought tolerance conditioning genes identified thus far are related to metabolism hence mainly contribute to plant survival rather than to productivity and may even have a negative effect on productivity (Parry *et al.*, 2005). This calls for further search for methods that may improve selection in

existing germplasm and identify QTL related to improved grain yield and general productivity under moisture stress conditions.

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CHAPTER THREE

3.0. FARMER PERSPECTIVE OF CRITICAL ATTRIBUTES NEEDED IN MAIZE GENOTYPES TARGETED FOR DROUGHT PRONE AREAS

3.1 Introduction

Maize is a crop that easily fits into the food security and livelihood strategies of farmers in SSA because diverse varieties have been bred and made available for high to low productivity potential areas. More than 80% of the farmers in southern Africa grow maize almost exclusively on a rain-fed basis yet have limited capacity to irrigate the crop especially among smallholder farmers rendering them prone to drought stress. Concomitantly, as a safety net to this vulnerability, most smallholder farmers in Zimbabwe and most of sub Saharan Africa put a big proportion of their farms under maize as a compensatory measure for low productivity at the disadvantage of other crops (Chikobvu *et al.*, 2010). Thus developing drought-tolerant varieties can boost harvests in many African countries by 10 to 34% and generate more than US\$1.5 billion in benefits for producers and consumers (La Rovere *et al.*, 2010). In order to enhance adoption of drought tolerant varieties, participatory variety development is needed to capture farmer perspectives and assure ownership of new varieties (Bänziger and Cooper, 2001).

Conventional maize breeding programs are mostly effective at developing varieties in fairly homogeneous farming systems but less effective when farmer reality is more complex and risk prone (Almerkinders *et al.*, 2001). Traditional breeding approaches, have not considered farmer preferences especially in the semi-arid or moisture stress environments (Bänziger and Cooper, 2001). For example, in Kenya, a country where maize production occurs in marginal and semi-arid zones, farmers prefer early maturing varieties in preference to increased yield potential, indicating that farmer preferences may not be congruent to conventional breeding objectives (De Groote *et al.*, 2002). Such trait trade-offs are often overlooked in traditional

breeding and may account in part for low adoption rates of some very ‘good varieties’. This lack of involvement of farmers in breeding processes has led to poor adoption of varieties by farmers as cited by Langyintuo et al. 2008. Smallholder farmer involvement in Participatory Plant Breeding (PPB) and Participatory Variety Selection (PVS) has been done on pearl millet in Namibia (Monyo *et al.*, 2001) and maize using the Mother Baby Trials in the Southern Africa Development Community (SADC) region (Bänziger and de Meyer, 2002). Participatory research with farmers generates the right research questions for scientists to address and provides a reality check on the scientists’ conceptions of system constraints and the performance of various technologies (Almerkinders *et al.*, 2001). Therefore, the aim of this study was to establish the factors that farming households consider when making decisions regarding selection of maize varieties for production in drought prone areas since low adoption current varieties has been confirmed by Langyintuo *et.al.* 2008, Chiko.

3.2. Methodology

3.2.1 Description of the Survey Area

Zimbabwe is divided into five ecological zones with the majority of smallholder farmers found in the drought prone ecological zones III, IV, and V that are marginal for maize production (Figure 1). This study was conducted in the agro-ecological regions III and IV which on average receive less than 600 mm of rain per annum and where most of Zimbabwe’s smallholder farmers live and depend largely on rain-fed agriculture (Figure 1). The study specifically targeted smallholder farmers in drought prone areas and marginal soils where selection of varieties is very detailed. Two drought prone districts namely Mutoko and Zimuto were selected for the study. Mutoko communal lands (Mashonaland East Province) are located in the North Eastern part of Zimbabwe. Mutoko district lies between latitude 18 - 19.5° S and longitude 31-32° E and is 1244m above sea level. Zimuto communal lands

(Masvingo Province), lie between latitude 19.5° S and longitude 30.8° E and is 1094m above sea level.

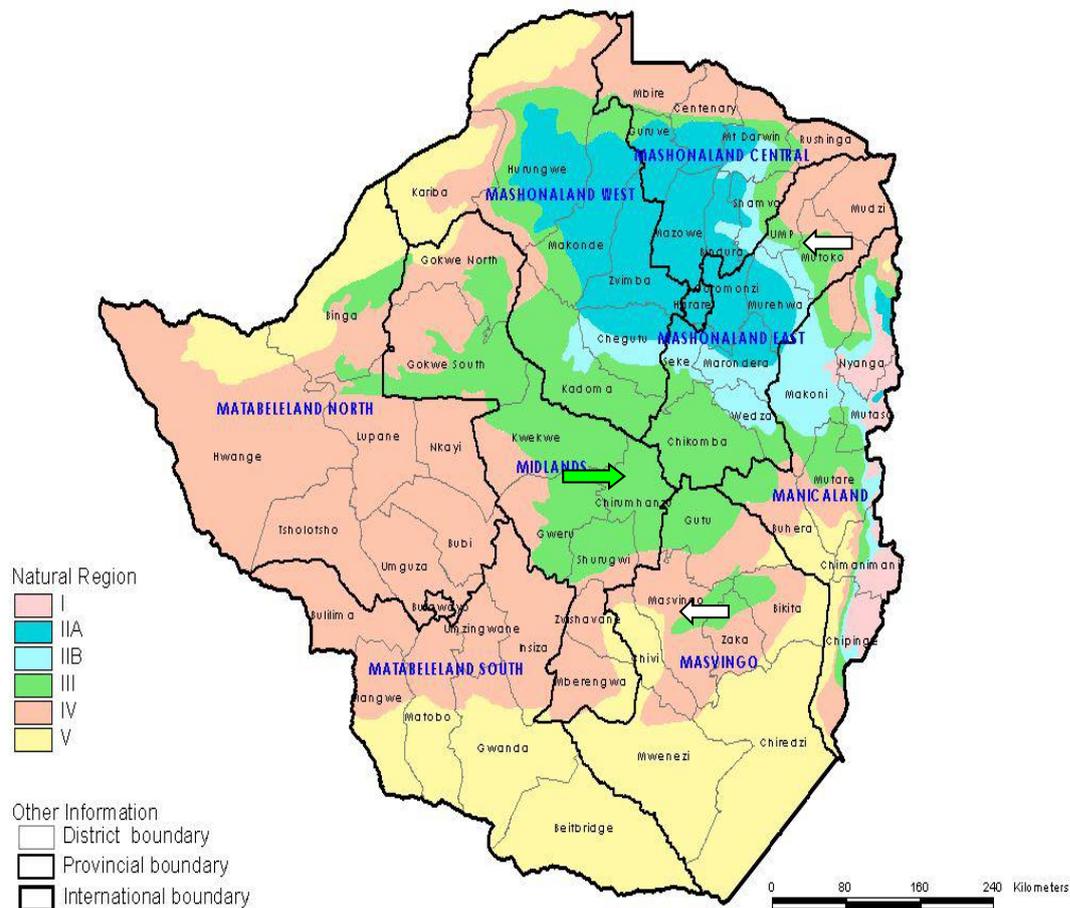


Figure 1: Map showing the survey area: White arrows show the actual area where the surveys were conducted while green arrow shows area where the pre-testing of the questionnaire was done

The selected communal areas experience a unimodal rainfall pattern with the bulk of the rains coming as sporadic heavy convectional storms annually from November - March. In Zimuto, the average rainfall as at nearby Makoholi Research Station is 425mm, ranging from 200-700 mm. The area experiences a 30-50% of mid-season droughts occurring in January. It has an average daily temperature of 27°C but temperatures can go up to $\pm 35^{\circ}\text{C}$ during the summer months. Most of the soils are granite derived sands with very little organic matter. Mutoko communal lands have an average rainfall of 450 mm, daily average temperature of

29⁰C with a more than 50% chance of a mid-season drought occurring in January. The average farm size per household in both study areas is 2.7 ha with granite derived sands dominating.

3.2.2 Sampling procedure and data collection

The selection of wards and villages was done through simple random sampling procedure. However for the focus group discussion, purposive sampling was used with one key informant being selected to represent one village.. After site selection, preliminary site visits with the help of the local extension staff were done prior to conducting the appraisal. Prior to data collection, a questionnaire was prepared and pretested in Chirumhanzu District (Midlands Province), a drought prone area.

Data collection was done using both a formal structured questionnaire and informal means such as focus group discussions with opinion leaders and local key informants. Whole community data were collected with the aid of local extension officers at ward level through transect walks.

Focus group discussions to get communally shared information were done using representatives from each of the 16 villages in a district. The group discussions included, problem listing of production constraints, varieties grown and identifying the preferred traits of drought tolerant cultivars. Primary data was collected through a formal household survey with triangulation being done using local key opinion leaders that included teachers, businessmen, headmen, and agriculture extension staff. Random sampling was done for households in the selected villages with a total of 202 heads of households being interviewed in the two districts.

Public announcement of activities through traditional structures mainly the chief, headman and kraal heads were done during the key informants, identification process In the focus group discussions individuals and group members would at times be asked to write their

contribution before a group oral discussion. Additional relevant data were also collected from secondary sources which included published and unpublished literature about agriculture production in the study areas.

3.2.3 Data Analysis

The data generated were analysed using descriptive statistics to characterise the sample households in terms of different socioeconomic and biophysical features, while mean separation using least square means of quantitative data was done using the Statistical Package for Social Scientists (SPSS) version 17. Principal Component Analysis (PCA) was used to generate the household level wealth indices based on asset and livestock endowments.

3. 3. Results

3.3.1 Household and demographic characteristics

The wealth indices of 202 households interviewed ranged from -5.47 to 24.23. About 52.4% of the households were poorly endowed (below index 0) across the study areas (Figure 2). Zimuto households (average wealth index of 2.72) were better endowed than those in Mutoko (average wealth index of -0.25). On the categorisation, about 20% of the sampled households were well-off, 55% scored average and 25% were poor. The mean wealth index of the poor class was -3.13; the average class showed a mean of 0.71 whilst the well-off mean was 8.53.

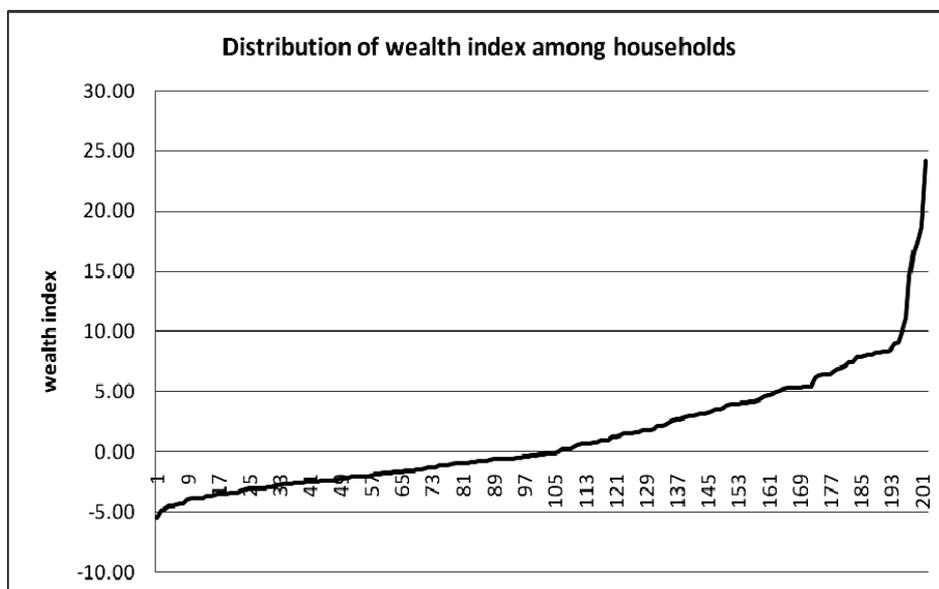


Figure 2: Distribution of wealth index among households in Zimuto and Mutoko.

More than half (51.5%) of the households were female headed. The average age of household heads in both Zimuto and Mutoko was about 50 years, with a 20 to 86 age range. The level of literacy was reasonably high, with 53% of the household heads having attained secondary education while about 4% of household heads were illiterate. Significant differences were observed for family size ($P \leq 0.024$) with Mutoko (6) and Zimuto (5) as average number of people per family. The mean farming experiences for the two districts were significantly different ($P \leq 0.003$) with Zimuto having a higher average of 18.59 years than Mutoko with an

average of 13.58 years. Zimuto district had the largest number of farmers owning animal drawn carts, oxen, chickens ($P < 0.000$), TV/Radio ownership ($P \leq 0.005$) compared to Mutoko.

Farm holdings under maize in the study sites ranged from 0.2 ha to 3 ha. In both districts there was a significant ($P \leq 0.001$) proportion of total cropped area and land put to maize production (Appendix 1). The study also showed that drought frequency over the last 5 years was significantly higher ($P \leq 0.000$) for Zimuto (average of 2.55) than for Mutoko (average of 1.34). Land holding in the survey areas ranged from 0.2 hectares to 5 hectares with a mean of 1.4 hectares. Appendix 2 shows the distribution of land size by wealth class where the 1 to 4 ha range dominated among the three wealth categories.

3.3.2 Maize production constraints and variety selection

Six major constraints were highlighted by the farmers as the critical factors affecting maize production and food security in the surveyed area. These included drought, timely availability of seed and fertilizers, draught power constraints, low soil fertility, lack of knowledge on the best varieties for their respective areas and the cost of inputs. Drought as a production constraint was ranked first by 40% of the total households thus highlighting it as the main factor limiting production. Inputs and animal draught power related constraints were cited as the second and third most limiting factors to increased maize production by the farmers (Figure 3). Farmers also indicated a number of factors they consider when buying maize seed. The highest ranked factor was variety's yield potential as shown by 89% of the farmers involved in the study (Appendix.3). Early maturity (< 125 days to maturity) and drought tolerance traits were ranked second and third attributes considered respectively. Very few farmers (8%) cited field pest and diseases resistance as traits considered when selecting a variety. However farmers in the two districts had different views on the ranking of traits they consider of importance when selecting varieties for stress prone environments. Zimuto farmers listed yield potential, storage pests, grain size, maturity and drought tolerance as the

most important traits to consider in order of importance. The Mutoko farmers considered yield potential, drought tolerance, maturity, palatability and quality of the staple ‘sadza’ as the traits of importance (Appendix 4).

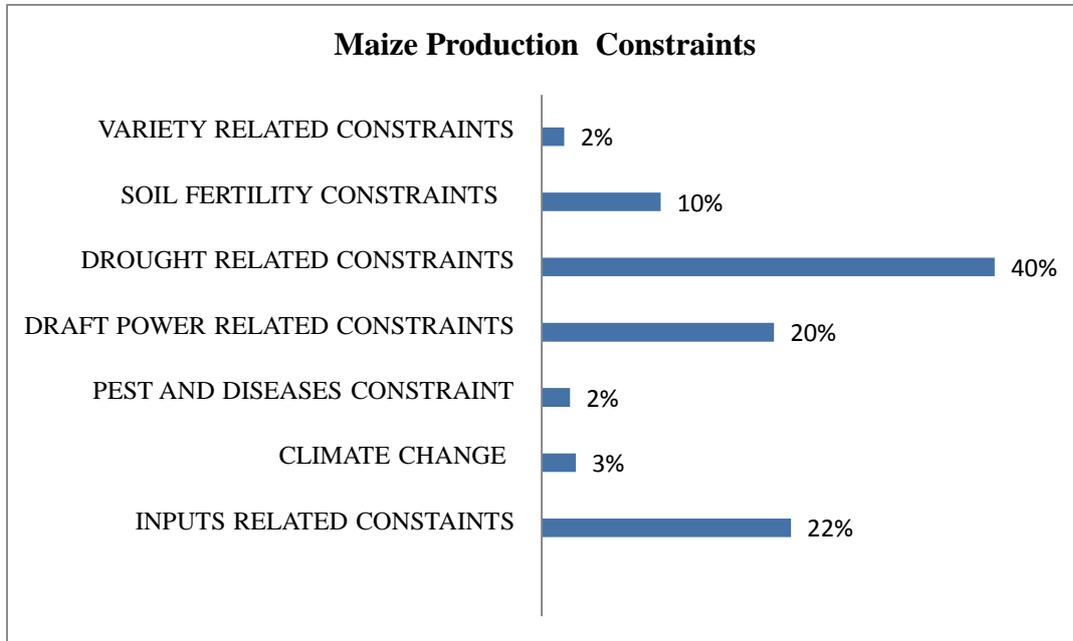


Figure 3: Factors affecting maize production and food security in the survey areas of Mutoko and Zimuto districts of Zimbabwe

Farmer to farmer exchange of information about varieties was significantly important for Zimuto farmers ($P < 0.001$) than in Mutoko. In Mutoko, information on variety performance and characteristics came predominantly through the government extension staff and seed company personnel. This was contrary to Zimuto, where non- governmental organisations (NGOs) had significant contribution ($P < 0.003$), to information dissemination. More than 80% of the farmers interviewed had some knowledge of drought tolerant maize varieties. About 80% of the farmers cited at least one perceived drought tolerant variety which included hybrids like R201, SC513, SC403; OPVs ZM421 and ZM521 and local landraces like ‘Eight Lines’ and Hickory King. Availability of seed for planting in time and knowledge of the best varieties for specific areas were indicated as major concerns. Group discussions highlighted

the need to have new varieties with pyramided traits for drought stress tolerance. Currently farmers plant these available varieties because they have no choice and select these ‘mediocre’ varieties by ranking of traits they consider most important and making trade-offs.

3.3.4 Traits that need improvement in current varieties

Drought tolerance was ranked top by 82% of farmers as a trait that needed improvement in the current varieties, (Figure 4). This was followed by improving yield potential followed by the need for early maturing varieties. Farmers preferred drought tolerance traits such as stay green characteristics, leaf unrolling ability or recovery from stress, non abortion at grain filling when under stress and tassels that stay alive for a long period under stress

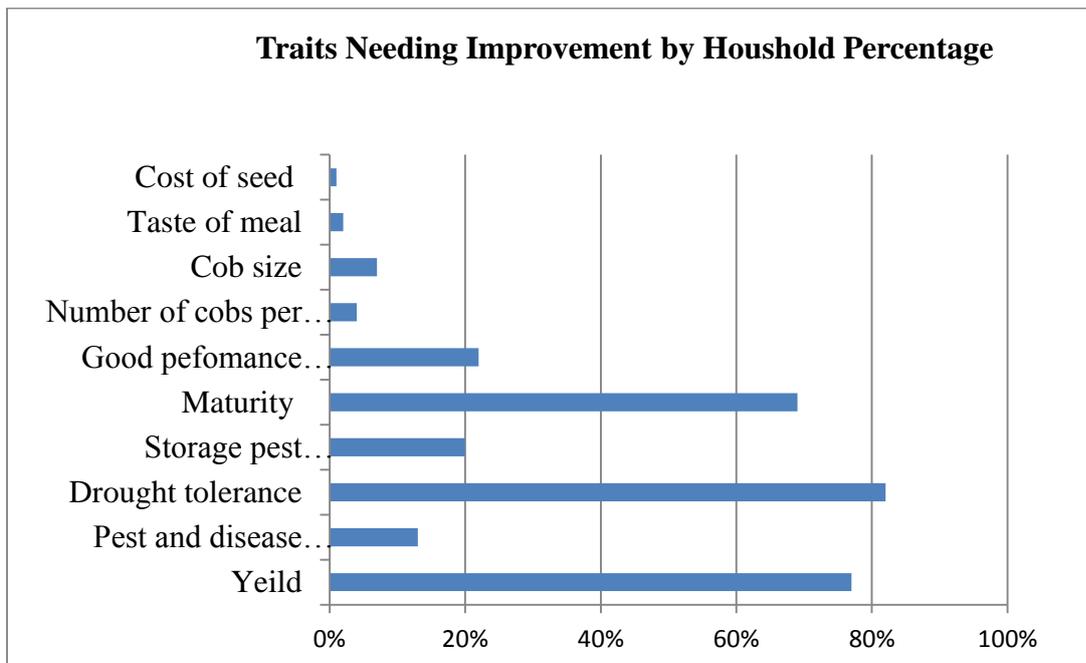


Figure 4: Traits needing improvement in current varieties as highlighted by farmers during the survey in Mutoko and Zimuto Districts in 2010.

3.3.5 Correlation Analysis of Demographic Attributes and Crop Traits

Weak but positive and significant correlations were observed for maize area planted and draft power, yield and area planted to maize, and drought and variety maturity period/duration. Education, age and experience were negatively correlated to each other.. Positive correlations were also recorded between variety maturity and maize area ($r = 0.183^*$), drought tolerance and early maturity ($r = 0.143^*$) and yield and maturity ($r = 0.633^{**}$), (Table 1). Overall in the study area, variety maturity time was a factor farmers considered very important when selecting a maize varieties. Most of the varieties grown were very early maturing, or drought escapees which would utilize the short growing season in the areas under study.

Table 1. Household and agro- morphological traits correlations of the surveyed areas

TRAITS	Age	Gender	Experience	Education	Draught Power	Total Area (ha)	Maize Area (ha)	Maturity	Grain Yield (t/ha)	Lodging	Drought
Age	1										
Gender	-0.094	1									
Experience	0.684**	0.013	1								
Education	-0.451**	-0.176*	-0.231**	1							
D. Power	0.211**	-0.164*	0.145*	-0.016	1						
T. Area	0.164*	0.005	0.263**	0.093	0.223**	1					
M. Area	0.129*	-0.009	0.194**	0.110	0.246**	0.879***	1				
Maturity	0.223**	0.189**	0.263**	-0.045	-0.083	0.286**	0.183*	1			
G. Yield	0.148*	0.143*	0.283**	-0.072	-0.097	0.276**	0.244**	0.633**	1		
Lodging	0.075	-0.052	0.099	0.049	0.119	0.043	0.052	-0.152*	-0.165*	1	
Drought	0.062	0.127	0.110	-0.157*	-0.137*	0.040	-0.032	0.143*	-0.137*	0.509*	1

P < 0.05 *; P < 0.01 ** ; P < 0.001

Data Source: Table of data generated from correlation of household and secondary traits of the surveyed area

3.4. Discussion

3.4.1 Household and demographic characteristics

In Zimbabwe traditional land tenure system characterised by communal ownership of land by clans, families or related individuals is still common. The land owners have user rights that may be transferred through inheritance or by the chief to another beneficiary. Under this land tenure system, wealth is measured by ownership of assets such as cattle, goats, sheep, poultry and farm implements and machinery for tilling and traction purposes. The two study districts had more goats than cattle per household because goats are generally better adapted to drier conditions than cattle. The goats in these communities are a source of food (milk and meat).

However, cattle are a sign of wealth in rural Zimbabwe and are the dominant source of draught power in most households. They also provide milk, meat and manure. Television sets or radio ownership is considered very important in the rural set-up apart from being a source of entertainment, because agricultural training programs and talk-shows are aired and hence radios are also used as sources of agriculture information. Family size and age of members is critical in the rural households in Zimbabwe as it determines the amount of labour available per household as farm labour is mostly provided for by the family members.

The house wealth and demographic- approach, was adopted in this study because findings by CIMMYT (1993) showed that resource endowments, size of family labour, livestock ownership and access to information through extension services were critical in helping farmers in variety adoption. In other studies, Almekinders *et al.* (2001) similarly showed that ‘a farmer’s decision in adopting or rejecting a new technology is influenced by a combination of factors related to farmer’s objectives and constraints such as: farmer’s socio-economic circumstances (age, formal education), and farmer’s resource endowments (e.g. size of family labour, farm size and livestock ownership).

Grain yield, total area planted and area planted to maize had a significant positive correlation ($P < 0.01$) implying that farmers who put more land to cropping in general also planted more maize and thus had significantly higher harvests during drought. This was also supported in the group discussion where farmers with more land planted more than one variety to spread the risk of crop failure

3.4.2 Maize production constraints and variety selection

In the districts under study, maize is the staple crop and is therefore both economically and politically important. To support maize as an economic crop a comparison of the wealth classes on the land allocated to maize showed that the average group dominated in the area under maize in both surveyed areas. The poor group had more people (21%) with maize area under 1 hectare as compared to more than 1 hectare (Appendix 2). A decline in maize production due to drought and economic factors has seen the country's food basket price increase as seen in the 2008/9 season decline in maize production to below the ten year (2000- 2010) average of 0.87 t/ha (FAO 2010).

The uncertainty of drought occurrence in the area of study and Zimbabwe in general results in farmers putting maize under bigger acreage as a compensatory measure, thus disadvantaging other crops. This is also evident from the strong, positive and significant correlation between total land area and area planted to maize (Table 1). This finding is similar to Makanda *et al.* (2009) who reported that maize takes more than half of the land area grown to crops in the smallholder sub-sector since it is considered a food security crop. However, in the large holdings farmers at times intercropped maize with legumes such as beans or cowpeas or other vegetables crops like pumpkins.

Foliar diseases are not a factor of economic importance in the smallholder agriculture sub-sector because of the low rainfall hence reduced or no disease pressure. In the group

discussions the farmers highlighted other factors considered when assessing a variety for stress tolerance which includes drought stress traits such as; stay green characteristics, leaf unrolling ability or recovery from stress, non abortion at grain filling when under stress and tassels and silks that stay alive for a long period under stress. Recovery ability was emphasized with farmers suggesting a maize variety that could have ‘sorghum drought stress recovery traits’. In other studies conducted in eastern districts of Save Valley and Mutare West in Zimbabwe, similar findings were reported in which farmer requested for ‘sorghum maize’ and ultra early (less than 90 days to mature varieties) to avoid late season drought stress (Derera *et al.*, 2006). Storage pest resistance was highlighted as a very important factor to consider in Zimuto due to a previous incident where farmers grew a variety that was high yielding but was completely destroyed by weevils under storage in granaries. Susceptibility to the maize weevil, accounted for many farmers in the area abandoning the variety despite it having a good yield.

In Southern Africa, farmers similarly preferred varieties with hard endosperm which is considered to confer resistance to storage pests and high maize flour production when pounding (Bänziger and de Meyer, 2002). Farmers expressed willingness to make trait trade-offs for some major traits with lodging and disease resistance being considered less critical for a good yielding variety for example. Similar results have been reported on farmers’ preferences on traits in eastern Kenya (Groote *et al.*, 2002). In general, one can see that despite the two districts being drought prone ranking of production constraints were different. This implies that farmers perceive issues differently depending on their environment and hence require specific solution i.e. breeding or variety development should also be for specific environments rather than broad.

3.4.3 Sources of information on the drought tolerant maize varieties

Access to information through extension services from the ministry of agriculture personnel was the major source of variety information with 90% of the respondents mentioning extension staff as vital sources of information on varieties and general agriculture (Appendix 5). This is the general trend in most of sub Saharan Africa where the main source of information for maize production technologies is the extension service (Zegeye *et al.*, 2001). However information exchange among farmers also referred to as farmer to farmer knowledge transfer, is a common mechanism for sharing communally owned wisdom and any approval or otherwise of technology introduced in the area. The Zimuto case where the entire farming community abandoned a good yielding hybrid strengthens this point. Having identified drought as the main maize production constraint, the farmers were asked to identify causes of drought and they highlighted three causes in their order of importance namely climate change, indiscriminate cutting down of trees and failure to hold traditional ceremonies.

Farmers were also asked to relate drought experience and its effects to which they associated drought with hunger, migration to urban area, selling of vegetables, survival on wild fruits, selling of livestock and failure of children to attend school. This is similar to responses reported by Mutasa *et al.* (2010) from a survey done in Buhera district which is in the eastern part of the country. Farmers interviewed showed that they were employing more reactive than pro active coping mechanisms in their response to drought. In spite of the information exchange among farmers lack of knowledge on the true/ real drought tolerant was very apparent. This is because landraces such as ‘Eight Lines’ and Hickory King identified as drought tolerant by most farmers are late maturing and do not fully fit in the short rainy seasons of the marginal areas under study. This finding is supported by Langyintuo *et al.*, (2008) who concluded that one of the major bottlenecks in the seed industry of Eastern and

Southern Africa was lack of awareness of the availability and value of existing improved varieties.

3.4.4 Low adoption of drought tolerant maize varieties

Eighteen percent (18%) of the respondents stated that they would continue growing the same seed varieties that they were currently growing (Appendix 6) despite their poor yields due to lack of other options. Sticking to the old varieties in spite of their weaknesses is in line with the threat rigidity hypothesis (Straw *et al.*, 1981), where farmers even under stress would stick to old solutions to their problems. In the group discussions a number of farmers highlighted the use of landraces such as ‘Hickory King’, ‘*Mukadzi Usaende*’ and ‘Eight Line’ for *sadza* palatability and farm saved seed. In Mutoko, farmers highlighted the growing of hybrid seed maize despite poor drought tolerance for commercial reasons where in a good season they would sell the grain to the national grain marketing board (GMB) while growing small plots of the landraces such as ‘eight line’ for home consumption because of its good taste or palatability.

This implies that there is need to improve the current varieties through introduction of stress tolerant traits by conventional or biotechnological methods. There is also need for breeders to tap into the farmer germplasm for developing new varieties or improving the old landraces through participatory plant breeding and variety selection. Policies on seed costs and possible subsidies might also be considered in view of 41% of respondents failing to afford improved seed varieties.

3.4.5 Correlation analysis of demographic characteristics and crop traits

Significant and positive correlation ($r = 0.648^{**}$) between age and experience shows that the older farmers had more experience than the younger ones in maize production and general agricultural practice. Generally, experience and education levels are expected to influence knowledge and the farming enterprises undertaken in rural households. This is also supported

by findings where variety use or adoption is influenced by the farmer's socio-economic circumstances such as age and formal education (Doss 2006; Chikobvu *et al.*, 2010). Draught power, age and experience are also significant ($P < 0.05$) and positively correlated. This is because farmers accumulate wealth over years hence they can afford to have animals for draught power.

This statistic implies that the older the household head, the better the experience and the more the chances of him or her having, draught power for timely operation. Draught power (traction) had a significant ($P < 0.01$) and positive correlation with total area cropped and area under maize. Total area planted and area planted to maize have a significant and positive correlation ($r = 0.879^{**}$). Accordingly, farmers with draught power capacity planted bigger areas ploughed and planted to maize the strategic crop. The experienced farmers also had more area planted to maize ($r = 0.194^{**}$) and were more knowledgeable about drought related traits such as maturity ($r = 0.263^{**}$) and yield ($r = 0.286^{**}$). Similar findings in Ethiopia show that farmer resource endowments such as farm size and livestock ownership influence varieties farmers grow (Zegeye *et al.*, 2001). Farmer preference for early maturity is consistent with previous findings, that farmers preferred these varieties because they escaped late season drought securing the harvest against crop losses (Bänziger and de Meyer, 2002).

In other semi arid zones of Zimbabwe, such as Chipinge and Chimanimani farmers grow short season cultivars such as SC403 and PAN413 (Derera *et al.*, 2006). However the Mutoko farmers were unique in that they preferred early maturing open pollinated varieties (OPVs) such as ZM421 and ZM521 to hybrids. Preference of OPVs in Mutoko can be explained by the fact that the area is drier than Zimuto and under such circumstances, OPVs are more resilient hence the demand for ZM series of synthetics. This preference for OPVs highlights the need for production and marketing of OPVs at community level. The group

discussions also revealed that some farmers in Mutoko in the 2008/09 cropping season were forced to grow recycled (F2) hybrid seed due to severe seed shortages. This shows the need to breed for synthetics and also train farmers on how to produce seed from OPVs for use when certified seed is not readily available

3.4.6 Traits that need improvement in current varieties and possible trade offs

The three drought tolerance associated traits; good yield potential and early maturity are a priority for any variety to be considered suitable in the survey area, hence the need for more breeding effort on the three traits. The farmers' emphasis on drought tolerance improvement shows that drought tolerant varieties are currently either not satisfactory or non-existent. Early maturity is preferred by most farmers because of the need for drought escape due to the short season nature of the rains or the need to give the farmer food in the hunger season i.e. period prior to the main season harvest. Disease and pests resistance traits while ideal for full season varieties or in good seasons with increased rain and disease pressure are not a priority for the farmers of Mutoko or Zimuto. Farmers also emphasized the need for improving the current varieties for better performance in poor soils (Figure 4). In the group discussions farmers identified three factors critical for variety characterization namely, trait degree of importance (rank), range of acceptability of a trait and trait trade-off they were willing to make when selecting desirable variety.

Farmers interviewed used reactive strategies when confronted with problems of drought and seed shortage such as selling of livestock or sowing less area where seed shortages occurred. Proactive strategies such as conservation agriculture practices like winter ploughing, fallowing, and stable-mulching were however highlighted in some instances. Orientation for coping during drought includes praying for rain, traditional ceremonies and social support. However, innovative strategies that may be effective when dealing with drought such as early or dry planting and planting drought tolerant landraces need to be promoted.

3.5. Conclusions

Farmers in both districts listed grain yield potential, early maturity and drought tolerance as the main traits to consider when selecting a variety to grow in drought stress prone areas. Other traits considered included variety performance under poor soil fertility conditions especially low nitrogen, storage pests resistance and variety recovery ability from stress. Survey data shows that low adoption of current varieties in Mutoko and Zimuto is due to their failure to meet the farmers' level of expectation on the yield potential and drought tolerance level. In the two areas farmers had different views and preferences in terms of traits to consider when selecting a variety. Farmers' preferences or choices on varieties and their traits varied with area and are informed by farmer experiences and circumstances. This therefore calls for participatory agro-ecological intensification in target areas with farmer involvement in early stages of the breeding process. Diverse information sources for farmers in the surveyed area shows that there is need for closer cooperation and exchange of technical information with the NGOs, government and seed companies in order to give farmers better information and technical skills to cope with the ever increasing drought conditions especially under the threat of climate change.

3.6 Recommendations

Breeding and evaluation for drought stress tolerance needs to be done in the target areas where the varieties are to be deployed upon release. Landraces need be improved over time through participatory breeding and variety selection and this should be coordinated by the public sector breeding programmes. More targeted training on variety characteristics, drought tolerant varieties and conservation techniques also needs to be done through joint private and public extension services. There is also need to package information on variety characteristics and create a central information dissemination system for easy information access for the farmer.

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CHAPTER FOUR

4.0 SECONDARY TRAITS, SELECTION INDICES, GENETIC ACTION AND STABILITY ANALYSIS OF GENOTYPES FOR DROUGHT TOLERANCE SELECTION

4.1 Introduction

Sub-Saharan Africa is the most severely affected region where almost half of the land surface is exposed to a high risk of meteorological drought (FAO 2010). The drought effects are mainly pronounced among the low income peasant and subsistence farmers who are found in the marginal environments and cannot afford supplementary irrigation (Tuberosa *et al.*, 2005). Future projections indicate that maize production will face a reduction in irrigation volumes, even in regions where supplemental water is essential for securing a profitable harvest as a result of climate change (Rosegrant *et al.*, 2002). Although appropriate irrigation and/or other agronomic practices may mitigate the reduction in yield caused by drought their effects largely depend on crop response which is mainly influenced by its genetic make up.

Low heritability for drought tolerance, genotype x environment effects and lack of effective selection approaches limit development of genotypes that are tolerant to water stress. Grain yield is a complex trait controlled by several interacting genotypic and environmental factors. However, in maize there are yield components which are less complex, highly heritable and less influenced by environmental effects (Malosetti *et al.*, 2008). Indirect selection of drought tolerance in maize has been successfully done using highly heritable traits that correlate with drought tolerance such as anthesis-silking interval, ears per plant and senescence rate (Araus *et al.*, 2008). Exploitation of these highly heritable yield components that are correlated to grain yield is an effective option for selecting drought tolerant genotypes than direct selection of yield (Kashiani and Saleh, 2010). By utilising genetic correlations, secondary traits can be used to improve primary traits that have low heritability or are difficult to measure with correlation analysis being used to determine efficacy or efficiency.

Traits such as leaf senescence, anthesis-silking interval, and plant height have been traditionally used as secondary traits for grain yield (Cooper *et al.*, 2006). The significance of delayed senescence or stay green characteristics has been extensively studied (Zheng *et al.*, 2009). A positive correlation of grain yield with the functional stay green trait has been reported, suggesting that senescence can be an indirect measure for drought tolerance (Zheng *et al.*, (2009).

To evaluate response to water stress, selection indices based on mathematical relations between optimum and stress conditions have been developed. The inter-relationship between yield and its contributing components can significantly improve efficiency of crop breeding programs through the use of proper selection indices (Mohammadi *et al.*, 2003). The most efficient index for the selection of stress tolerant cultivars is one that has a relatively high correlation with grain yield under both stressed and non-stressed conditions (Choukan *et al* 2006). Indices such as stress tolerance index, geometric mean productivity, yield index, yield stability index and others have been traditionally used for selection. Stress tolerance index is a criterion for selection of varieties tolerant to drought stress and higher values for this index represent high stress tolerance capability and high yielding potentiality (Fernandez, 1992). Geometric mean productivity (GMP) and stability tolerance index (STI) can be used in selecting genotypes with high yield in both water stressed and stress free environments (Khalili *et al.*, 2004).

Secondary traits and selection indices have been used separately in maize studies before but this study was informed by the need to have the two selection methods used in tandem for even better selection efficiency as well as the need to see their effectiveness when used in germplasm of diverse background namely lowland tropics (CIMMYT Mexico) and African adapted sub tropical germplasm.

Genotype by environment interaction is a challenge when evaluating and selecting hybrids suitable for different target environments. To overcome this challenge the use of stability assessment methods such as Genotype x Environment (GE), Genotype x Genotype x Environment (GGE) biplots, regression analysis and Additive Main effects and Multiplicative Interaction (AMMI) are normally used to handle both the main effects and G x E interactions in multi-location yield trials making it easier to select good performing hybrids (Bondari 2003).

Knowledge of gene action of secondary traits correlated with grain yield is also of paramount importance if one needs to make progress in selecting for lines that can then be used in developing synthetics or hybrids targeting the both stress prone and non stress environments. Therefore the objective of this study was to assess the reliability of tandem use of selection indices and secondary traits, understanding the modes of gene action of moisture stress related traits and genotype x environment effects in an effort to improve selection efficiency under stress environments.

4.2 Materials and Methods

4.2.1 Germplasm and experimental design

Fifteen white grained tropical inbred lines (10 lines and 5 testers) obtained from the International Maize and Wheat Improvement Centre, (CIMMYT) were crossed using the North Carolina Design II. The fifty resultant single cross hybrids, their parental lines and four hybrid checks were evaluated across five sites using the alpha 0.1 lattice design (Appendix 7). Hybrids and their parents were evaluated separately in two trials planted adjacent to each other in the 5 sites (Table 2). All the trials under study were conducted in Zimbabwe.

Table 2: Description of experimental site and evaluation characteristics

Site	Longitude	Latitude	Altitude	Evaluation Environment
ART Harare	31.50 ⁰ E	17.43 ⁰ S	1500m	Optimum
RARS Harare	31.30 ⁰ E	17.35 ⁰ S	1496m	Optimum
Kadoma	30.90 ⁰ E	18.32 ⁰ S	1149m	Optimum
Chiredzi	31.58 ⁰ E	21.02 ⁰ S	429m	Optimum/Managed Drought
Chisumbanje	33.00 ⁰ E	20.00 ⁰ S	421m	Optimum/ Managed Drought

The testcross evaluation was done using the alpha (0.1) lattice design. At each site trials were replicated twice, with each entry being planted in single row plots 5 m long, at a spacing of 75cm x 25cm. Two seeds were planted per hill and later thinned to one plant per hill population to give a plant population of 53000 plants ha⁻¹. The hybrid SC727 obtained from Seed Co Zimbabwe was used as drought susceptible check. The parental lines used in the study and the primary criteria for selections are shown in Table 3.

4.2.2 Optimum evaluation and stress management of trials

Managed drought stress studies were done in the cold rain-free season using irrigation. Moisture stress was induced by withdrawing irrigation water four weeks before and after flowering period. Water was withdrawn from the crop to concede with a stress of below 50% of field capacity during the flowering period as measured by neutron probe and calculated by the evaporation pan records (Banziger et al., 2006). In this study the moisture stress intensity was on average 40% of well watered conditions. Inbred line parents were planted in a different trial but in the same environment as the testcross hybrids. In all trials, data for flowering dates (at 50% anthesis and 50% silking), plant and ear height, plant root and shoot stand, leaf senescence, disease and grain weight were recorded. Some derived traits such as anthesis-silking interval (ASI), lodging percentage, ears per plant (EPP) and yield per hectare (at 12.5% moisture adjustment) were also calculated.

Table 3: Pedigrees, heterotic groups of parental material and selection criteria used in testcross development

Name	Pedigree	Heterotic Group	Selection Criteria
T1	CML312-B	A	MSV and Drought
T2	CML442-B	A	MSV and Drought
T3	CML537	A	MSV and Drought
T4	CML538	A	MSV and Drought
T5	CKL05005	B	MSV
L1	[CML445/ZM621B]-2-1-2-3-1-B*8	B	MSV and Drought
L2	CML312/TUXPSEQ C1F2/P49SRF2	A	MSV and Drought
L3	MSRXPOOL9]C1F2-205-1	A	Drought
L4	TS6C1F238-1-3-3-1-2-#-BB	A	MSV and Drought
L5	P501SRc0-F2-47-3-1-1-BB	A	MSV and Drought
L6	ZM521B-66-4-1-1-B*5	B	MSV and Drought
L7	SYN-USAB2/SYN-ELB]12-1-1-1-	B	MSV
L8	SYN-USAB2/SYNELB2]-35-2-3-1-	B	MSV
L9	Z97SYNGLS(B)F2-188-2-1-3-B*4	B	MSV and Drought
L10	MAS[206/312]-23-2-3-B*5	A	MSV and Drought

Selection criteria were based on moisture stress or drought tolerance and resistance to maize streak virus disease a major challenge to maize production. Heterotic classifications were done according to the CIMMYT A and B heterotic patterns.

4.3 Data management and analysis

Analyses of variance were computed for each trial using the PROC MIXED procedure from SAS (SAS 2009) with hybrids and inbreds being considered as fixed effects, while replications and blocks were considered random effects. However, this study reports on the across site analyses of the data in the two environments. The adjusted means were used to estimate GCA and SCA effects, while heritability was calculated as the proportion of genetic

variance over the total phenotypic variance. Relative importance of general and specific combining ability on progeny performance was estimated using the Baker's ratio.

$$2\delta^2_{gca} / (2\delta^2_{gca} + \delta^2_{sca}) \dots\dots\dots (\text{Baker, 2002})$$

Where, δ^2_{gca} and δ^2_{sca} are the variance components for GCA and SCA. Simple phenotypic correlation coefficients among test environments for the same trait were also computed.

Drought tolerance indices were calculated using the formulae below:

Yield Stability Index (YSI)

$$YSI = Y_{si} / Y_{pi} \text{ (Lin et al., 1986)} \dots\dots\dots (i)$$

Stress Susceptibility Index (SSI)

$$SSI = [1 - YSI] / SI \text{ (Fischer and Maurer, 1978)} \dots\dots\dots (ii)$$

Yield Index (YI)

$$YI = Y_{si} / Y_s \text{ (Gavuzzi et al., 1997)} \dots\dots\dots (iii)$$

Stress Tolerance Index (STI)

$$STI = (Y_{pi} \times Y_{si}) / Y_p^2 \text{ (Fernandez, 1992)} \dots\dots\dots (iv)$$

Geometric Mean productivity (GMP)

$$GMP = \sqrt{Y_{pi} \times Y_{si}} \text{ (Fernandez 1992)} \dots\dots\dots (v)$$

Drought Tolerance Index (TOL)

$$TOL = Y_{pi} - Y_{si} \text{ (Hossain et al., 1990)} \dots\dots\dots (vi)$$

Mean Productivity (MP)

$$MP = (Y_{pi} + Y_{si}) / 2 \text{ (Hossain et al., 1990)} \dots\dots\dots (vii)$$

Stress Intensity (SI)

$$SI = 1 - (Y_s / Y_p) \dots\dots\dots (viii)$$

- **Where;**
- **Y_{si}** = yield of cultivar under stress condition
- **Y_{pi}** = yield of cultivar under optimum condition
- **Y_s** = total mean yield under stress condition
- **Y_p** = total mean yield under optimum condition

4.4 Results

4.4.1 Gene action under drought and optimum conditions

Significant and positive GCA values for grain yield were observed for inbred lines Tester 1, Line 2 and Line 7 while Tester 5, Line 4 and Line 10 had significant but negative GCA values under drought (Table 4). Under optimum conditions, GCA values for grain yield were positive and significant for Tester 2, Tester 3, Line 3, Line 7 and Line 9 while negative and significant for Tester 5, Line 5 and Line 10. Line 7 had significant ($P < 0.01$) and positive, while Tester 5 and Line 10 had negative and significant GCA values for grain yield in both environments. There was a significant and positive correlation ($P < 0.05$) between additive genetic variance (GCA) and grain yield of inbred lines under drought conditions ($r = 0.541^*$). Plant height GCA values had positive correlation ($r = 0.822^{**}$) for both stress and well-watered environments. Most inbred lines contributed to a decrease in plant height as shown by the GCA values which were mostly negative in both environments.



Figure 5: Inbred line growing under managed moisture stress in the trials at Chiredzi research station, Zimbabwe

Table 4. Inbred lines grain yield and secondary traits GCA values across four managed drought and six optimum environments

Name	GRAIN YIELD				AD		ASI		EPP		SEN		PH	
	Drought (Drt)		Optimum (Opt)		Drt	Opt	Drt	Opt	Drt	Opt	Drt	Opt	Drt	Opt
	Inbred	GCA	Inbred	GCA	GCA		GCA		GCA		GCA		GCA	
T1	2.01	0.33*	2.73	0.06	0.13	0.43*	0.06	0.06	0.05	0.01	-0.11	-0.06	7.88	7.19
T2	1.77	0.06	3.07	0.21*	-0.29*	0.73**	-0.20	0.18	-0.06	-0.02	-0.24*	0.36*	-0.13	-0.92
T3	1.64	0.06	2.45	0.25*	0.95**	0.39*	0.00	0.05	0.01	0.01	0.39*	0.11	4.13	0.58
T4	2.06	-0.02	3.05	0.14	-0.34*	-0.32*	0.20	-0.17	0.01	-0.01	-0.08	0.00	-1.25	-1.07
T5	1.11	0.32*	1.92	0.75**	0.41*	1.41**	-0.07	-0.04	-0.01	0.01	0.04	-0.56*	-4.88	-6.22
L1	1.48	0.01	2.27	-0.10	-0.65**	-0.49*	0.39*	0.33*	0.02	0.23*	0.18	0.13	1.50	2.47
L2	2.70	0.42**	3.24	0.17	-1.30**	-0.43*	-0.76**	-0.47*	0.07	-0.27	-0.11	-0.06	-7.50	7.07
L3	1.82	0.05	3.39	0.63**	0.65**	-0.03	0.07	0.29*	-0.02	0.29*	-0.10	-0.11	-8.50*	-6.32
L4	1.14	-0.23*	1.52	0.01	-1.02**	-0.01	0.09	0.11	0.00	0.11	0.35*	0.09	7.50	5.09
L5	1.21	0.05	2.28	-0.37*	0.78**	0.04	0.46*	0.01	0.01	0.01	0.34*	0.22	0.25	4.67
L6	1.72	-0.02	3.05	-0.01	-0.85**	-0.19	0.43*	0.61*	-0.01	0.41*	0.25*	0.09	4.75	0.24
L7	1.90	0.52**	2.83	0.61**	0.78**	0.39*	0.14	-0.27	0.05	-0.25*	-0.15	0.01	-11.5*	4.22
L8	1.49	-0.16	3.05	-0.10	0.86**	0.39*	0.28*	-0.23	0.00	-0.28*	0.04	0.03	-8.5*	7.88
L9	1.52	0.05	3.62	0.27*	0.05	0.14	-0.28*	0.37*	-0.01	0.37*	0.06	-0.15	-0.75	4.03
L10	1.27	-0.64*	1.96	-1.11***	0.95**	1.26**	0.14	0.17	-0.07	0.19	0.30*	0.21	7.75	-4.42
Mean	1.66		2.70	0.04	0.01	0.03	0.06	0.05	0.03	0.02	0.06	0.08	3.22	4.16
GCA SE		0.13		0.23	0.56	0.24	0.37	0.19	0.03	0.02	0.17	0.21	1.51	2.08
Correlation		0.541*		0.428		0.477		0.418		-0.446		-0.046		0.822**

†This data was obtained from 4 drought evaluations 6 and optimum evaluations; * = P<0.05 ; ** = P< 0.01

4.4.2 Genotype x Environment interaction

The trial testcross hybrid trial mean was 2.84 t/ha, with a parental inbred line trial mean of 1.66 t/ha under drought. Under well watered conditions the hybrid trial mean was 7.01 t/ha, while the inbred line trial mean was 2.7 t/ha. The stress intensity index (SI) for the testcross hybrids was 0.59 implying that the drought yields were approximately 40% of the optimum yields. Significant differences were also observed for GCAf, GCAM, GCAf x environment SCA and SCA x environment for grain yield across optimum and managed drought environments (Table 5). Per plot and across environment heritability values for this trait were higher under stress than under optimum conditions. The Baker's ratios show that there was a consistent increase in the additive variance contribution for grain yield, anthesis date, ears per plant and leaf senescence from optimum to drought stress environments, while grain yield and plant height values were higher under well watered environments (Table 5). Significant GCAM and GCAf were observed in both environment for grain yield, anthesis date and plant height, while GCAM was significant for ears per plant and leaf senescence under moisture stress and GCAf significant for anthesis silking interval under optimum and for leaf senescence under drought.

The coefficients of additive genetic variability which estimate narrow sense heritability (h^2) were higher for anthesis silking interval, ears per plant and leaf senescence under drought compared to optimum environments, while for grain yield, anthesis date and plant height heritability reduced under moisture stress. There was a general decline in heritability from per plot values to across environment values in all traits except for anthesis-silking interval, ears per plant and leaf senescence under moisture stress conditions.

Table 5. Across sites mean squares for grain yield and secondary agro-morphological traits under moisture stress and optimum conditions

Source of Variation	DF		GY		AD		ASI		EPP		PH		SEN	
	Drt	Opt	Drt	Opt	Drt	Opt	Drt	Opt	Drt	Opt	Drt	Opt	Drt	Opt
Environment	3	5	96.47**	258.95**	4695.95**	8814**	254.06**	67.73**	4.92**	0.51**	3075.2**	9332**	2151**	714.1**
Rep/ Env	4	6	2.61*	8.19*	81.72**	68.73**	8.30	3.53*	0.114*	0.28**	804.54**	723.04**	1.34*	0.99
GCAf	9	9	4.09**	14.93**	31.25**	15.81**	5.28	6.05*	0.04	0.06	1055**	1728.6**	1.04*	0.54
GCAm	4	4	4.25**	19.82**	24.79**	85.11**	1.52	1.82*	0.09*	0.02	1043.7**	2688.3**	2.29*	6.45*
GCAf x Env	27	45	0.79*	3.44**	14.02**	3.89**	6.09*	1.91*	0.03	0.13*	299.78	288.53*	0.63*	0.51
GCAm x Env	12	20	0.57	0.77	10.24*	0.00	10.94**	1.08	0.06	0.14*	114.50	333.71*	1.78*	0.00
SCA	36	36	1.36**	10.34**	12.35*	19.58**	4.15	1.94*	0.05	0.18**	665*	781.61**	0.29	1.05*
SCAxEnv	108	180	0.93**	2.04**	7.16	0.68	2.81	0.98	0.15*	0.13*	235.89	209.95	0.24	0.25
Error	196	294	0.475	1.364	5.743	2.249	3.765	0.722	0.085	0.022	170	191.85	0.265	0.578
Per Plot h ²			0.331	0.459	0.460	0.976	0.142	0.148	0.11	0.007	0.683	0.825	0.604	0.516
Baker's Ratio			0.554	0.336	0.515	0.366	0.169	0.474	0.334	0.076	0.353	0.478	0.441	0.142
Across Env h ²			0.154	0.271	0.269	0.357	0.245	0.070	0.247	0.000	0.268	0.394	0.577	0.160

* = P < 0.05; ** = P < 0.01; *** = P < 0.001 ; Drt = Drought ; Opt = Optimum ; GY = grain yield; AD = Anthesis Date; ASI = Anthesis -Silking Interval; EPP = Ears Per Plant; SEN = Leaf Senescence; PH = Plant Height

4.4.3 Pearson's Correlation coefficients of secondary traits and drought tolerance indices

Stress tolerance index, geometric mean productivity, mean productivity, yield index were significantly positively correlated to each other ($P < 0.01$) and positive correlations with total mean yield under both optimum (Y_p) and stress (Y_s) environments (Table 6). Anthesis silking interval had strong and negative correlation with total mean yield under stress, stability tolerance index, ears per plant. Ears per plant had significant correlation with total mean under stress, total mean yield under optimum and yield stability index but had strong negative correlation with anthesis silking interval. Yield stability index had significant negative correlation ($r = -0.62^{**}$) under optimum but significant and positive correlation (0.431^{**}) under drought. Yield stability and stress susceptibility indices had a perfect negative correlation (-1.00^{**}).

Grain yield under drought was significantly ($P < 0.01$) correlated to SCA and ($P < 0.05$) to plant height, ears per plant while negatively correlated to anthesis silking interval ($r = -0.38^*$) and leaf senescence ($r = -0.63^{**}$) (Table 7). Anthesis-silking interval was positively correlated to leaf senescence ($r = 0.29^*$) under drought. Grain yield was positively correlated to ears per plant ($r = 0.34^*$) and plant height ($r = 0.65^{**}$) and SCA ($r = 0.80^{**}$). SCA and mid parent heterosis (MPH) were positively correlated ($r = 0.42^{**}$) and both were significantly ($P < 0.01$) correlated to ears per plant. Grain yield and SCA had positive and significant ($P < 0.01$) correlation across both environments while grain yield and MPH had a non significant correlations ($r = 0.11$) under optimum and ($r = 0.13$) under drought conditions. Comparison across the two environments shows that grain yield and leaf senescence had changes in correlation direction with ($r = 0.27^*$) under optimum to ($r = -0.63^{**}$) under drought conditions.

Table 6. Correlation coefficients between optimum and moisture stress environments mean yields, secondary traits and drought tolerance indices

	Yp	Ys	SSI	STI	TOL	GMP	MP	YI	YSI	ASI	EPP	SEN
Yp	1.000											
Ys	0.298*	1.000										
SSI	0.618**	-0.433**	1.000									
STI	0.732**	0.848**	0.001	1.000								
TOL	0.849**	-0.251	0.866**	0.273*	1.000							
GMP	0.762**	0.841**	0.072	0.985**	0.308*	1.000						
MP	0.913**	0.662**	0.299*	0.938**	0.560**	0.958**	1.000					
YI	0.296*	1.000**	-0.435**	0.847**	-0.253	0.839**	0.660**	1.000				
YSI	-0.62**	0.431**	-1.000**	-0.002	-0.866**	-0.074	-0.302*	0.433**	1.000			
ASI	-0.008	-0.379**	-0.527**	-0.144	-0.379**	-0.230*	-0.300*	-0.006	0.528**	1.00		
EPP	0.356**	0.282**	-0.472**	0.273*	0.260*	0.360*	0.357**	0.182	0.471**	-0.405**	1.00	
SEN	-0.227	-0.117	-0.141	-0.179	-0.165	-0.205	0.229	-0.116	-0.135	-0.077	-0.16	1

* = P<0.05; ** = P<0.01 Yp: Total mean yield under optimum, MP: Mean Productivity, Ys: Total mean yield under stress, YI: Yield Index, SSI: Stress Susceptibility Index, YSI: Yield Susceptibility Index, STI: Stress Tolerance Index, ASI: Anthesis Silking Interval, TOL: Drought Tolerance Index, EPP: Ears per Plant, GMP: Geometric Mean Productivity, SEN: Leaf Senescence

Table 7: Correlation of hybrids grain yield and secondary agro-morphological traits under optimum and managed drought conditions

	GY	AD	ASI	EPP	PH	SEN	SCA	MPH
GY	1.00	0.22	-0.14	0.34*	0.65**	0.27*	0.80**	0.11
AD	-0.18	1.00	0.47**	-0.53**	-0.06	0.47**	-0.38**	-0.42**
ASI	-0.38*	0.31*	1.00	-0.27*	-0.17	0.09	-0.17	-0.06
EPP	0.45*	-0.13	-0.43**	1.00	0.31*	-0.12	0.42**	0.39**
PH	0.24*	0.23*	-0.17	0.17	1.00	0.29*	0.59**	0.13
SEN	-0.63**	-0.12	0.29*	-0.32*	-0.19	1.00	0.12	0.25*
SCA	0.69**	-0.02	0.03	-0.15	0.38*	-0.28*	1.00	0.42**
MPH	0.13	-0.02	-0.04	0.24*	0.47**	-0.33*	0.47**	1.00

Optimum Environments (Above); Drought Environments (Below); * P<0.05; ** P< 0.01; ***P<0.001. GY = grain yield; AD = anthesis date; ASI = anthesis -silking interval; EPP = ears per plant; SEN = leaf senescence; PH = plant height; SCA = specific combining ability; MPH = mid parent heterosis.

4.4.4 Across environments testcross hybrids performance and stress related indices

Under moisture stress conditions the best yielding testcross hybrid was Line 2 x Tester 2, while the worst was Line 10 x Tester 3. Line 2 and Tester 1 had positive effects on grain yield performance of the resultant testcross hybrids as each contributed 3 of the best 5 best yielding testcross hybrids (Table 8). The significant and negative GCA values for Line 4 and Tester 5 had some negative effects on grain yield performance. Positive correlation between grain yield and SCA can be seen where best yielding hybrids had positive SCA values while poor performing hybrids had significant negative SCA values. However mid parent heterosis trend in relation to grain yield was undefined in the best or worst performing hybrids.

Table 8: Grain yield performance of testcross hybrids and their respective stress indices responses under drought conditions

Rank	Testcross	Grain Yield Drought					Grain Yield Optimum				
		(t/ha)	GCAf	GCAm	SCA	MPH	(t/ha)	STI	YSI	TOL	
1	L2xT2	3.61	0.42	0.06	0.30	105	7.45	0.55	0.48	3.84	
2	L2xT4	3.56	0.42	-0.02	0.31	47	7.90	0.57	0.45	4.34	
3	L8xT1	3.52	-0.16	0.33	0.52	38	7.04	0.50	0.50	3.52	
4	L7xT1	3.51	0.52	0.33	-0.17	- 9	7.93	0.57	0.44	4.42	
5	L2xT1	3.44	0.42	0.33	-0.14	30	5.90	0.41	0.58	2.46	
46	L4xT5	2.04	-0.23	-0.32	-0.26	82	4.70	0.20	0.43	2.66	
47	L10xT1	2.03	-0.64	0.33	-0.49	-46	5.36	0.22	0.38	3.33	
48	L3xT5	1.98	0.05	-0.32	-0.60	6	5.93	0.24	0.33	3.95	
49	L4xT2	1.96	-0.22	0.05	-0.71	70	6.71	0.27	0.29	4.75	
50	L10xT3	1.47	-0.64	-0.06	-0.67	-53	2.87	0.09	0.51	1.4	
Mean		2.84					7.01				
SE		0.08	0.13	0.08	0.29						

T3 had a positive effect on GY performance of the testcross hybrids since it contributed in 3 of the best 5 yielding hybrids under optimum conditions (Table 9). On the contrary Tester 5 had a negative effect on GY performance of hybrids under well watered environments. Poor performing hybrids had negative GCA and SCA values while good yielding hybrids had positive GCA and SCA values.

Yield stability index showed a general decline from the best to the worst yielding testcross hybrid except for Line 10 x Tester 3 which had a high yield stability index due its very low yielding nature under optimum conditions. Testcross hybrids with high stress tolerance index had good grain yield performance and those with low stress tolerance index had poor grain yield performance. A negative trend between yield stability index and tolerance index was

observed where a high yield stability index showed a low drought tolerance index and vice versa.

Table 9: Grain yield performance of testcross hybrids and their stress indices under optimum conditions

Rank	Testcross	Grain Yield Optimum				Grain Yield Drought				
		(t/ha)	GCAf	GCAm	SCA	MPH	(t/ha)	STI	YSI	TOL
1	L3xT3	8.53	0.63	0.26	0.63	74	3.09	0.54	0.36	5.44
2	L3xT4	8.46	0.63	0.14	0.68	48	3.40	0.59	0.40	5.06
3	L7xT3	8.34	0.61	0.26	0.46	92	3.38	0.57	0.41	4.96
4	L4xT1	8.16	0.01	0.08	1.07	190	3.31	0.55	0.41	4.85
5	L4xT3	8.09	0.01	0.26	0.82	184	3.03	0.50	0.37	5.06
46	L10xT1	5.36	-1.11	0.08	-0.61	48	2.03	0.22	0.38	3.33
47	L5xT5	5.35	-0.37	-0.76	-0.53	156	2.36	0.26	0.44	2.99
48	L1xT5	5.30	-0.09	-0.76	-0.86	104	2.05	0.22	0.39	3.25
49	L4xT5	4.70	0.01	-0.76	-1.57	182	2.04	0.20	0.43	2.66
50	L10xT3	2.87	-1.11	0.26	-3.28	- 21	1.47	0.09	0.51	1.40
Mean		7.01					2.84			
S.E.		0.19	0.23	0.07	0.35					

4.4.5. Stability analysis using additive main effects and multiplicative interaction

AMMI analysis of variance for the testcross hybrids and four checks and the parental inbred line were done separately but are presented in one table as shown in Table 10. In the hybrid trials the environment relative magnitude was higher than the genotype effect, with 34.1% of the total sum of squares attributable to environment effects, 9.9% to genotypic effects and 56% to genotype x environment effects (Table 10). The two interaction components IPCA1 and IPCA2 were significant ($P < 0.01$) and explained 68.9 % of the interaction.

Table 10: Across environments AMMI analysis of variance for parental lines, testcross hybrids and checks

Source of Variation	DF		SS		MS		Explained %	
	H	I	H	I	H	I	H	I
Environment (E)	9	9	215.55	11.18	23.95***	1.2425*	34.1	15.2
Genotype (G)	53	14	63.22	19.47	1.1928**	1.391*	9.9	26.4
E x G	477	126	354.39	43.09	0.7429*	0.342*	56.0	58.4
IPCA 1	158	22	161.33	23.98	1.0211*	1.0901*	45.5	55.7
IPCA 2	126	20	83.13	9.01	0.6598*	0.4504*	23.4	20.9
Residual Error	193	84	109.93	10.10	0.5696	0.1203	31.1	23.4

H = Testcross hybrids I: Parental inbred lines

The magnitude of genotype x environment sum of squares was 5.6 times more than that of genotypes indicating that there were distinct differences in genotypic response across environments. However under parental inbred lines, sum of squares attributable to genotypic effects (26.4%) were greater than the environmental effects (15.2%) with genotype x environment contributing 58.4%.

The drought and optimum environments were orthogonal and clustered separately (Figure 7). The four managed drought stress sites (E3, E4, E5 and E8) were clustered on their own with a site average of > 2t/ha. The drought sites were also positively correlated as seen by the relative acute angles from the origin. Environments E1, E2, E7 and E9 were clustered together as high yielding environments under optimum conditions and were also positively correlated.

Genotypes with IPCA scores near zero such as G15 and G18 had little interaction across environments, while genotype G48 had the highest yield in the best optimum environment E7 which is discriminated as indicated by the longest distance between its marker and the

origin. Genotypes G4, G22 and G27 were good under drought and optimum environments as they had positive IPCA scores in both environments. Hybrids had 68.9% of the genotype x environment interaction of the multiplicative interaction explained.

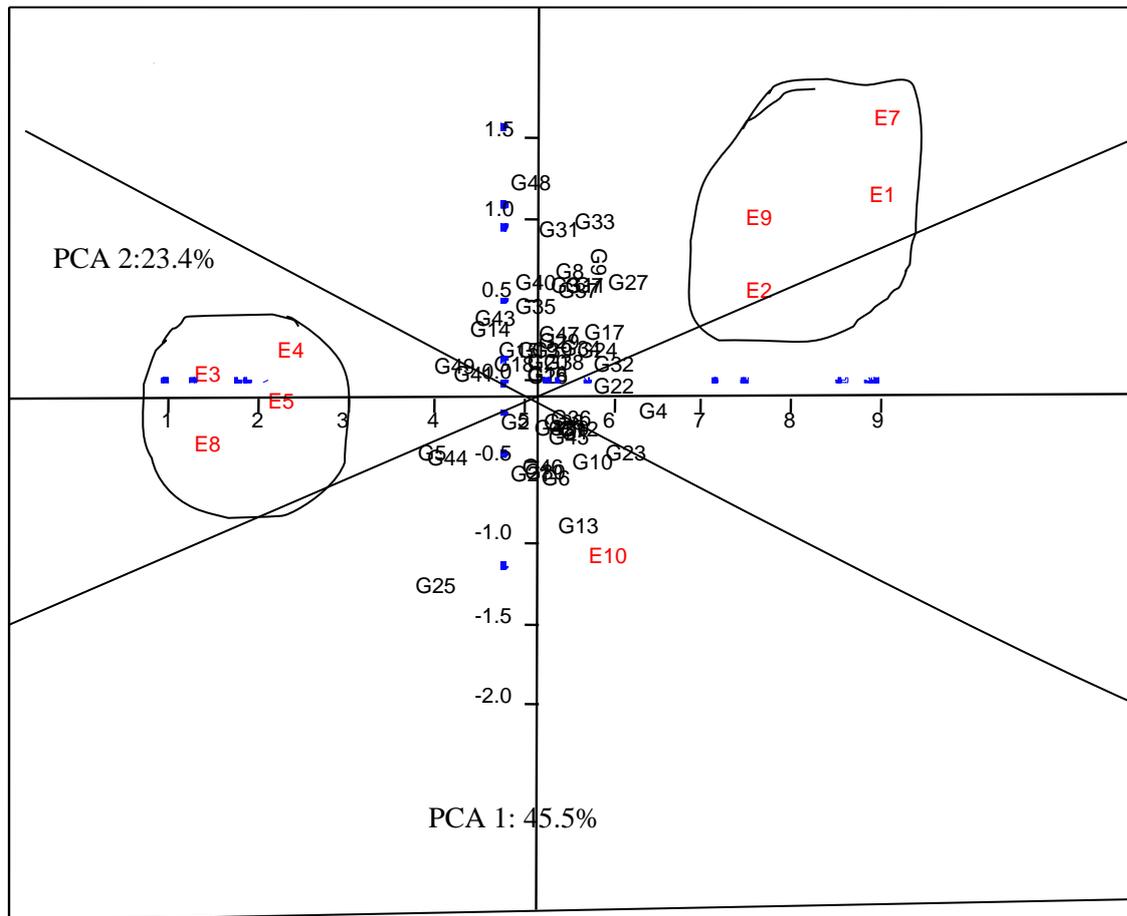


Figure 6: AMMI Biplot for genotype grain yield and across ten environments with 68.9% genotype x environment of the multiplicative interaction explained showing orthogonality and clustering of moisture stress (encircled left) and non stress (encircled right) environments.

4.5 Discussion

4.5.1 Gene action under drought and optimum conditions

The positive correlation of GCA values and grain yield performance under drought conditions shows that GCA values can be used to estimate contribution of inbreds in hybrid combinations targeting good yield performance under drought conditions. In this study tester 1, line 2 and line 7 can therefore be used in developing drought tolerant hybrids. Anthesis dates were significantly reduced implying hastened physiological maturity. Early maturity implies a shorter growing cycle for genotypes under moisture stress hence the reduction in yield. This early maturity might also imply drought escape or avoidance more than drought tolerance *per se*. A significant correlation of grain yield with plant height in both environments implies significant genotype x environment effects, showing inbred lines responded differently to above ground plant biomass accumulation. GCA values can act as indicators to trait performance and therefore when one considers grain yield, anthesis to silking interval, ears per plant and leaf senescence GCA values across the environment inbred lines T1 and T2 were the best line testers, whereas inbred line L2 and L7 were the best among the female lines. These lines can be used in the development of drought tolerant hybrids or segregating population for better adapted inbred lines.

4.5.2 Genotype x Environment Interaction (GEI)

Grain yield GCAM, GCAf and SCA significant effects show that additive and non additive effects are important under both optimum and managed drought conditions. Thus parental selection and combination for drought or optimum environment yield performance can exploit both non additive and additive gene affects. The significance of SCA x environment interaction for grain yield, under both environments implies that testcross hybrids responded differently to moisture stress and optimum conditions. The environmental influence on genotype performance therefore calls for need of separation of interaction factors through

other means other than normal analysis of variance. Significant GCAf x environment might suggest maternal influence in the grain yield response of hybrid in both environments. In addition grain yield per plot heritability was higher than across environments implying that more grain yield heritability precision is obtained with an across environment analysis. The Baker's ratio for grain yield was significantly higher under drought implying that additive gene action was predominant for this trait under drought. This finding is also confirmed by the GCA: SCA variance ratio which was greater than 1 with similar findings from Araus *et al.* (2008) and Derera *et al.* (2008) working on subtropical maize germplasm.

Significant GCAm of anthesis date under drought and its non-significance under optimum conditions imply that additive paternal effects had a bearing to maturity under drought conditions. A higher anthesis date heritability estimate under optimum conditions shows that male flowering prediction can be done more effectively under optimum conditions where a per plot heritability of 97.6% was recorded. Across site anthesis date heritability declines due to high environmental variability errors while Baker's ratio shows that additive gene effects for this trait increase with stress intensity.

The significance of GCAm and GCAf interaction with environment and non-significance of SCA and SCA x environment under drought for anthesis silking interval implies that additive gene effects control this trait. Anthesis silking interval heritability was higher under moisture stress conditions implying a higher additive gene action contribution for the trait under drought. Across environments anthesis silking interval heritability was higher than per plot implying that higher precision of measuring this trait is obtained with an across site assessment. This implies that ASI can be used reliably as a proxy to yield under drought as its heritability increases with stress intensity hence selection and higher breeding progress can be realised.

Leaf senescence GCAM and GCAf significances under drought indicate that parental choice is important in determining functional stay green traits in genotypes under drought conditions since this trait is mainly controlled by additive gene effects. The Baker's ratio also shows that additive genes had the greatest contribution to the phenotypic variance of hybrids for this trait under stress conditions. This therefore implies that senescence can be reliably used for selection of genotypes under stress conditions.

Significance of ears per plant at ($P < 0.05$) for SCA x environment in both environment is an indication that additive and non additive (dominance and epistatic effects) contribute to genotype prolificacy. Increase in additive genetic variance for anthesis silking interval, ears per plant and leaf senescence with increase in stress intensity implies that breeding progress can be made when selecting for these traits under drought to produce genotypes that are drought tolerant. The significance of plant height GCAM and GCAf in both environments shows that parental choice has an effect on plant height. SCA effects for plant height were also significant in both environments indicating that both additive and non additive effects contributed to plant height of genotypes under study. However the non significance of SCA x environment for this trait shows that in this study environments did not influence plant height. This is however contrary to findings by Bänziger *et al.* (2006) and Cooper *et al.* (2006) who found a general reduction in height with increase in moisture stress.

4.5.3 Selection indices and trait correlations

Positive and significant correlation between total mean yield under stress (Y_s) and total mean yield under optimum (Y_p) implies that there were significant differential among genotypes to enable effective selection for drought tolerance. Ears per plant had significant and positive correlation with Y_s and Y_p , stress tolerance index and geometric mean productivity showing that this trait is reliable and can be used in determining genotype performance across both stress and non stress environments. In this study geometric mean

productivity was used because it is more reliable and robust since it's relative to genotype performance across environments and seasons.

Anthesis silking interval (ASI) had significant and negative correlation with total mean yield under stress and tolerance index an indication that this trait is reliable for use under stress environments as low values of ASI are ideal when selecting genotypes for stress tolerance. The anthesis silking interval to tolerance index negative relationship implies that ASI low value genotypes have low yield reduction under stress environments hence the two can be used in selection for genotypes that perform across environments. Anthesis silking interval and ears per plant were significant negatively correlated hence genotypes with high ears per plant values have low ASI values under moisture stress. The significantly negative correlation ($r = 0.433^{**}$) for stress susceptibility index and mean yield under stress moisture stress conditions shows that the plant environment has a decisive factor in yield performance. This further demonstrates that evaluation and reliability of the indices and the secondary traits used depends on the level or severity of stress under which the genotypes are exposed. Yield stability index and stress susceptibility index had a negative perfect correlation (-1.00^{**}) and implying the two indices can be used interchangeably depending on the direction of selection a breeder might want to follow.

A strong and negative association of grain yield with anthesis silking interval (ASI) under moisture stress implies that testcross hybrids with negative values for ASI had a good grain yield performance. Reduced or negative ASI values or protogynous testcrosses are desirable under drought stress as silking delay is the main cause of poor pollination, kernel setting and grain filling in maize under drought (Muraya *et al.*, 2006; Pswarayi and Vivek 2008).

Results show a very strong but negative correlation between grain yield and leaf senescence under moisture stress implying that direction of selection for these two traits is opposing,

showing that an increase in grain yield is a result of reduced leaf senescence. This concurs with results obtained by Zaidi *et al.*, (2004) and Moreno *et al.*, (2005) who found out that increased senescence led to reduced grain yield under drought conditions. Therefore reduced senescence under drought stress is a desirable selection condition as this may result in increased functional stay green and consequently more assimilates to ear and kernel formation. Under moisture stress conditions this negative association of grain yield with anthesis silking interval and leaf senescence is desirable for selection purposes.

A very strong and positive association between grain yield and SCA values implies that SCA has good predictive value for grain yield of F1 testcross hybrids in both environments. However there was a weak and insignificant association between grain yield and mid parent heterosis implying that in this evaluation mid parent heterosis could not be used as a predictor of grain yield performance. This finding is however contrary to Geetha (2001) and Amaregouda (2007) who found a strong correlation but were both working on Indian subcontinent germplasm though my finding is similar to Bänziger *et al.* (2004)'s work on subtropical germplasm. This might also imply that use of mid parent heterosis as grain yield performance predictor is germplasm source specific. A positive correlation of ears per plant to specific combining ability and mid parent heterosis implies same directional selection among the three traits and this is desirable for breeding and selecting genotypes under moisture stress conditions.

Specific combining ability and mid parent heterosis had a very strong positive correlation which implies that an improvement in selection for SCA which is a good grain yield predictor will result in an indirect improvement of mid parent heterosis of the hybrids under moisture stress environments. Another breeding and selection desirable outcome was the negative association of leaf senescence and SCA under moisture stress since grain yield performance will be boosted by increasing the functional stay green traits of the hybrids. Grain yield and

plant height were positively correlated suggesting that under moisture stress selecting for genotypes with increased plant biomass could result in improved grain yield performance.

A significant and positive correlation of anthesis date with leaf senescence and anthesis silking interval observed in this study is undesirable since it implies that late maturity results in increased leaf senescence and anthesis silking interval hence very limited selection can be done in late maturing genotypes under stress conditions. Reduction in maturity could also imply reduced grain yield, due to an early maturity grain yield reduction penalty. Ears per plant and grain yield had a very strong positive association implying that prolificacy and increased kernel number is ideal for increased genotype grain yield performance. This also implies that when ears per plant heritability, is high it can be used as an ideal proxy for grain yield under stress.

Grain yield had positive and strong associations with ears, SCA and plant height implying that the three were good predictors of grain yield under optimum conditions. SCA and MPH had strong positive correlation with ears per plant which confirms the use of ears per plant as a good grain yield proxy. The low correlation of grain yield and mid parent heterosis under stress might be due to the low inbred line genetic variability due to low grain yield and high error variability associated with moisture stress. This further confirmed that MPH had poor predictive value for grain yield performance of testcross hybrids under study in both environments. A change in correlation direction of grain yield and senescence across the 2 environments from a positive under optimum conditions to a significant negative under drought stress is a desirable condition for selection purposes. Increase in leaf senescence under optimum conditions can be explained by the translocation of assimilate from older lower leaves to the upper leaves and the ear but under moisture stress senescence is undesirable since increase in grain yield requires a genotype that has functional stay green characteristics to increase photosynthates for grain filling.

The general trend was that negative GCA values for both male and female resulted in poor grain yield performance of the testcross hybrids in both environments. This implies that grain yield performance can be predicted by the GCA of the parental lines. The two parents must have positive grain yield under drought stress conditions for the hybrid to have a good grain yield predictive value. In addition, SCA was negative for testcross hybrids involving inbred lines with the same genetic background. However the Line 2 x Tester 2 hybrid exhibited complementarity and positive additive x additive epistatic effects on grain yield. The testcrosses Line 4 x Tester 5 and Line 10 x Tester 3 had significant negative SCA effects in both environments and were among the worst grain yield performers in both environments. This therefore means that SCA was a good predictor of grain yield performance in testcross hybrids under study. Testcrosses Line 4 x Tester 5 and Line 10 x Tester 3 had poor grain yield performance due to the similarity in gene composition of the parental lines. This also helps to confirm that the parental lines are in the same heterotic group.

Stress tolerance index also had significant positive correlation with grain yield where testcrosses Good yielding hybrids had high stress tolerance index confirming this index can be used to select hybrids that have good grain yield performance under drought conditions. This implies that stress tolerance index is positively associated with grain yield performance and can be used as a reliable stress indicator when selecting for drought tolerance. In addition stress tolerance index is a more reliable predictor of grain yield than yield stability index as seen by the low yielding testcross hybrids in Table 8.

4.5.4 GEI and AMMI Stability Analysis

Orthogonality of the drought and optimum environments implies that selection of hybrid performance criteria and hybrids selected under optimum are not usually the same under drought conditions. The clustering of the drought environments and acute angles separating them shows that the sites were positively correlated and almost similar for stress intensity to

warrant an across site analysis. The hybrid G4 (Line 7 x Tester 1) was stable and high yielding across environments. This testcross had both parental lines with positive and significant GCA values for grain yield under drought. This implies that there is a possibility of selecting testcross hybrids that are high yielding in both environments. However for high grain yield performance under moisture stress crosses using inbred lines with significant positive GCA values under moisture stress are made. This is also confirmed by the poor grain yield performance of the hybrids Line 4 x Tester 5 (G25) and Line 10 x Tester 3 (G49) which had parents with significant negative GCA values for grain yield.

The SCA values for hybrid G4 were positive and significant since Line 7 (heterotic B group) x Tester 1 (heterotic A group) are divergent group and hence heterosis was exploited explaining the high yields and stability across environments. G27 (Line 6 x Tester 2) had none significant GCA values for the parents across the 2 environment but had high yields under optimum conditions. The hybrid G27 exploited the heterosis of Line 6 (B group) x Tester 2 (A group) but was only adapted to high yielding well watered environments. This might imply that divergent line heterosis can be exploited resulting in poor general combiners having good specific combiners under well watered environments.

4.6 Conclusion

Stress tolerance index and geometric mean productivity were the most reliable among the selection indices used in this study and therefore we recommend that they be used in selection. Stress susceptibility index and tolerance index had a negatively significant correlation with the yield under stressed conditions hence drought tolerant genotypes can be selected based on either higher values of stress tolerance index, mean productivity and geometric mean productivity or lower values of stress susceptibility and tolerance indices. In this study phenotypic variation explained by additive genetic effects increased with increase in severity of stress. Additive effects were more important under drought conditions as shown by the consistent increase of additive variance contribution for anthesis silking interval, ear per plant, and leaf senescence from optimum to drought stress environments. Anthesis silking interval, ears per plant and leaf senescence had higher across environment than per plot heritability estimates. Thus the three traits can reliably be used for selection of genotypes under stress conditions. However the severity of the stress (selection intensity) will also determine the level of contribution of the different traits and the usefulness of a given index.

The study showed that there is a change in correlation direction of grain yield and leaf senescence across the 2 environments from a positive under optimum conditions to a significant negative under drought stress for a desirable selection outcome. SCA had good while mid parent heterosis had poor predictive values for grain yield performance of testcross hybrids in both moisture stress and non stress environments.

Overall, this study shows that it is possible to develop hybrids that are both drought tolerant and respond positively giving good grain yield under optimum or well watered environments. Line 2 x Tester 2 testcross hybrid exploited the additive x additive epistatic effects resulting in high performance in both environments.

4.7 Recommendations

Stress tolerance index and geometric mean productivity were the most reliable indices for use under drought stress conditions. The two indices together with secondary traits (anthesis silking interval, ears per plant and leaf senescence) can be used in tandem for increased genotype selection efficiency under drought.

This L2 x T2 testcross hybrid is therefore recommended for use as a single cross tester in the A heterotic group or for generation of even better drought tolerant inbred lines through conventional means or using marker assisted selection.

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CHAPTER FIVE

5.0 MAPPING OF QUANTITATIVE TRAIT LOCI FOR MOISTURE STRESS RELATED SECONDARY TRAITS

5.1 Introduction

Drought or moisture stress limits maize production, reducing crop yields especially in water-limited areas where many of the world's poorest farmers are found. As a result, development of drought tolerant maize varieties can contribute to food production and improved livelihoods of farmers in these areas. Conventional breeding approaches are the main crop improvement approaches but take long time for product development (Andjelkovic and Thompson, 2006). One strategy for breeding complex traits such as drought requires the dissection of the trait into a number of physiological component traits, that have simple genetic basis (Kulwal *et al.*, 2011). The complex trait is then introgressed via improvement of component traits. In the case of drought tolerance in maize, use of secondary morphological traits have been explored through genetic dissection of QTL associated with yield components as well as secondary morphological traits of interest such as anthesis-silking interval, plant height and many others (Semagn *et al.*, 2006c).

Several loci on different chromosomes are involved in the expression of grain yield and secondary traits for drought tolerance in maize. Mapping of QTL will elucidate contribution of such loci and this will improve breeding efficiency. The mapping and tagging of QTL for drought tolerance in temperate maize has been reported in many studies (Li *et al.*, 2003, 2004; Lu *et al.*, 2006). This study was done to validate the existence and eventual use of similar QTL in lowland tropics and sub tropical mid altitude maize germplasm used in Mexico and sub Saharan Africa respectively.

Tagging of QTL using molecular markers facilitates introgression of such loci from the donors using marker assisted backcross selection or marker assisted recurrent selection. Ribaut *et al.*, 2006 cited by Guo *et al.*, 2008 reports four genomic regions were identified for the expression of both grain yield and the anthesis -silking interval. In three of these regions, the allelic contributions were for short anthesis silking interval and an increase in grain yield. Mapping of most secondary traits is done under different circumstances and for different purposes. In the present study, mapping of quantitative trait loci (QTL) for grain yield and its secondary morphological traits such anthesis date (AD), anthesis silking interval (ASI), ears per plant (EPP), leaf senescence (SEN), stem lodging (SL), root lodging (RL) and plant height (PH) were done with the aim of utilizing the putative QTL in advanced cycles of selection using marker assisted recurrent selection (MARS).

5.2 Materials and Method

5.2.1 Plant Materials

In this study the QTL for moisture stress related traits such as anthesis date, plant height, ears per plant, anthesis silking interval, leaf senescence, and diseases of economic importance in maize namely maize streak virus, grey leaf spot (*Cercospora zea maydis*), maize ear rots and grain yield were characterized with F2:3 family lines derived from the cross between CML505 (P1) and CZL00009 (P2) both moderately drought tolerant lines under managed moisture stress and well-watered regimes.

5.2.2 Phenotypic evaluation

The F2:3 population and the testcrosses were evaluated in the winter rain free season under managed moisture stress and well watered conditions at Chiredzi and Muzarabani Research Stations in Zimbabwe. A total of 1000 F2:3 plants were planted under managed moisture stress and the two inbred line parents were also included in the field evaluation. Selected 252

F3 families were testcrossed using single cross testers of the opposite heterotic group. The hybrids were phenotyped under managed drought stress and well watered (optimum) growing conditions and genotyped using the 1536 SNP marker assay (<http://www.KBioscience.co.uk>)

The SNPs were later reduced to 300 SNPs specific for this study. Drought tolerance of genotypes was assessed after withdrawal of irrigation for four weeks before and after flowering with water withdrawal being informed by the maturity of the inbred parental lines, and only survival irrigation being given until harvesting. The F2:3 testcross families were planted using an alpha lattice design with 2 replicates each trial under managed drought and well watered conditions. An entry plot consisted of one 4-m row with a 0.75m space between rows and 0.25m within row. Morphological traits such as anthesis date (AD), silking date (SD) (50% flowering), plant height (PH) in cm, ear height (EH) in cm, percentage plant stem lodging (SL) percentage plant root lodging (RL), maize streak virus (MSV) score, were recorded while derived traits such as anthesis silking interval (ASI) in days, number of ears per plant (EPP) and grain yield (GY) in t/ha were computed with grain yield

being adjusted for moisture at 12.5%.

5.3 Data Analyses

Grain yield and secondary traits were evaluated on a plot basis under the managed moisture stress and well watered conditions. A test for data normality was done using the SAS PROC UNIVARIATE programme before doing PROC GLM using SAS version 10.1 for the general analysis of variance

5.3.1 Molecular evaluation and QTL Analysis

An F2:3 population derived from a cross CML505 and CZL00009 both moderate drought tolerant lines was used to construct the genetic linkage map using 278 SNP markers. A total

of 252 F2:3 testcross families were evaluated for drought response under managed drought stress in a rain free season as well as under optimum conditions.

DNA extraction was done by harvesting 10 healthy plants per family and bulked about 3 weeks after planting at Kiboko research station. The samples were stored in perforated polythene self sealing bags and kept cooled on dry ice in a Styrofoam box. The leaf samples were then transported to Biosciences for East Africa (BecA) hub in Nairobi, cut into pieces and transferred into 1.2ml strip tubes that contained two 4mm stainless steel grinding balls (Spex CetriPrep USA). The leaf samples were then freeze dried for 4 days using a Labconco freeze dryer (Labconco corporation, Kansas City) (<http://www.labconco.com>) as described in the user manual. The lyophilized leaf samples were ground into fine powder at 1500 strokes per minute for 2 minutes using GenoGrinder 2000. Genomic DNA was extracted using a modified version of the high throughput mini prep Cetyl Trimethyl Ammonium Bromide (CTAB) method (Mace *et al.*, 2003). The quality of the isolated DNA was checked after running aliquots of DNA samples on a 0.8% agarose gel that contained 0.3µg/mL Gel-red-Biotium. DNA concentration was measured using NanoDrop-ND-1000 Spectrophotometer, (Thermo Scientific, Wilmington, DE 19810, USA)

SNP genotyping was done on 248 testcross families as referenced on <http://www.KBioscience.co.uk> website. The linkage analysis was done using JoinMap, version 4. The mapping of QTL and estimation of their effects were performed using composite interval mapping (CIM) by PLABQTL software (Utz and Melchinger, 2003). The composite interval mapping approach was conducted using the following options: automatic cofactor selection, model to determine additive effects at individual QTL and additive x additive epistatic interactions as implemented in PLABQTL. In this study, QTL that explained < 10% and > 10% of the total phenotypic variation were classified into minor and major QTL, respectively with a likelihood of odds (LOD) score of 3 being used for declaring a putative

QTL significant as determined after 1000 permutation tests (type I error level $\alpha = 10\%$).

Genetic maps and QTL graphs were drawn using the MapChart

(Voorrips, 2002)

5.4 Results

The traits exhibited continuous variation in both well watered (optimum) and managed moisture stress conditions. Log transformation was done for stem lodging (SL), root lodging (RL) and maize streak virus (MSV) which were not normally distributed.

5.4.1 Mapping of QTL

A total of 278 SNP markers on 248 testcross families were polymorphic and were assigned to the 10 linkage groups (chromosomes) that covered 1238.8 cM giving an average interval length of 4.6 cM. The eight traits assessed recorded a total of 31 significant QTL on eight of the ten chromosomes and are summarized in Tables 11- 13. One to eight QTL were detected for each trait with each QTL having a phenotypic variance range of 5.5 % to 19.9%. One QTL position on chromosome seven (7) mapped for both anthesis date and stem lodging.

5.4.1.1 QTL for grain yield

A total of three QTL were detected on chromosome 5 and 7 (Table 11). Under optimum conditions a major QTL contributing 11.4% of the phenotypic variation was found on chromosome 5 with P2 being the parental source. This QTL is constitutive and stable as it was also found under stress albeit with a reduced effect. The moisture stress conditions stimulated two minor QTL with a combined phenotypic variance of 16.4%. These QTL were found on chromosomes 5 and 7 and both came from CZL00009 which is drought tolerant. The additive effect showed that these 3 QTL had a net effect of reducing grain yield.

Table 11: QTL influencing grain yield and its positively correlated traits in 248 F2:3 families

Trait/ Regime	Link Grp	Left Marker	Right Marker	Position +	LOD	Phenotypic Variance %	Parental Source
Grain Yield							
Moist	5	Pza01371_2	Pza030092-7	8	6.45	11.4	-0.082
Dry	5	Pza01796_1	Pza02641_2	46	5.07	9.0	-0.116
	5	Pza01371_2	Pza030092-7	28	3.24	5.9	-0.017
	7	Pza06116_13	Pza63583_1	38	4.12	7.4	-0.017
Anthesis Date							
Moist	2	Pza01991_3	Pza01352_5	154	3.06	5.5	-0.324
	5	Phm12992_5	Pza00222_7	34	6.76	11.8	-0.448
	7	Pza02722_1	Pza02260_2	64	3.09	5.6	-0.316
	8	Pza00460_8	Pza00904_1	102	3.13	5.7	+0.304
Dry	2	Pzb00901_4	Phm6111_5	50	4.65	8.3	-0.513
	2	Pza02680_1	Pza02453_1	144	3.02	5.5	-0.370
	4	Phm687_25	Pza02457_1	46	4.26	7.6	-0.437
	5	Phm12992_5	Pza00222-7	34	3.33	5.6	-0.374
	7	pza02722_1	Pza02260_2	64	3.10	5.6	-0.387
	8	Pza00429_1	Pzb01454_1	78	3.48	6.3	+0.304
Ears Per Plant							
Dry	1	Phm5306_1	Pza00944_1	66	3.11	5.6	+0.043
Plant Height							
Moist	4	Pza01810_2	Pza02779_1	94	4.25	7.6	-2.770
	6	Pza00440_1	Pza03069_8	2	7.79	13.7	+4.099
Dry	1	Pza02186_1	Phm5293_11	122	3.18	5.7	+2.758
	5	Pza02633_4	Pza02383_1	62	3.36	6.1	+3.483

5.4.1.2 QTL for anthesis date

One major QTL explaining 11.8% of the phenotypic variation was found on chromosome 5 with the parental source CZL00009 under well watered conditions (Table 11). In addition CZL00009 contributed 2 other minor QTL that were on chromosome 2 and 5 with phenotypic variances of 5.5% and 5.6% respectively. CZL00009 also had a minor QTL found on chromosome 8. The 4 QTL explained a total phenotypic variance of 28.6%. However under moisture stress a total of 6 minor QTL were found deriving from CZL00009. Two of these QTL, were on chromosome 2 with chromosomes 4, 5, 7 and 8 contributing one QTL each. All the six QTL accounted for a combined phenotypic variance of 39.3%. CML505 contributed a minor QTL (6.3%) on chromosome 8 which increased male flowering under moisture stress conditions. The QTL on chromosome 5 and 7 are constitutive since they were found in both stress and non stress moisture conditions (Table 11).

5.4.1.3 QTL for number of ears per plant

One minor QTL contributing to 5.6 % of the phenotypic variance was found on chromosome 1 from CML505 under moisture stress conditions with no ears per plant QTL being found under optimum well watered conditions. This QTL increased the number of ears per plant ratio of the progenies.

5.4.1.4 QTL for plant height

A total of 4 QTL were observed for both water stressed and well watered regimes. In the well watered environment two QTL were found on chromosome 4 and 6. The QTL on chromosome 6 is a major QTL (13.7%) and was contributed by CML505. Chromosome 4 QTL came from CZL00009 and it reduced plant height. The water stress environment had 2 QTL obtained from chromosome 1 and 5 with both QTL coming from CML505. The total phenotypic variance of the 2 QTL under moisture stress was 11.8%.

5.4.1.5 QTL for anthesis siking interval

One QTL was found on chromosome 7 under optimum water condition, and it contributed 8.0 % of the phenotypic variance with P1 being the source (Table 12). Under water stress three minor QTL were found and these explained a total 17.7% of the phenotypic variation. Two of the QTL on chromosomes 4 and 6 are drought adaptive QTL, while the QTL on chromosome 7 is stable and constitutive.

Table 12: QTL influencing grain yield negatively correlated traits in 248 F2:3 Families

Trait/ Regime	Linkage Group	Left Marker	Right Marker	Position ⁺	LOD	Phenotypic Variance	Parental Source
Anthesis Silking Interval							
Moist	7	Pza00695_3	Pza01044_1	110	4.41	8.0	-0.374
Dry	4	Pza00030_2	Pza00193_2	90	3.53	5.6	-0.058
	6	Pza01029_1	Iac1_3	36	3.32	6.0	-0.713
	7	Pza00695_3	Pza01044_1	110	3.76	6.1	-0.134
Leaf Senescence							
Dry	1	Pza02284_1	Pza03551_1	30	3.30	6.0	+0.096
	2	Pza02727_1	Pza03577_1	176	3.22	5.9	-0.090
Shoot Lodging							
Dry	1	Pza03741_1	phm759_24	116	4.23	7.6	+2.612
Dry	7	Pza02722_1	Pza02260_2	60	3.21	5.8	-5.183
Root Lodging							
Dry	5	Phm13942_7	Phm4647-8	32	4.83	8.6	-3.320

Position⁺: QTL peak position on the respective chromosome in centimorgan (cM)

5.4.1.6 QTL for leaf senescence

Two QTL with a phenotypic variance of 11.9% were identified on chromosomes 1 and 2 coming from CZL00009 and CML505 respectively. The QTL on chromosome 1, increased leaf senescence while that on chromosome 2 reduced senescence under drought conditions.

5.4.1.7 QTL for stem and root lodging

Two QTL for plant lodging contributed a total 13.4% of the phenotypic variance were found on chromosome 1 and 7 under water stress. However the QTL on chromosome 1 came from CML505 and it resulted in increased stem lodging while the QTL on chromosome 7 reduced stem lodging under water stress conditions. A QTL for root lodging resistance with a phenotypic variance of 8.6% was found on chromosome 5 coming from CML505 and had a reduction effect on root lodging.

5.4.1.8 QTL for disease resistance

Maize streak virus (MSV) resistance QTL was found on chromosome 8 and had a 6.4% phenotypic variance coming from CML505 (Table 13). A major QTL with 11.1% phenotypic variance for grey leaf spot (GLS) resistance was found on chromosome 3 and another with a 5.5% phenotypic was also found on chromosome 5 coming from CZL00009. A major (19.9%) and minor (5.8%) QTL for ear rots (ER) resistance were found on chromosome 7 and 1 respectively and both came from CML505.

Table 13: QTL influencing MSV, GLS and ER resistance in 248 F2:3 Families

Trait/ Regime	Linkage Group	Position	Left marker	Right marker	LOD	Phenotypic Variance%	Parental Source
Moist							
MSV	8	56	Pza00908_2	Pza02748_3	3.57	6.4	-0.049
GLS	3	16	Pza02098_2	Pza00210-9	6.29	11.1	+0.074
GLS	5	106	Pza01680_3	Pza02480_1	3.03	5.5	+0.053
ER	1	18	Phm13094_8	Pza02284_1	3.17	5.8	-1.133
ER	7	34	Pza00153_7	Pza00616_13	11.97	19.9	-2.478

Position⁺: QTL peak position on the respective chromosome in centimorgan (cM). MSV = maize streak virus; GLS = grey leaf spot (*Cercospora zea maydis*); ER = ear rots

5.5 Discussion

Understanding the genetic basis of drought tolerance in maize is critical in helping breeders identify or develop new methods of selection. Breeding using molecular markers can improve accuracy and efficiency of the process especially for complex traits (Wang *et al.*, 2003). The difference in the number and type of QTL identified in this study show that several putative regions were involved in the expression of traits under the two water conditions. In general, less than 25% of the reported QTL in this study were found in non stress conditions. Majority of the QTLs detected had small effects, explaining less than 10% of the phenotypic variation. However pyramiding of these small effects QTL in MARS will have an additive effect that will help improve these traits of interest. Cumulative effects of minor ASI under moisture stress was 17.7% and if these minor QTL are pyramided into parental inbred lines in subsequent cycles of selection using MARS marked genetic improvement could be achieved for germplasm response to drought stress.

Five major QTL were associated with grain yield, anthesis date, plant height, gray leaf spot and ear rots disease resistance. It should also be noted that all the five major QTL were identified under non water stress conditions. Similar findings have been reported (Li *et al.*, 2005 ; Guo *et al.*, 2008).

5.5.1 Grain yield and its positively correlated morphological traits

Negative anthesis date additive effect, implies that CZL00009 resulted in the reduction in the male flowering time under both stress and non stress conditions. Two constitutive QTL were observed on chromosome 5 and 7. Two QTL were also identified under stress conditions while there was a QTL co-localisation and/ or overlap on position 150 -154 of chromosome 2 coming from CZL00009 which resulted in reduction of male flowering time. Chromosome

8 QTL had positive additive effects, which shows that the QTL came from CML505 and it caused increase in male flowering time.

The QTL for number of ears per plant under water stress conditions had a positive additive effect implying its parental source was CML505. This QTL though minor increased the number of ears in the segregating lines. Under non stress conditions one major QTL for increased plant height was observed on chromosome 6 deriving from CML505. A negative additive effect QTL on chromosome 4 coming from CZL00009 resulted in reduced plant height under non stress conditions. Two QTL were found on chromosome 1 and 5 and they resulted in an increase in plant height coming from CML505 under drought stress.

5.5.2 Secondary traits negatively correlated to grain yield

Negative anthesis silking interval additive values for QTL under both stress and non stress conditions imply that all QTL resulted in the reduced values of this trait in their progenies and they were coming from CML505. This is a desirable selection conditions since anthesis silking interval is phenotypic negatively correlated to grain yield. Desirable genotypes are those that have smaller or negative anthesis silking interval values or those that exhibit protogyny. The most stable QTL across the 2 water regimes was detected on position 110 of chromosome 7. This implies that this QTL can be reliably used in reducing ASI using MAS methods such MABC or MARS.

Two QTL for leaf senescence were found on chromosome 1 and 2 under stress while non were observed under non water stress conditions. This implies that both QTL are adaptive since the genes are only triggered when plants are under moisture stress albeit the need for a larger QTL effect for such conclusions to be made. Putative QTL for standability traits of stem lodging and root lodging were only found under drought stress conditions. The 3 QTL were found on chromosome 1 and 7 for stem lodging and chromosome 5 for root lodging.

QTL on chromosome 5 and 7 had negative additive values implying they reduced lodging under moisture stress conditions.

All disease scores reported were recorded under non stress water conditions since there were no significant phenotypic differentials observed under drought stress. Two major QTLs for grey leaf spot and ear rots resistance were observed on chromosome 3 and 7 respectively. The grey leaf spot major QTL had positive additive effect implying that this QTL resulted in grey leaf spot susceptibility in the progenies. The major ear rots QTL came from CML505 and had negative additive effects meaning increased ear rot resistance to the progenies which a desirable trait. A minor QTL for maize streak virus resistance was from CML505 on chromosome 8. This QTL had a negative additive effect implying it's a desirable QTL which increases resistance to maize streak virus disease in progenies.

5.5.3 QTL co-localisation and clustering

There was co-localisation of anthesis date and stem lodging on position 60-64 of chromosome 7 under drought stress conditions. This co-locality of QTL for different traits implies possible presence of QTL pleiotropic effect or very close linkage between the QTL that control these traits. There was also some clustering of QTL on chromosome 5 for grain yield (28), root lodging (32) and anthesis date (34). Co-localisation and clustering are desirable since they signify the plural selection efficiency by selecting marker(s) that are closely associated with these traits. Similar results of clustering and co-localization in maize have also been reported by Tuberosa *et al.* (2005) and Messmer *et al.* (2009).

5.6 Conclusion

Several minor and major QTL for secondary morphological traits were detected under both water regimes using SNP markers. The presence of QTL for secondary morphological traits of anthesis silking interval, ears per plant and leaf senescence and their high heritability under

drought stress shows that these traits are reliable and may be used in any MAS program for improving drought tolerance and grain yield. Stability of QTL such ASI on chromosome 7 is desirable as it will enable their transferability or introgression in other backgrounds for drought tolerance improvements using conventional or molecular breeding methods such as MABC or MARS. Detection of QTL governing many traits is ideal as it increases the efficiency of MAS and consequently lead to breeding progress as it will enable their transferability or introgression in other backgrounds for drought tolerance improvements using conventional or molecular breeding methods such as MABC or MARS. Co-localisation and or clustering of QTL is also desirable since it signifies the plural selection efficiency by selecting marker(s) that are closely associated with these traits.

5.7 Recommendations

Therefore given the outcome in this study the stable anthesis silking interval QTL on chromosome 7, co-localised anthesis date and stem lodging QTL on position 60-64 of chromosome 7 and clustering of QTL on chromosome 5 on position 28 -34 for grain yield root lodging and anthesis date, maize streak virus QTL on chromosome 8 and ear rots QTL on chromosome 7, can be used in the MARS for pyramiding these traits in the subsequent generations thereby accumulatively improve trait overall effect and germplasm response to stress conditions. Since QTL allele effect can be influenced by genetic background of parent lines and association mapping cannot be employed in bi-parental crosses the use of adhoc selection indices in each MARS subsequent generation will help in improving the selection process. Uniform management of the moisture stress conditions and robust statistical analysis method also will need to be employed as these may also affect the range of the QTL position.

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CHAPTER SIX

6.0 GENERAL DISCUSSION

Drought tolerance in crop plants is an important trait especially for crop plants that are grown in drought prone environments. Drought tolerance in plants is conditioned by both physiological and behavioral attributes that improve resilience to adverse weather. Many crop varieties have been bred and released for adoption by farmers but their adoption has been slow due to a number of reasons that range from socioeconomic to biological differences in selection criteria between farmers. In this thesis, effort is made to investigate these challenges in order to develop better breeding strategy and potentially adopted varieties. These issues and their inter relatedness are presented and discussed below.

6.1 Morphological traits of importance to farmers for variety selection

In this study farmers were aware of the effects and challenges posed by drought to maize production. Farmers were also aware of the important traits that influence tolerance to moisture stress when selecting for drought tolerance in maize. Interestingly, farmers and breeder selection ranking of priority traits were different. Foliar diseases resistance such grey leaf spot and maize leaf blights are high priority traits of breeding programmes, yet this trait was ranked very low by farmers in the surveyed area. This discrepancy could be due to the fact that in the study areas folia diseases are not a major concern because the dry weather is not favorable for epidemics hence reduced or no disease pressure in the drought prone smallholder communities. Yet breeders in general consider diseases as major threats to production. The survey data also shows that farmers make decisions based on relative trait ranking and trait trade-off when selecting desirable varieties. Thus farmers appear to have a priority list that is used to guide selection and ultimately adoption of suitable varieties. Awareness of such a list or the issues that farmers consider critical can help breeders better

target variety development for specific agro-ecological needs and specifics. Similar findings have been reported in eastern Kenya (Groote *et al.*, 2002).

It should be noted that whereas hybrids yield higher than OPVs under ideal conditions, farmers in the dry areas of Mutoko preferred the ZM series of synthetics because they were more drought tolerant and therefore higher yielding than most of hybrids grown in this area. This finding shows that the universal drive to promote hybrid use by farmers irrespective of their adaptation to agro-ecological needs while noble, needs in depth situational analysis especially in dry and resource poor farmer communities where inputs such fertilizer use is minimal and yields are below 1 t/ha. Hybrids in general are designed for high input use agriculture and their nature limits robustness depending on parental background, whereas synthetics are more robust due to wide parentage and presumably robust genotype.

While some drought tolerant varieties have been developed by private seed companies the surveyed area showed that there is lack of such information by farmers hence the need for a public private partnership in packaging extension messages and variety catalogues for use by farmers, government extension agents and non-governmental organizations involved in agricultural activities. Farmers' emphasis on the need to improve maize for drought tolerance shows that drought tolerant varieties are currently either not satisfactory or non-existent. Overall, therefore, this study demonstrates the need for continued interactions between farmers and plant breeders as part of a process to improve success of breeding and adoption of drought tolerant crop varieties.

6.2 Gene action and breeding value of traits for genotypes selection

Ability to predict grain yield performance for stress environments such as drought is one of the major challenges to breeding. Prediction of yield has been advocated for due to low heritability values obtained especially under suboptimal conditions (Araus *et al.*, 2008). In

this thesis investigations were made to address this problem focusing on gene action, drought tolerance related morphological traits and yield. The data shows that grain yield GCA values and grain yield performance were positively and significantly correlated under drought conditions implying that GCA values can be used to estimate grain yield performance contribution of inbred lines. The significant and positive GCA values for grain yield and cobs per plant, are desirable for selection under stress and stress free environments, while negative GCA values for leaf senescence and anthesis silking interval are desirable in selection of drought stress tolerant parental genotypes. Tester 1 and 2 and line 2 in heterotic group A and line 7 in heterotic group B of maize were the best performing lines that could be used to develop within heterotic group drought tolerant testers or for development of even better drought inbred line.

In this study, significance of SCA x environment interactions for grain yield, under moisture stress and optimal environments implies that testcross hybrids responded differently to moisture stress and optimum conditions. This interaction is expected for quantitative traits such as tolerance to drought requires that confounding effects of the environment are elaborated via other means other than analysis of variance such as regression analysis or use of additive main effects and multiplicative interaction (AMMI) or genotype x genotype x environment (GGE) biplot methods (Bondari, 2003).

Among the morphological traits assessed, plant height and grain yield were the only traits with reduced heritability because estimated genetic effects for these traits have limited transferability in moisture stress environments. The results found are consistent with most work done under moisture stress where biomass is reduced due to increased partitioning of assimilates to grain formation rather than apical dominance (Banziger *et al.*, 2006 ; Messmer *et al.*, 2009).

Under drought there were no significant differences observed for disease among genotypes. This also helps to explain the non significance of this trait under farmer selection priorities as most diseases of economic importance in maize do not fully express in dry conditions. However heritability of leaf blight resistance was high implying that disease resistance can easily be incorporated into hybrids as a safety net as the resistance genes are either mono or oligogenic. In general therefore, when breeding for drought tolerance, small or negative anthesis silking interval, high ears per plant or reduced bareness, reduced senescence or increased functional stay green are the traits of choice. However breeders have also to take into consideration, the trait trade - offs which are specific to specific environments hence the need for ecosystem breeding.

6.3 Secondary morphological traits and selection indices correlations

Equally important for development of effective screening of maize to drought tolerance is the need to use effective secondary traits that are part of a plants mechanism to respond to drought stress. In this study, positive and significant correlation between total mean yield under drought stress and total mean yield under optimum condition was found, indicative of the fact that genotypes performed differently under sub-optimal and optimal environments to enable effective selection for drought tolerance. Anthesis date and grain yield were negatively correlated suggesting that late maturity reduced grain yield under moisture stress conditions. A significant and positive correlation of anthesis date to leaf senescence and anthesis silking interval (ASI) observed shows that late maturing plants will experience increased leaf senescence and ASI. As such ASI and anthesis date have limited use in late maturing genotypes under moisture stress conditions. This is undesirable since selecting for earliness results in reduced grain yield performance, due to an early maturity grain yield reduction penalty.

Anthesis silking interval had significant and negative correlation with mean yield under moisture stress showing that this trait is also reliable for use under water stress environments whilst low ASI values are ideal when selecting genotypes for moisture stress tolerance. In other studies it has been shown that reduced or negative ASI values or protogynous testcrosses are desirable under drought stress, because a delay in silking causes poor pollination, kernel setting and grain filling in maize under drought (Muraya *et al.*, 2006; Pswarayi and Vivek, 2008). Significant negative correlation of ASI and ears per plant found in this study is desirable for selection condition for superior genotypes under moisture stress conditions.

Ears per plant and grain yield had a very strong positive association showing that increased kernel number is ideal for increased genotype grain yield performance. This also suggests that when the number of ears per plant, has high heritability, it can be used as an ideal proxy for grain yield under stress. Significant positive correlation of the number of ears per plant with mean yield under stress and optimum, stress tolerance index and geometric mean productivity suggests that this trait is reliable and can be used to determine genotype performance under stress and non stress environments. Plant height and grain yield were positively correlated suggesting that under stress, selecting for genotypes with increased plant biomass improves grain yield performance.

Stress tolerance index had significant and positive correlation with grain yield in both environments, indicating its possible use it as a reliable stress indicator when selecting for drought tolerance. Geometric mean productivity was strong and positively correlated to grain yield, stress tolerance index and tolerance index suggesting that it can be used to identify genotypic differentials in the 2 environments. Geometric mean productivity is more reliable and robust since it's relative to genotype performance across environments and seasons hence can be used to predict yield performance. The significantly negative correlation for stress

susceptibility index and grain yield under drought conditions shows that environment can adversely affect yield. This further demonstrates that evaluation and reliability of the indices and the secondary traits used depends on the level or severity of stress under which the genotypes are exposed. Yield stability and stress susceptibility index had a negative perfect correlation and can therefore be used interchangeably depending on the direction of selection a breeder might want to follow.

Specific combining ability and mid parent heterosis had a very strong and positive correlation suggesting that the use of SCA which is a good grain yield predictor might result in an indirect improvement of mid parent heterosis of the hybrids under moisture stress conditions. The low correlation of grain yield and mid parent heterosis under moisture stress might be due to the low inbred line genetic variability due to low grain yield and high error variability associated with drought stress environments. The general trend was that negative GCA values for both male and female accounted for poor grain yield performance among the testcross hybrids in both environments. Thus grain yield performance can be predicted by the GCA of the parental lines. However, the two parents must have positive grain yield under drought stress conditions for the hybrid to have a good grain yield predictive value.

6.4 Quantitative trait loci mapping

In this study QTL were mapped using populations derived from bi-parental crosses. The aim was to map QTL for tolerance to drought among tropical maize germplasm to underpin marker assisted recurrent selection (MARS). Several QTL were mapped suggesting that several putative loci were involved in the expression of traits under the two water stress conditions. The mapped QTL had positive and negative additive effects implying that QTL allele effects could be influenced by genetic background of accessions used in the breeding programme. The QTL for anthesis silking interval (ASI) had negative additive values under

both stress and non stress conditions suggesting that these QTL reduced ASI values in the progenies. Given that ASI is phenotypic negatively correlated to grain yield, this finding is a desirable and suggests that it can and should be used as a selection index. The most stable QTL across the 2 water regimes was detected on position 110 of chromosome 7. These QTL can thus be reliably used when breeding confirming anthesis silking as a reliable selection trait for drought tolerance. Two QTL for leaf senescence were observed on chromosome 1 and 2 under moisture stress only as was QTL for stem and root lodging. These results show that these QTLs are adaptive in nature and are desirable for selection under drought stress and can be targeted for generation selection in the marker assisted recurrent selection.

All disease scores reported were recorded under non stress water conditions since there were no significant phenotypic differences observed under moisture stress conditions. Grey leaf spot major QTL with positive additive effect was detected implying that this QTL accounted for grey leaf spot disease susceptibility in the progenies. The major QTL for ear rots had negative additive effects meaning increased ear rot resistance to the progenies which a desirable trait. A minor QTL for maize streak virus resistance was found on chromosome 8 and it had a negative additive effect implying it's a desirable QTL which increases maize streak virus resistance to progenies hence ideal and desirable for selection.

This study found co-localisation of anthesis silking and stem lodging on position 60-64 of chromosome 7 under drought stress conditions. There was also clustering of QTL on chromosome 5 for grain yield (28), root lodging (32) and anthesis date (34). Co-localisation and clustering are desirable since they signify the plural selection efficiency by selecting marker(s) that are closely associated with these traits (Tuberosa *et al.*, 2005; Messmer *et al.*, 2009). In this study the co-localisation of anthesis date and stem lodging under moisture stress is ideal for selection since the two traits have negative additive effects implying reduced maturity and low stem lodging under drought conditions.

In general the desirable QTL of anthesis date, plant height, anthesis silking interval, leaf senescence, ears per plant, maize streak virus and ear rots can be exploited in further improving the progenies for drought tolerance and general agronomic performance using marker assisted recurrent selection where the desirable QTLs are pyramided in subsequent cycles. However, the study shows that no major QTLs were observed for the drought related traits of anthesis silking interval, ears per plant and leaf senescence. However since marker assisted recurrent selection will be used in subsequent selections, minor QTL can be pyramided to give significant trait effects in the final inbred lines.

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6.6 General Conclusions and Recommendations

6.6.1 Improving end user targeting of breeding programmes

This study shows that maize varieties currently on the market are not meeting the needs of the majority of the farmers in the drought stress prone environments. Recovery from stress, non abortion at grain filling when under moisture stress, duration of tassel and silk survival under stress and stay green characteristics are some of the attributes that inform farmers' choice of a drought tolerant variety. Contrary to breeders' high prioritization of disease resistance, the study showed that foliar disease tolerance was not high on the farmers' priority list of traits. Therefore breeders should engage farmers in participatory agro-ecology intensification in the target areas with farmer involvement in the early stages of the breeding process. This will increase farmer ownership of the process and improve adoption of the varieties. Consequently there should now be a shift from general breeding to eco-specific variety development targeting small communities or regions for effect results despite the costs involved.

6.6.2 Strengthening selection for drought tolerance

In this study phenotypic variation explained by additive genetic effects increased from optimum to drought stress conditions. Additive effects are more important under drought conditions as shown by the consistent increase of additive variance contribution for anthesis silking interval, ears per plant and, leaf senescence from optimum to moisture stress environments. Stress tolerance index and geometric mean productivity were the most reliable among the selection indices used in this study and therefore can be used in tandem with anthesis silking interval, ears per plant leaf senescence and SCA for one to make an informed decision on selecting the best and most ideal genotypes under moisture stress conditions. The study also showed that severity of the stress also determines the level of contribution of the different traits and the usefulness of a given index.

The predominance of GCA effects over SCA effects for the three secondary traits under moisture stress, confirmed they can be used for effective selection as proxies for grain yield under for both stress and non stress environments. GCA values of traits can be used for selection especially under moisture stress conditions making it possible to use inbred line information to predict hybrid performance under moisture stress. Reduced or negative anthesis values or protogynous genotypes are desirable under drought stress.

6.6.3 Mapping of drought tolerance loci to underpin marker assisted selection

In the study several minor and major QTL for secondary morphological traits were detected under both water regimes using SNP markers. Each of the measured traits was controlled by at least one QTL detected in one or both environments. The presence of QTL for secondary morphological traits of anthesis silking interval, ears per plant and leaf senescence and their high heritability under drought stress shows that these traits are reliable and can be used in any marker assisted selection programme for improving drought tolerance and grain yield. Stability of QTL such as ASI on chromosome 7 is desirable as it will enable their transferability or introgression in other backgrounds for drought tolerance improvements using conventional or molecular breeding methods such as marker assisted recurrent selection.

Detection of QTL governing many traits is ideal as it increases the efficiency of marker assisted selection and consequently breeding progress as it will enable their transferability or introgression in other backgrounds for drought tolerance improvement. Co-localisation and or clustering of QTLs was found for some traits and could be used for multiple trait selection.

Overall data generated from farmer trait preference, genetic control of traits and the QTL identified in the target traits can now be used in augmenting the development of drought stress tolerant inbred lines.

6.7 Future Perspectives

Maize breeding selection for smaller tassels and improvement of other morpho-physiological traits that have been neglected, such root architecture and size, efficiency of CO₂ fixation, and other physiological traits that influence availability of photosynthates and sucrose metabolism may help to increase grain yield under moisture stress environments in the near future (Varshney *et al.*, 2009). Indeed, carbohydrate metabolism can influence drought tolerance, because the diminished supply of carbohydrates to the developing floral and seed organs that occurs under drought reduces seed set and overall yield. Therefore dissecting carbohydrate metabolism and linking it to drought can be very important for the development of a drought resistant maize plant. Choosing these genes within pathways that may have the greatest effect on drought tolerance becomes very critical. This is an area that was not covered in this thesis and will need further investigation.

In addition, the low heritability of quantitative traits, difficulty in phenotyping and ineffectiveness of MAS when many alleles of small effects are involved, justifies the need for deploying genome-wide approaches to better characterize their genetic basis and more effectively improve their value in elite germplasm. This is because genome wide selection uses all marker data as predictors of performance and consequently delivers more accurate predictions. Genome wide selection makes use of individuals that have been both genotyped and phenotyped to develop a model that takes genotypic data from a ‘candidate population’ of untested individuals and produces genomic estimated breeding values (Lee *et al.*, 2008).

Association mapping applied directly to breeding populations is the next option proposed to mitigate the limitations of biparental populations in QTL identification (Collins *et al.*, 2008) Association mapping involves QTL molecular profiling and phenotypic characterization of multi-parent intercross populations or unrelated accessions (Rakshit *et al.*, 2012). Three

approaches namely the candidate gene based, genome wide association mapping and genotyping by sequencing can be used with the aid of high density consensus maps. Association mapping employs the random effects model where extrapolation of results to other populations can be done as opposed to bi-parental crosses which are specific to the given population (fixed effects model).

There is need for increased integration of physiology, genomics and quantitative genetics since the successful exploitation of MAS and other genome-wide approaches to enhance drought tolerance will only be possible within a multidisciplinary context. The current challenge is to make our research relevant and articulate the connections between researchers' activities and priorities and finding suitable products for the farmers in moisture stress environments.

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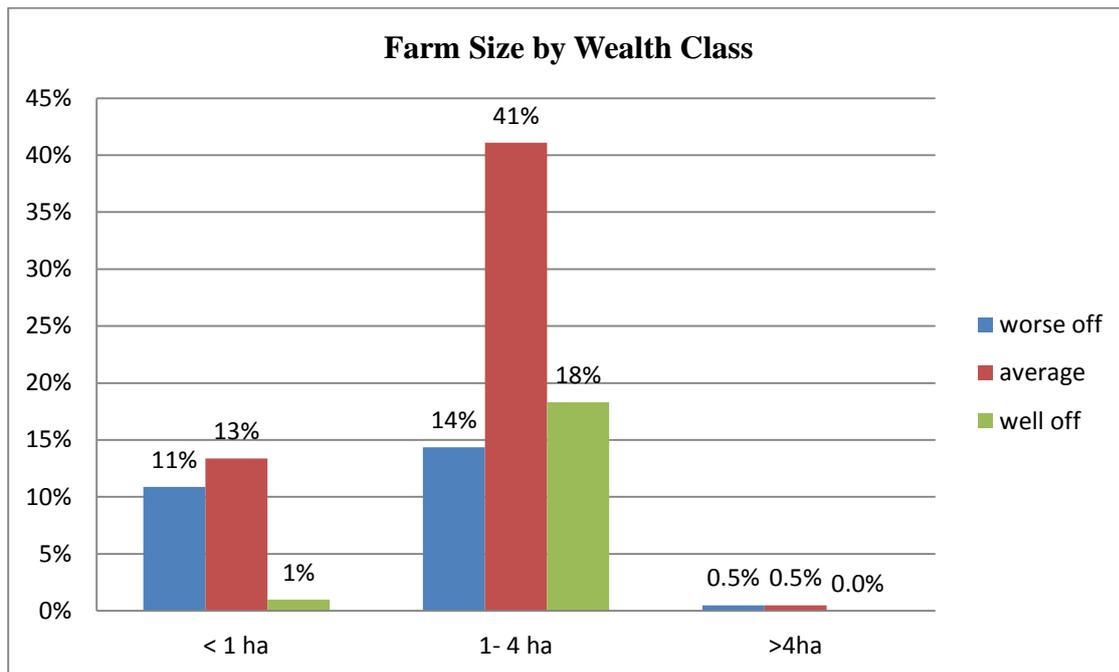
www.maizegenetic.net accessed on 2/10/2012

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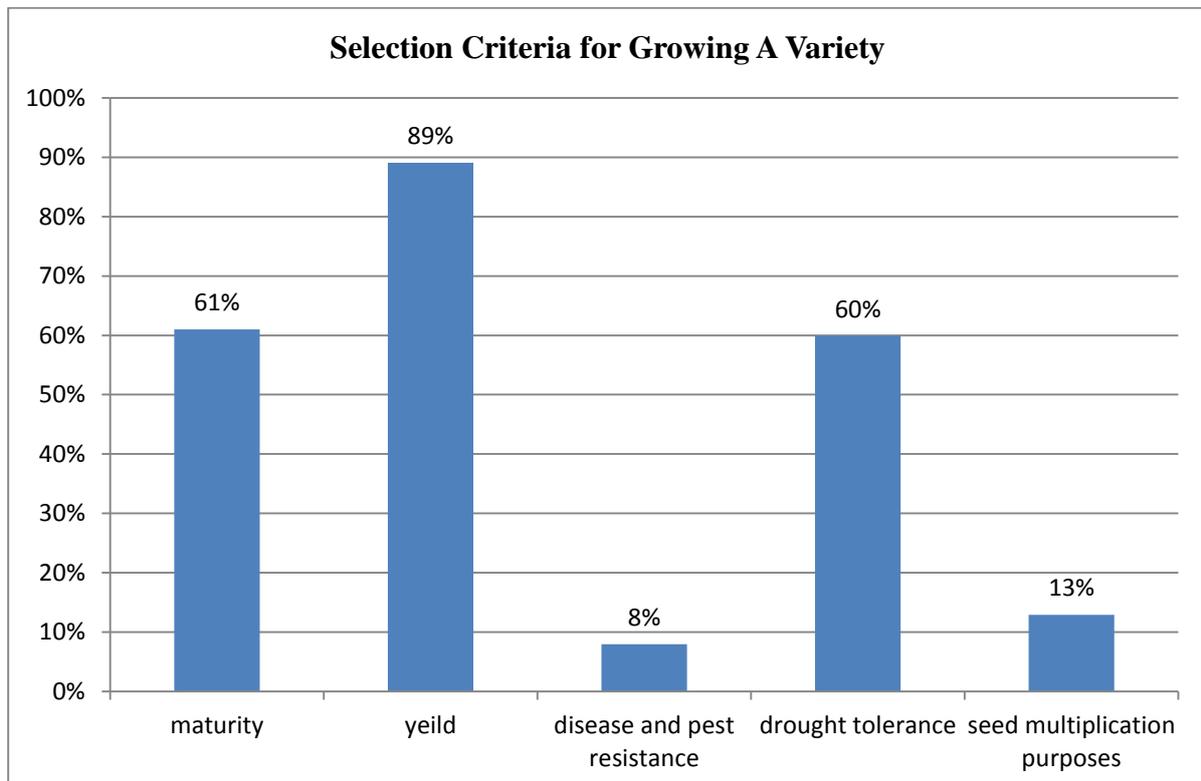
Appendix 1: Demographics and Wealth Ranking

General Information		Masvingo	Mash East	P value	
		Zimuto	Mutoko		
Number of Households Interviewed (n)		103	99		
Average number per household (n)		4.99	5.72	0.024	
Modal age range of farmers					
	Years				
	Male	15-65	1.04	1.41	0.006
	Female	15-65	1.53	1.44	0.532
Household Head Gender					
	Male	38	60	0.000	
	Female	65	39	0.000	
House Head Average Age (years)		48.4	47.6		
House Head Age Range (years)		22-77	20- 86		
House Head mean years in farming (years)		18.59	13.58	0.003	
House Head Marital Status					
	Married	62	77	0.050	
	Single	0	4		
	Divorced	4	0		
	Widowed	37	18	0.000	
House Head Education Level					
	Illiterate	1	7	0.020	
	Primary	40	40		
	Secondary	58	49	0.001	
	Tertiary	4	3		
Assets Owned					
Vehicle		0.06	0.00	0.088	
Motorcycle		0.01	0.00	0.328	
Bicycle		0.25	0.21	0.512	
Animal Plough		0.72	0.71	0.892	
Animal Drawn-cart		0.43	0.20	0.000	
TV/Radio		0.56	0.33	0.005	
Water Source		0.69	0.19	0.000	
Water Tank		0.06	0.01	0.062	
Mobile/ Fixed Phone		0.57	0.37	0.015	
Oxen		0.57	0.09	0.000	
Goats		2.04	2.75	0.332	
Sheep		0.00	0.07	0.063	
Transport Animals		0.35	0.03	0.185	
Draught Animals		0.88	0.79	0.595	
Pigs		0.12	0.30	0.070	
Chicken		10.16	6.81	0.000	
Cropping Data					
Mean Household area cropped (2009)		1.80	1.00	0.000	
Mean Household area under maize (2009)		1.16	0.62	0.000	
Mean Drought years in last 5 years		2.55	1.34	0.000	

Appendix 2: Graph Showing Proportion of Farm Size By Wealth Class



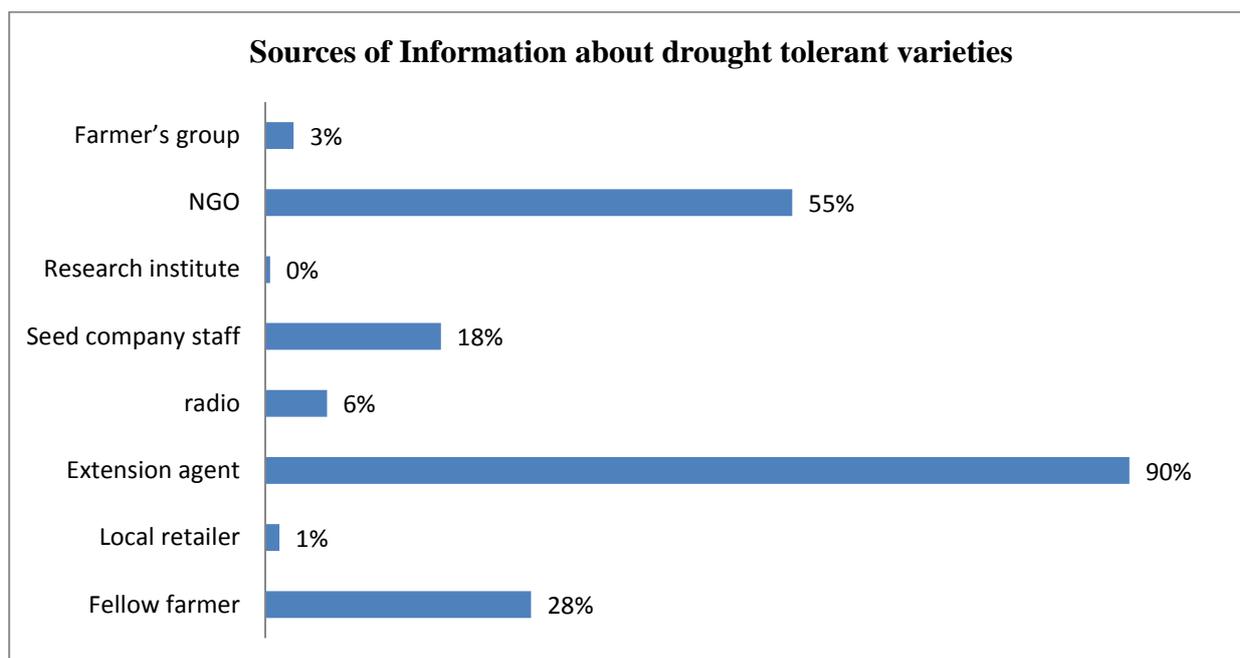
Appendix 3: Factors farmers considered when buying maize seed



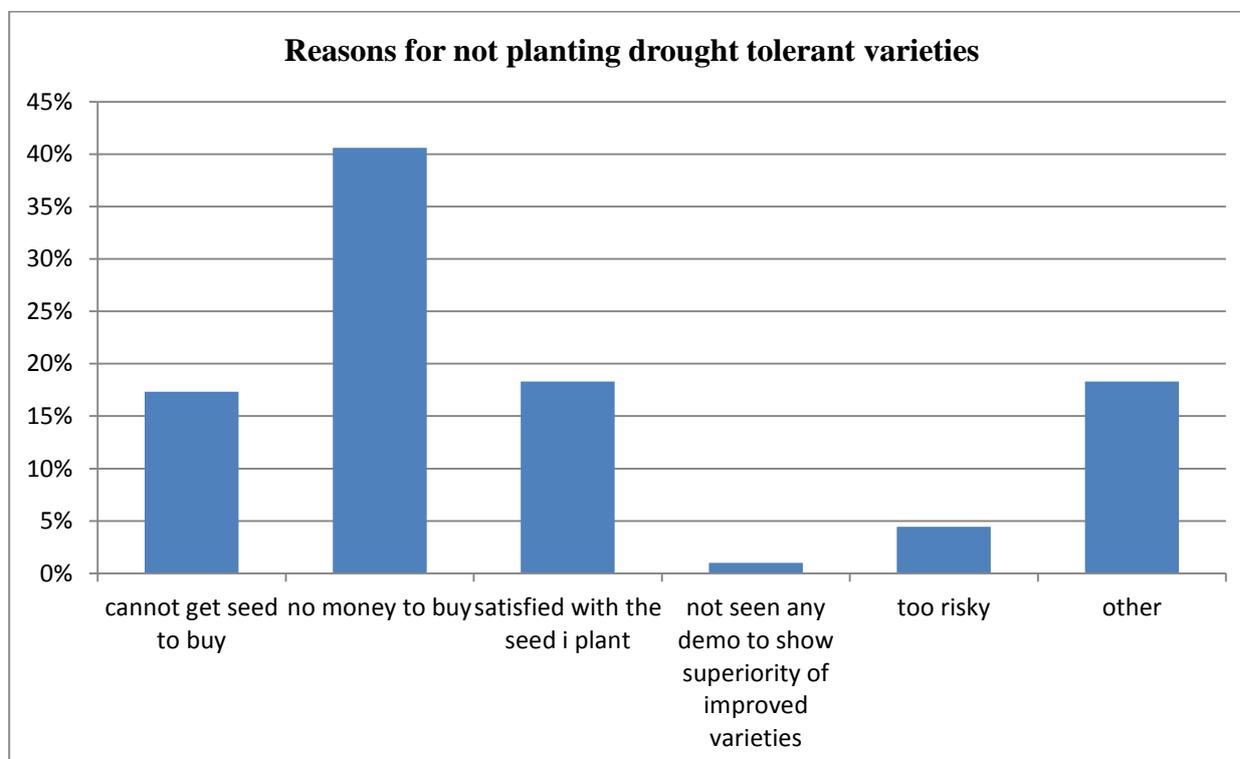
Appendix 4: Variety Information and Selection Criteria using Least Mean Squares

General Information	Masvingo	Mashonaland East	P value
	Zimuto	Mutoko	
Source of Variety Information			
Fellow Farmers	1.86	1.58	0.000
Local Retailer	1.97	2.00	0.088
Ministry Extension Agent	1.00	1.19	0.000
Seed Company Staff	1.68	1.96	0.000
Research Institute Staff	2.00	1.99	0.309
NGO Staff	1.55	1.34	0.003
Radio/Television	1.98	1.97	0.622
Newspaper	1.99	1.98	0.540
Farmer Group	1.98	1.95	0.382
Why Grow Current Variety			
Maturity	1.50	1.27	0.001
Yield Potential	1.18	1.03	0.000
Pest & Disease Resistance	1.88	1.95	0.046
Drought Tolerance	1.53	1.26	0.000
Seed Multiplication Purposes	1.80	1.95	0.001
Storage Pest Resistance	1.34	1.90	0.000
Lodging Resistance	2.39	2.19	0.030
Cob Size	1.65	1.93	0.004
Husk Cover	2.25	2.28	0.729
Grain Size	1.44	1.45	0.830
Palatability	1.84	1.1	0.000
Sadza Quality	1.57	1.16	0.000
Traits that Require Improvement			
Yield Potential	1.12	1.34	0.000
Pest & Disease Resistance	1.85	1.87	0.770
Drought Tolerance	1.20	1.16	0.440
Storage Pest Resistance	1.77	1.83	0.281
Maturity	1.42	1.19	0.000
Husk Cover	2.00	2.00	n/a
Good Performance In Poor Soils			
Soils	1.81	1.75	0.322
Number of Cobs per Plant	1.95	1.97	0.509
Cob Size	1.93	1.93	0.939
Palatability	1.96	2.00	0.048
Seed Costs	2.00	1.98	0.149

Appendix 5: Information Source About Varieties Characteristics



Appendix 6: Reasons for not planting drought tolerant varieties



Appendix 7: Genotypes and their pedigrees used in the study. These materials were obtained from CIMMYT

- 1 [CML445/ZM621B]-2-1-2-3-1-B*8/CML312-B
- 2 [CML312/[TUXPSEQ]C1F2/P49-SR]F2-45-3-2-1-BB//INTA-F2-192-2-1-1-1-B*4]-1-5-1-1-2-B*6/CML312-B
- 3 [MSRXPOOL9]C1F2-205-1(OSU23i)-5-3-X-X-1-B//EV7992/EV8449-3-2-2-2-B*7/CML312-B
- 4 [TS6C1F238-1-3-3-1-2-#-BB/[EV7992#/EV8449-SR]C1F2-334-1(OSU8i)-10-7(I)-X-X-X-2-BB-1]-1-1-2-1-1-B*6/CML312-B
- 5 MAS[206/312]-23-2-1-3-B*5/CML312-B
- 6 ZM521B-66-4-1-1-B*5/CML312-B
- 7 [SYN-USAB2/SYN-ELIB2]-12-1-1-1-B*5/CML312-B
- 8 [SYN-USAB2/SYN-ELIB2]-35-2-3-1-B*4/CML312-B
- 9 P501SRc0-F2-47-3-1-1-BB/CML312-B
- 10 Z97SYNGLS(B)-F2-188-2-1-3-B*4/CML312-B
- 11 [CML445/ZM621B]-2-1-2-3-1-B*8/CML442-B
- 12 [CML312/[TUXPSEQ]C1F2/P49-SR]F2-45-3-2-1-BB//INTA-F2-192-2-1-1-1-B*4]-1-5-1-1-2-B*6/CML442-B
- 13 [MSRXPOOL9]C1F2-205-1(OSU23i)-5-3-X-X-1-B//EV7992/EV8449-3-2-2-2-B*7/CML442-B
- 14 [TS6C1F238-1-3-3-1-2-#-BB/[EV7992#/EV8449-SR]C1F2-334-1(OSU8i)-10-7(I)-X-X-X-2-BB-1]-1-1-2-1-1-B*6/CML442-B
- 15 MAS[206/312]-23-2-1-3-B*5/CML442-B
- 16 ZM521B-66-4-1-1-B*5/CML442-B
- 17 [SYN-USAB2/SYN-ELIB2]-12-1-1-1-B*5/CML442-B
- 18 [SYN-USAB2/SYN-ELIB2]-35-2-3-1-B*4/CML442-B
- 19 P501SRc0-F2-47-3-1-1-BB/CML442-B
- 20 Z97SYNGLS(B)-F2-188-2-1-3-B*4/CML442-B
- 21 [CML445/ZM621B]-2-1-2-3-1-B*8/CML537
- 22 [CML312/[TUXPSEQ]C1F2/P49-SR]F2-45-3-2-1-BB//INTA-F2-192-2-1-1-1-B*4]-1-5-1-1-2-B*6/CML537
- 23 [MSRXPOOL9]C1F2-205-1(OSU23i)-5-3-X-X-1-B//EV7992/EV8449-3-2-2-2-B*7/CML537
- 24 [TS6C1F238-1-3-3-1-2-#-BB/[EV7992#/EV8449-SR]C1F2-334-1(OSU8i)-10-7(I)-X-X-X-2-BB-1]-1-1-2-1-1-B*6/CML537

25 MAS[206/312]-23-2-1-3-B*5/CML537

26 ZM521B-66-4-1-1-B*5/CML537

27 [SYN-USAB2/SYN-ELIB2]-12-1-1-1-B*5/CML537

28 [SYN-USAB2/SYN-ELIB2]-35-2-3-1-B*4/CML537

29 P501SRc0-F2-47-3-1-1-BB/CML537

30 Z97SYNGLS(B)-F2-188-2-1-3-B*4/CML537

31 [CML445/ZM621B]-2-1-2-3-1-B*8/CML538

[CML312/[TUXPSEQ]C1F2/P49-SR]F2-45-3-2-1-BB//INTA-F2-192-2-1-1-1-B*4]-1-5-1-1-2-B*6/CML538

32

[MSRXPOOL9]C1F2-205-1(OSU23i)-5-3-X-X-1-B//EV7992/EV8449-3-2-2-2-B*7/CML538

33

[TS6C1F238-1-3-3-1-2-#-BB/[EV7992#/EV8449-SR]C1F2-334-1(OSU8i)-10-7(I)-X-X-X-2-BB-1]-1-1-2-1-1-B*6/CML538

34

35 MAS[206/312]-23-2-1-3-B*5/CML538

36 ZM521B-66-4-1-1-B*5/CML538

37 [SYN-USAB2/SYN-ELIB2]-12-1-1-1-B*5/CML538

38 [SYN-USAB2/SYN-ELIB2]-35-2-3-1-B*4/CML538

39 P501SRc0-F2-47-3-1-1-BB/CML538

40 Z97SYNGLS(B)-F2-188-2-1-3-B*4/CML538

41 [CML445/ZM621B]-2-1-2-3-1-B*8/CKL05005

[CML312/[TUXPSEQ]C1F2/P49-SR]F2-45-3-2-1-BB//INTA-F2-192-2-1-1-1-B*4]-1-5-1-1-2-B*6/CKL05005

42

[MSRXPOOL9]C1F2-205-1(OSU23i)-5-3-X-X-1-B//EV7992/EV8449-3-2-2-2-B*7/CKL05005

43

[TS6C1F238-1-3-3-1-2-#-BB/[EV7992#/EV8449-SR]C1F2-334-1(OSU8i)-10-7(I)-X-X-X-2-BB-1]-1-1-2-1-1-B*6/CKL05005

44

45 MAS[206/312]-23-2-1-3-B*5/CKL05005

46 ZM521B-66-4-1-1-B*5/CKL05005

47 [SYN-USAB2/SYN-ELIB2]-12-1-1-1-B*5/CKL05005

48 [SYN-USAB2/SYN-ELIB2]-35-2-3-1-B*4/CKL05005-BB

49 P501SRc0-F2-47-3-1-1-BB/CKL05005

50 P501SRc0-F2-47-3-1-1-BB/CKL05005-BB

51 CML312/CML442

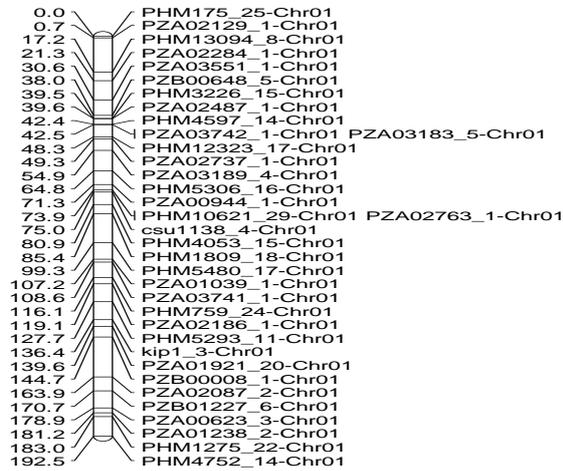
52 CML539/CML442

53 SC727

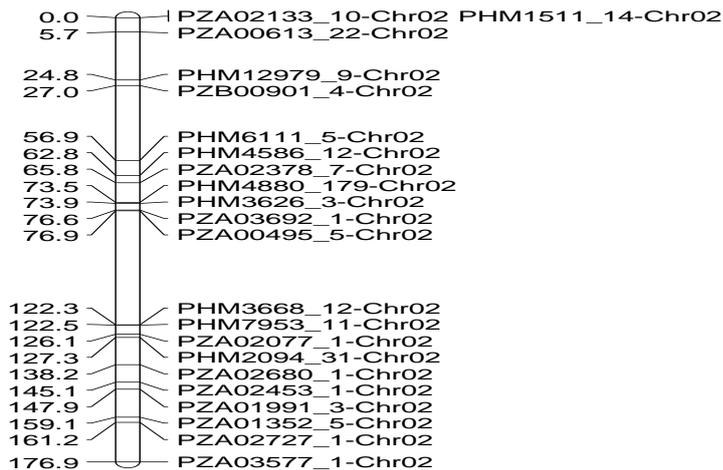
Hybrids used as checks are in bold letters

Appendix 8 : SNP marker distribution showing regions with major QTL on the linkage maps

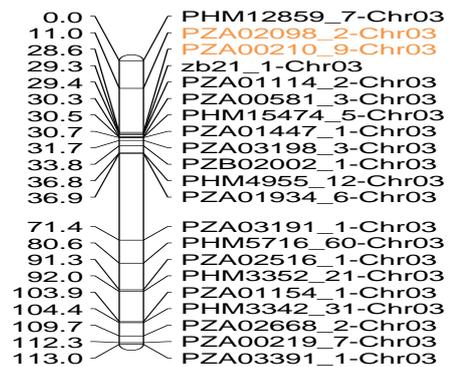
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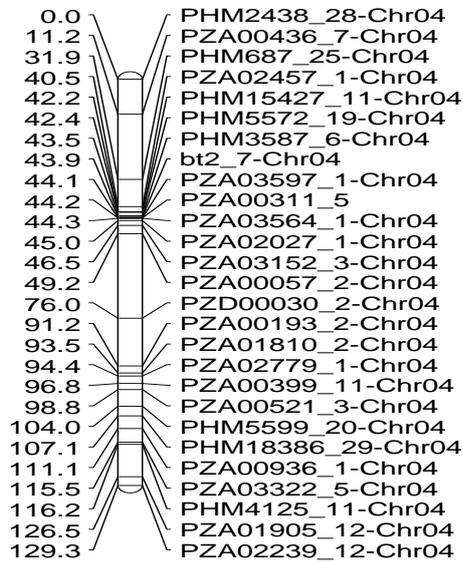
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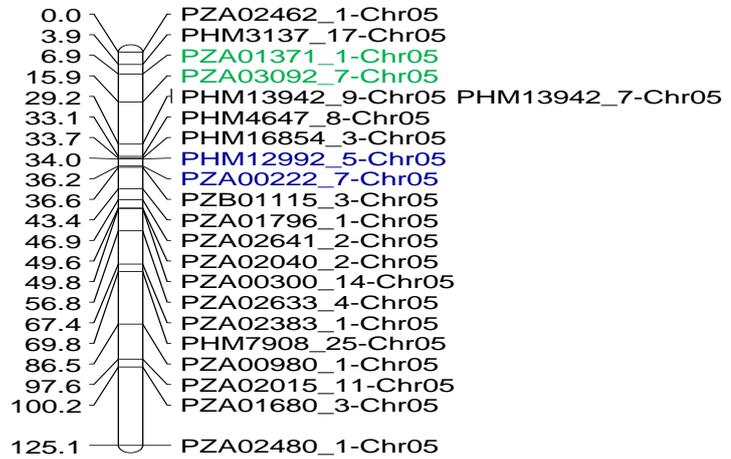
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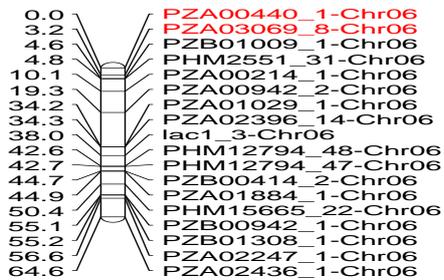
Chromosome-04



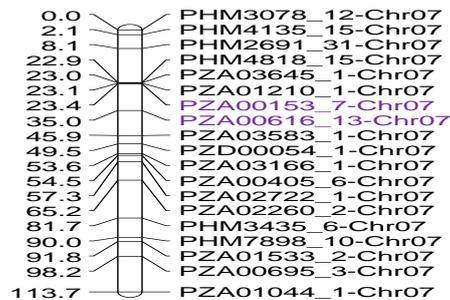
Chromosome -05



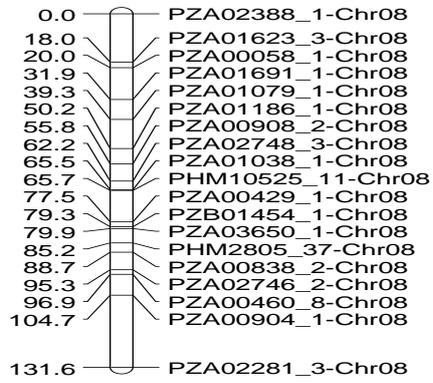
Chromosome-06



Chromosome-07



Chromosome-08



Chromosome -09

