GENETIC DIVERSITY AND PERFORMANCE OF MAIZE VARIETIES FROM ZIMBABWE, ZAMBIA AND MALAWI

A Dissertation

by

COSMOS MAGOROKOSHO

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

December 2006

Major Subject: Plant Breeding

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Approved by:

Chair of Committee, Javier F. Betrán
Committee Members, William L. Rooney
C. Wayne Smith
Thomas DeWitt
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ABSTRACT

Genetic Diversity and Performance of Maize Varieties from Zimbabwe, Zambia and Malawi. (December 2006)

Cosmos Magorokosho, B.S.; M.S., University of Zimbabwe

Chair of Advisory Committee: Dr. Javier F. Betrán

Large scale and planned introduction of maize (*Zea mays*) in southern Africa was accomplished during the last 100 years. Since then, smallholder farmers and breeders have been selecting varieties best adapted to their specific growing conditions. Six studies were conducted to generate information on the current levels of genetic diversity and agronomic performance of both farmer-developed and commercially-bred maize varieties in Zimbabwe, Zambia and Malawi to help in the identification of sources of new alleles for improving yield, especially under the main abiotic stresses that prevail in the region. In the first study, 267 maize landraces were collected from smallholder farmers in different agro-ecological zones of the three countries for conservation and further studies. Passport data and information on why smallholder farmers continue to grow landraces despite the advent of modern varieties were also collected along with the landraces. The second study revealed considerable variation for phenological, morphological and agronomic characters, and inter-relationships among the landraces and their commercial counterparts. A core sample representing most of the diversity in the whole collection of landraces was selected for further detailed analyses. The third study revealed high levels of molecular diversity between landraces originating from different growing environments and between landraces and commercially-bred varieties. The Simple Sequence Repeat (SSR) data also showed that the genetic diversity introduced from the original gene pool from the USA about 100 years ago is still found in both the descendant landraces and commercially-bred varieties. The fourth study showed that in general, commercially-bred varieties outyielded landraces under both abiotic stress and nonstress conditions with some notable exceptions. Landraces were more stable across environments than improved varieties. The most promising landraces for pre-breeding and further investigation were also identified. The clustering patterns formed based on

agronomic data were different from SSR markers, but in general the genotype groupings were consistent across the two methods of measuring diversity. In the fifth study, the more recently-bred maize varieties in Zimbabwe showed consistent improvement over older cultivars in grain yield. The apparent yearly rate of yield increase due to genetic improvement was positive under optimum growing conditions, low soil nitrogen levels and drought stress. The sixth study revealed that in general, genetic diversity in Zimbabwean maize has neither significantly decreased nor increased over time, and that the temporal changes observed in this study were more qualitative than quantitative.

The results from the six studies confirm the origin of maize in southern Africa and reveals that considerable genetic variation exists in the region which could be used to broaden the sources of diversity for maize improvement under the current agro-ecological conditions in southern Africa.

DEDICATION

I would like to dedicate this work to my father, Mr. Tobias Atiyakoni Magorokosho.

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CHAPTER I

INTRODUCTION

In 2001, maize (*Zea mays* L.) became the number one production crop in the world, and current world maize production surpasses that of either wheat or rice. Data from the United Nations (UN) Food and Agriculture Organization (FAO) shows that for 2005 world maize production was 692 million Mg, while that for wheat was 626 million Mg and for rice it was 614 million Mg (FAOSTAT, 2005). Africa's share of maize production for 2005 was 47 million Mg or just about 7% of world production. In southern Africa production and consumption of maize is high reflecting its role as the primary food staple for the majority of rural households. It ranks first in Zimbabwe, Zambia and Malawi in total production and yield per hectare, and is the most important food crop grown and consumed by Africans. The three countries each plant more than half a million hectares of maize yearly and annual production for 2005 was 900,000 Mg for Zimbabwe, 1.2 Mg for Zambia and 1,75 Mg for Malawi (FAOSTAT, 2005). Maize accounts for about 30% of total calories consumed in southern Africa. In this region, per capita annual consumption of maize averages more than 100 kg in several countries including Malawi (181 kg), Zambia (168 kg) and Zimbabwe (153 kg) (Aquino, 2001).

The environments and farming systems in Zimbabwe, Zambia and Malawi where maize is grown are extremely diverse with production varying considerably between years, and showing a close dependence on rainfall and soil fertility (Aquino, 2001). In this region, maize is grown mainly under rainfed conditions from sea level to 2,400 meters above sea level (masl). Over 90% of maize produced in the three countries is grown by small and medium scale farmers who cultivate 10 ha or less (DeVries and Toenniessen, 2001). Production technology varies greatly with agro-ecology, cultural background, resource availability, and stresses, but is generally traditional, resulting in low productivity in most agro-ecological zones except on the commercial farms that have access to the appropriate inputs.

This dissertation follows the style and format of Crop Science.

Average yields on smallholder farms are currently about 1.2 Mg ha^{-1} (DeVries and Toenniessen, 2001), among the lowest in the developing world, even though yields in the same environments can be higher (Banziger et al., 1997). Low maize yields on smallholder farms reflect frequent use of low plant densities either in monocrops or in intercropping systems, but are also the result of stresses arising from low soil fertility, weeds, drought, pests and diseases. Therefore, despite its importance as the main source of calories in the three countries, average productivity of maize is low on smallholder farms. Breeding of maize varieties suited to the diverse and unpredictable growing conditions in this region is thus urgent.

Maize is one of the most diverse crops both genetically and phenotypically. Current genetic diversity in the crop today is the product of a long selection process practiced by native Americans in central America before the spread of the crop to other parts of the world (Manglesdorp, 1974). The Portuguese introduced maize in Africa beginning in the 16th century and since then the crop has replaced sorghum and millet as the main staple in most of the continent (McCann, 2005). Early maize introductions in Zimbabwe, Zambia and Malawi consisted mostly of flint and soft floury types originating mainly from Brazil and the Caribbean (McCann, 2005) followed later in the twentieth century by open pollinated dent types from the USA (Weinamann, 1972). Local maize populations have now been cultivated and submitted to natural and human selection in different environments and cultural methods in the three countries for the past 100 years. Smallholder farmers in the three countries traditionally collect seeds from the best plants with preffered ear and seed traits. Each year selection of seeds is limited to a few chosen individuals and seeds are bulked and kept for the next planting. Thus the local varieties grown in the various countries have adapted to local conditions and farmer's practices, and represent unique sources of genetic diversity. Many useful traits have developed in these areas following natural and farmer's selection over the years. These local populations or landraces could offer new alleles for abiotic stress tolerance in maize.

Most maize diversity remains undescribed, poorly understood and under utilized in modern plant improvement largely because of the difficult of identifying useful genetic variants hidden in the background of low yielding local varieties or lines (Tanksley and McCouch, 1997). Although local varieties have not been extensively used by breeders

because of their other undesirable agronomic traits, they can serve as sources of new desirable traits to enhance performance of germplasm under abiotic stresses such as drought, low soil fertility and acid soils (Beck et al., 1997). Information about the impact of smallholder farmer selection on abiotic stress tolerance of maize is mostly lacking. Farmers' local varieties collected from marginal environments may possess some unique physiological attributes that may not be present in germplasm not exposed to abiotic stress (Blum and Sullivan, 1986).

The large-scale inland spread of maize in southern Africa is related to the history of British white settlement in the region. In Zimbabwe, white settlers started producing maize as early as the 1890s after the arrival of American white dent varieties (Weinamann, 1972). These dent varieties became the most important progenitors of southern and eastern African commercial maize types over the course of the twentieth century. Many of these varieties were characterized by large dent kernels, and could tolerate poor soils and out-yield the older flint types (McCann, 2005). Research on hybrid maize in Zimbabwe was initiated at Harare Research Station in 1932 following the news of the success of hybrid maize varieties in the US. The first hybrid, Southern Rhodesia 1 (SRI) was released after 17 years of research and thereafter breeders continued to develop improved hybrids up to this date, including the famous 1960 release of SR-52, the first commercial single cross in the world (Mashingaidze, 1994).

Average maize grain yield per area on commercial farms in Zimbabwe increased dramatically during the second half of last century (Mashingaidze, 1994). Commercial farmers in Zimbabwe commonly produced 10 Mg ha^{-1} or more, some the highest cereal yields in the world (DeVries and Toenniessen, 2001). The large yield gain has been attributed to genetic improvement as well as improvements in crop management practices (Mashingaidze, 1994). Maize grain yield increases due to genetic improvement have been reported extensively for temperate regions of the world (Duvick, 1997; Russell, 1991; Tollenaar et al., 1994), but information is lacking for tropical maize, particularly in southern Africa. Knowledge of genetic gains in yield potential of tropical maize under varying growing conditions is essential to improve the understanding of yield-limiting factors and to inform future breeding strategies. Genetic gain of the time-series of varieties released over years in Zimbabwe should provide an indication of the relative

value of modern cultivars under drought, poor soil fertility and low yielding environments. This relative value should guide breeders in the types of cultivars to develop and deploy in the country.

With the advent of the first maize hybrids in Zimbabwe, the original open-pollinated landraces were substituted by a limited number of hybrids. Currently, the main maize hybrids cultivated in the country are thought to be restricted to a limited number of key inbred lines. Therefore, genetic diversity of those hybrids is almost certainly limited, in comparison to the large original genetic diversity that was available in open pollinated landraces.

The first objective of this study was to understand how smallholder farmers' selection under different agro-ecological conditions in Zimbabwe, Zambia and Malawi has shaped genetic diversity in maize and then relate the diversity to agronomic performance under different abiotic stress conditions. Specific aims to address this objective were to (i) assemble a diverse collection of local maize population grown by smallholder farmers in different agro-ecologies of Zimbabwe, Zambia and Malawi, and (ii) examine the current levels of genetic diversity and agronomic performance of the collected varieties through (a) morpho-phenological, (b) molecular, and (c) agronomic evaluations under various abiotic stresses. The second objective of this study was to determine genetic gain in yield and examine the impact of the development of hybrid varieties upon maize genetic diversity and erosion, and to determine the proportion of the original landrace gene pool transferred to modern hybrid varieties for improved maize varieties grown in Zimbabwe since the introduction of the crop in the country. To address this objective, a time series of key maize cultivars released and grown in the country from 1900 to 2004 was; (i) compared under different growing environments for agronomic traits, relative yields and apparent rates of yield increase due to genetic improvement, and (ii) fingerprinted using simple sequence repeat (SSR) markers to quantify genetic diversity among earlier and modern cultivars.

CHAPTER II

COLLECTION AND DOCUMENTATION OF MAIZE LANDRACES

INTRODUCTION

Scientific maize breeding to support and sustain increased production and productivity began as early as 1932 in Zimbabwe (Eicher and Kupfuma, 1997), followed by Malawi in 1954 (Hassan et al., 2001), and Zambia in 1964 (Hassan et al., 2001). Maize production and research during the last seventy years has been described as a 'green revolution' in Zimbabwe (Eicher and Kupfuma, 1997), as a 'stop-and-go revolution' in Zambia (Hassan et al., 2001) and, 'a green revolution in the making' in Malawi (Hassan et al., 2001). More than a hundred improved open pollinated varieties (OPVs) and hybrids have been released in the three countries since 1966 (Hassan et al., 2001). Although some improved hybrids such as the R200 series (R200, R201, R215) were developed to address the needs for farmers located in less favorable production ecologies in the three countries (Eicher and Kupfuma, 1997), it is thought that mainly commercial farmers have benefited from the bulk of the released varieties while smallholder farmers still lag behind. Yields can be as high as 14 Mg ha^{-1} under high input management systems on commercial farms in Zimbabwe, while yields obtained by smallholder farmers average 1.2 Mg ha^{-1} even in similar environments (DeVries and Toenniessen, 2001).

Research carried out by Hassan et al. (2001) in Zambia and Malawi, has shown that smallholder farmers in these countries consider many improved varieties as inferior to local maize populations or landraces, especially in production and performance under abiotic stress conditions. Smallholder farmers in these two countries have benefited little from the improved varieties and many still grow obsolete OPVs and local landraces. Even though the majority of smallholder farmers use hybrid seed in Zimbabwe, there are still areas where obsolete OPVs and landraces are still preferred and grown in the country. As examples, some smallholder farmers in Zimbabwe and Zambia still grow Hickory King, an OPV introduced in the country from the USA in 1905 (Weinamann, 1972), while others still grow Salisbury White, an OPV released in Zimbabwe in 1975 even though the seed industry has not produced or sold any seed of the variety for well

over three decades (Friis-Hansen, 1995). In all the three countries, local landraces with distinct characteristics are still grown (Hassan et al., 2001). It can therefore be hypothesized that most of the new improved OPVs and hybrids varieties released in these three countries have not met the needs and preferences of the majority of smallholder farmers and many of these farmers still depend on local varieties and landraces, especially those farming in marginal areas.

Since their introduction into southern Africa more than 100 years ago, maize landraces have been subjected to natural and farmer selection under different cultural and environmental conditions. This selection is thought to have resulted in many different types of varieties with varying levels of adaptation to specific agro-ecologies where the crop is grown. At present, little or no formal attempts have been made to examine the impact of smallholder farmer selection on adapting maize to different growing environments or evaluating the current diversity that has resulted from over a hundred years of farmer and natural selection in southern Africa. Farmers' local varieties collected from marginal environments may possess some unique physiological attributes that may not be present in germplasm not exposed to abiotic stress (Blum and Sullivan, 1986). Attempts such as these, especially when aided with the use of molecular characterization techniques, could result in better-targeted maize breeding programs for the production systems of Zimbabwe, Zambia and Malawi, particularly in marginal areas.

In this chapter, the objectives were to (i) report on a survey carried out to collect and conserve maize landraces, and document information related to their uses and maintenance in Zimbabwe, Zambia and Malawi, and (ii) classify the landraces according to agro-ecological conditions of the collecting sites for identification of landraces grown in areas with abiotic stresses related to potential breeding goals in southern Africa.

LITERATURE REVIEW

Origin and Introduction of Maize in Africa

Maize was domesticated from the wild grass *teosintle* in central America about 9,000 years ago and spread northwards and southwards and was particularly abundant in the Aztec and Inca empires in Central America at the time when the New World was

discovered (Manglesdorp, 1974). Due to its adaptability and productivity, maize spread rapidly around the world after the Europeans exported the plant from the Americas in the 15th and 16th centuries (McCann, 2005).

Despite some earlier controversy, it now seems clear that the Portuguese first introduced maize into Africa during the 16th century (Miracle, 1966; McCann, 2005). Early Portuguese merchants introduced maize into Africa though their trade networks along the western and eastern coasts of Africa starting in the 16th century. There are historical records of maize cultivation in Cape Verde in 1541, Angola in 1590, and Mozambique 1821 (McCann, 2005). The Dutch introduced maize along the southern African coast in 1658, but this maize is thought to have originated from West Africa's Guinea coast (Miracle, 1966). Caribbean and Brazilian flints, such as the yellow-to-orange *Cateto* variety or a blue flint were probably the first maize imports to southern Africa. These varieties had hard endosperm, were early maturiting, and had variegated bright colored grains. Flint maize adapted well to many of the same niches in which Africa's indigenous sorghums and millets had thrived. Later soft floury maize types arrived in southern Africa, possibly from Mexico via Brazil (McCann, 2005). The earliest spread of flint maize away from the coastal zones was associated with slave trade networks coupled with the movement of Christian missionaries inland. Maize was already being cultivated in northeastern Zambia in the late 1700s and in southern Malawi by the middle of the nineteenth century (McCann, 2005). In Zimbabwe, white settlers started producing maize as early as the 1890s (Weinmann, 1972). However, maize never fully replaced sorghum and millets as the staple crop of African people in southern Africa until well into the twentieth century (Hassan et al., 2001).

The large-scale spread of maize in southern Africa is related to the history of British white settlement in the region. American white dent maize arrived in southern Africa in the late 19th and early 20th centuries, with such names as Boone County, Leaming, Golden King, Iowa Silver Mine, Hickory King and Horsetooth (Weinamann, 1972). Many of these varieties were characterized by large dent kernels, and could tolerate poor soils and out-yield the older flint types (McCann, 2005). These varieties and others became the most important progenitors of southern and eastern African commercial maize types over the course of the 20th century, but their relative

contributions to the establishment of southern Africa maize genetic diversity has remained largely speculative.

The arrival and quick adoption of the dent maize by both African and white settler farmers was propelled by at least five driving factors: (i) the agronomic suitability of maize to climatic conditions and soil types of the region; (ii) the lucrative British starch market which preferred white maize over yellow maize from America; (iii) milling technologies in southern Africa that favored soft-dent types; (iv) the huge domestic demand for maize that arose due to the integration of African people in to the white settlers' wage economy; and (v) market and trade policies which promoted large scale commercial maize production by settler farmers (Weinmann, 1972). Ironically, the preferences of today's African consumers for white as opposed to yellow grain color began with the influence of the British starch market during the early twentieth century (Weinmann, 1972). By 1920, both smallholder and commercial farmers in the Zimbabwe and Zambia had largely replaced their flint cultivars with improved white dents while farmers in Malawi continued to grow mostly flint maize (McCaan, 2005). Maize gradually became a staple of the African population beginning with those who were most exposed to the white settlers' commercial activities. By the 1930s, maize was important in smallholder agriculture in Zimbabwe, Zambia and Malawi as both a subsistence and a cash crop (Weinamann, 1972).

Farmer Selection and Maintenance of Maize Landraces

Maize local populations, also called landraces, often exhibit high levels of phenotypic variability. Landraces are commonly identified by their local name or other unique traits they posses that are different from improved varieties. There are many definitions of landraces in literature. In a broad sense, landraces are crop genetic resources that have evolved continuously under natural and farmer selection practices rather than in the collections of gene banks or plant breeding programs (Zeven, 1998). Historically, landraces were the progenitors of the modern crop varieties. Landraces have certain unique phenotypic, morphological and phenological characteristics, a reputation for adaptation to local climatic conditions and cultural practices, and resistance or tolerance to diseases and pests (Zeven, 1998). As a result landraces usually have high

yield stability and intermediate yield levels under a low input agricultural system (Zeven, 1998). Over a long period of time, farmers have developed ways and means of maintaining the useful genetic diversity existing in maize landraces in many part of the world through selection.

Since crop domestication, farmers have traditionally kept aside part of the harvested crop as seed for the next planting. Farmers traditionally collect seeds from the best plants preffered ear and seed traits. Each year selection of seeds is limited to a few chosen individual plants and seeds are bulked and kept for the next planting. Plant maturity is an important selection criterion for maize seeds by smallholder farmers. Rigorous selection criteria can result in a quite uniform landrace (Bellon, 1991). In general, people in Central and South America associate maize landraces with light-colored kernels with long growing season whereas dark-toned cultivars are destined for short growing seasons (Zeven, 1998). A short growing period may be important in some environments where crops usually have to escape drought stresses occurring during the growing season. Time from planting to maturity is of importance to the farmers because this character is often positively correlated with flowering time. Therefore, selection for various periods of maturity also induces selection for flowering period. This spread in flowering time may reduce introgression of foreign genetic material. Consciously, or unconsciously, the farmers apply a method which promotes the maintenance of the landraces. In Ethiopia, Malatu and Zekele (2002) found anthesis-to-silking interval (ASI), compatibility to intercrop with sorghum, stalk thickness, absence of barren plants, and stover yield as other additional important plant selection criteria of maize varieties for smallholder farmers. The Western Apache and Navajo tribes in southern USA and Central America select the tallest plants with two or three ears for choosing seeds for next planting (Bellon, 1991). It has also been assumed that prolific plants produce a higher yield type, i.e. assure a good crop. This character occurs quite commonly in landraces grown in México and Guatemala, which may point to prolonged positive selection for this trait (Bellon, 1991).

The selection criterion of large ears is understandably, one of the major criteria for yield, as was described for the Central and South-America, and reported for farmers in the USA, north-Portugal, Lithuania, and Tanzania (Zeven, 1998). However, in Bénin

the Adja community maintains a landrace with small ears. In addition, farmers select ears with a minimum tapering, ears with more kernel rows, and the absence of insect damage and diseases on the ear (Zeven, 1998).

For kernels, the most important selection criteria include; kernels obtained from the largest cobs, the depth of the kernels on the ears, and larger kernels from the middle part of the ear (Bellon, 1991). Kernels at the top or bottom of the ear are only used in times of seed shortage as the farmers believe that they grow into weak plants (Manglesdorf, 1974). Farmers in Zimbabwe, Zambia and Malawi are known to favor large kernelled varieties - a preference that probably arose from the influence of the large dent maize varieties such as Hickory King and Horsetooth that were introduced into the region by the British from the late nineteenth century (Weinamann, 1972).

Depending on the farming area, different kernel textures are chosen by different groups of farmers. In Malawi and some areas of Zimbabwe and Zambia, flint kernels are preferred as the flint texture reduces insect damage and facilitates hand pounding to make maize flour, a job carried out by women. On the other hand, the majority of farmers in Zimbabwe prefer dent kernels which facilitate roller milling (Eicher and Kupfuma, 1997). Selection for kernel color is common in Central America where different kernel colors are used for specific purposes (Bellon, 1991). In southern Africa, the preferred grain type is white and farmers deliberately select against other grain colors as these colors cause a downgrading of the maize for the market in addition to not being liked for making the staple dishes (Weinamann, 1972). In general, for seed for the next planting, attention is paid to kernels with a uniform shape, plumpness and tightness, their color, large size, and physical damage by insects and diseases, and their provenance.

Different socioeconomic and cultural factors may contribute to the diversification of landraces. Adaptive and socio-economic selection for a particular type of maize by American Indians resulted in many landraces, which were propagated and maintained as separate entities and with a diverse array of purposes (Bellon, 1991). In some countries in Central America, ‘sweet varieties’ are maintained for native beer brewing, roasting, and others for preparing of native confectionaries. Black kernels of plants with deep purple rachis are used for making tortilla and beer, and for medicinal-ceremonial purposes such as offerings to gods (Zeven, 1998).

The growing of more than one landrace by farmers is a common practice in certain regions of the world where maize is cultivated. In Chiapas, Central México, maize farmers recognized at least 15 landraces cultivated by one farmer. Some original components of such landraces could have been derived from improved cultivars, of which some have become a 'criolized' *(acriollada)* landrace. At another village in Mexico, one farmer was growing four landraces, each having its own purpose. The varieties could be identified by the kernel color, length of growing period, relative yield, kernel taste and texture (Bellon, 1991). By planting the four landraces at the same time simultaneous flowering was avoided and, also of importance, simultaneous harvesting.

Gender and age also play an important part in seed selection for the next planting. With the Hopi Indians in Central America, care of seeds from harvest until the next planting is the responsibility of women. It appears that with increasing age of the farmer and his wife, more landraces were maintained. For instance, three farmers younger than 30 years maintained an average of 5 landraces, 12 farmers being 30-60 years old kept 6 landraces, and 30 farmers older than 60 years maintained 7 varieties (Zeven, 1998). The difference may not be statistically significant, but the figures may indicate a trend. They may either indicate a dying-out relic, i.e. the older farmers still maintain more landraces than younger ones. Or when a farmer becomes older she or he enjoys maintaining more landraces, whereas the younger farmers may not see the point of growing many landraces.

Environmental Conditions Shaping Maize Landrace Diversity

Maize is cultivated in a wider range of environments in Zimbabwe, Zambia and Malawi than most cereals because of its greater adaptability. The conditions where landraces are grown are usually associated with different patterns of genetic variability, reflecting processes of adaptation of germplasm to the environmental factors (Zeven, 1998). Resistance to abiotic stress may be found in germplasm previously exposed to the specific environmental stress (Beck et al., 1997). Farmers' local varieties acquired from abiotic stress-prone environments may possess some unique physiological attributes that may not be present in germplasm not exposed to abiotic stress (Blum and Sullivan, 1986). From a study of 5,072 wheat lines originating from different countries, Sayed (1985)

showed that the largest portion of salt tolerant lines came from regions considered salinized. In beans, the frequency of obtaining P-efficient genotypes was higher when landraces were obtained from geographic regions with acid soils than when the landrace was obtained from soils that were not acidic (Beebe et al., 1995). From these studies, the different authors recommended the screening of germplasm from abiotic stress regions if the aim was to obtain abiotic stress tolerant germplasm. Zeven (1998) cites the relationship between maize landrace performance and the geographical location where the landrace is grown; in Benin, the landrace 'Djongo' provides some yield on exhausted soils of the Adja Plateau while the landrace 'Bogan' tolerates floods in the flood-prone areas where it is grown. Beck et al. (1997) concluded that although local varieties have not been extensively used by breeders because of their other undesirable agronomic traits, they can serve as sources of new desirable traits to enhance performance of germplasm under drought conditions.

The importance of collecting environmental data related to germplasm collection sites has been discussed by Steiner and Greene (1996). Lack of precise data on collection site description and the lack of standardization of such data have hindered the interpretation and use of such information. However, over the years, the use of geographic information system (GIS) maps for germplasm collection has increased. With GIS, it is possible to estimate environmental conditions of collecting sites (Hartkamp et al., 2000). Steiner and Greene (1996) termed the application of GIS-based classification as 'retro-classification" when applied after germplasm classification. The standardization of ecological descriptors is greatly facilitated when such descriptors are obtained through the use of environmental maps and databases in GIS. Hartkamp et al. (2000) have produced a GIS based system for describing the environmental conditions associated with maize production zones worldwide. Geographical information can be obtained either locally during collecting expeditions, or later on, derived from GIS. GIS information may also be useful in the development of core collections and many studies carried out to develop core collections found the eco-geographic origin to be a good component for germplasm classification and stratification (Steiner and Greene, 1996). A routine methodology was developed to overlay the geographic sites of genetic resources collecting with different environmental maps, using GIS (Hartkamp et al., 2000). More

recently, GIS programs developed specifically to carry out studies on genetic resources have been made available, such as FloraMap (Jones and Gladkov, 1999) and DIVA-GIS (Hijmans et al., 2001).

MATERIALS AND METHODS

Landrace Collection and Documentation

Agriculture in Zimbabwe, Zambia and Malawi is divided into two distinct sectors: large-scale commercial agriculture which is mainly privately owned and the smallholder sector which is dominated by subsistence farming. The smallholder areas are predominantly found in regions with low rainfall coupled with poor soils and farmers in these areas frequently grow obsolete OPVs, traditional varieties and landraces. Smallholder areas formed the focus of this survey since large-scale commercial farmers predominantly use hybrid maize and crops on their farms are not frequently subjected to abiotic stress from rainfall or soil fertility. From June to August 2003, maize landraces were collected from smallholder farmers in Zimbabwe, Zambia and Malawi by an expedition team comprising CIMMYT and Ministry of Agriculture staff for each respective country.

The expedition team used a modification of the stratified sampling strategy recommended by Brown and Marshall (1995). The stratification involved a combination of agro-ecological and farming system parameters, and local knowledge by agriculture extension staff. A 1º longitude by 1º latitude grid system was superimposed on a map of the country (i.e. grid squares) and samples were collected in 150 grids across the three countries. The grids spanned across all the principal maize agro-ecologies defined for the three countries (Hartkamp et al., 2000). In each grid, samples were collected at 1-2 randomly chosen farms per agro-ecology. At each farm, 2 kg of seed or an equivalent amount of maize as ears was collected. This sample has been found to adequately represent most of the diversity in a maize population as recommended by Crossa (1989). Passport data and qualitative data on the characteristics of each sample were recorded on a survey form that had been prepared beforehand. Passport data included the name of the farmer, district where the landrace was collected, latitude, longitude, altitude, and soil

type. Qualitative data collected alongside the seed samples included the name of the variety, kernel color, kernel texture, main use of the landrace, unique traits of the variety, and the number of years that the farmers had been cultivating or had knowledge of the landrace. Kernel texture was rated on a scale from 1-5 where 1= flint, 2=semi-flint, 3= semi flint/semi dent, 4= semi dent and 5= dent. Once collected, the germplasm samples and the corresponding data entered the conservation procedures of the National Genebank of each of each respective county.

Landrace Classification Based on Environmental Data for Collecting Sites

In order to classify the maize landraces collected, the 267 samples from Zimbabwe, Zambia and Malawi were grouped into mega-environment regions by overlaying, in GIS Arc/Info and ArcView www.esri.com, the geographic data of the landrace collection points (origin as given by latitude and longitude) with the map of world maize mega-environments developed by Hartkamp et al. (2000). These mega-environment regions had been clustered based on altitude, average maximum temperatures during the growing season, seasonal precipitation, subsoil pH and risk of drought as given in Table 2.1.

Table 2.1. Main characteristics of the predominant maize mega-environments in southern Africa (Vivek et al., 2005).

Mega-environment	Mega-Environment Description	Maximum temperature (°C)	Seasonal precipitation (mm)	Sub-soil pH (water)	Risk of drought
A	Wet Upper Mid Altitude	24-27	> 700	<5.7	Low
B	Wet Lower Mid Altitude	24-27	> 700	>5.7	Low
C	Dry Mid Altitude	24-30	< 700		High
D	Wet Lowland	>30	>700		Low
E	Dry Lowland	27-30	> 700	>5.7	High
F	Highland	>30	> 700		

Lowlands = 0 to 500masl, mid-altitude = 800 to 1500masl; highlands = >1600masl

RESULTS AND DISCUSSION

Collection of Maize Samples

Across the three countries, a total of 267 maize landraces were collected. Local names of the landraces and passport data for the collection sites are presented in Table 2.2. Figure 2.1 shows the range of diversity of some of the ears of the landraces collected. Grain color was predominantly white although other colors were found in the collection (Figure 2.2a). The fact that most of the collected samples were white-colored is not surprising considering that most maize in southern Africa is consumed as food, and consumers strongly prefer white-colored varieties. The preferences of today's African consumers for white as opposed to yellow or other grain color began with the influence of the British starch market during the time when the British expanded maize production into the interior of southern Africa. During this time, the British starch market provided a premium for white maize, and local legislation was passed in some parts of southern Africa requiring that only white maize be accepted for export though both white and yellow maize varieties of maize were grown (Weinmann, 1972).

Across the three countries, national differences were evident in the grain texture of the collected samples (Figure 2.2).

Fig. 2.1. Some of the maize landraces collected from Zimbabwe, Zambia and Malawi.

Hard-grained varieties (flints, semi-flints) were mostly found in Malawi and Zambia and to a lesser extent in Zimbabwe, reflecting differences in post-harvest processing methods. In Malawi and Zambia, farmers prefer flinty grain types, which not only lend themselves more easily to traditional processing methods (hand pounding) but also store better (Hassan et al., 2001). From this survey, it was clear that farmers believe that the flour to bran ratio is higher for flintier versus dent maize after hand grinding or pounding to make maize meal. Soft-grained varieties (dents, semi-dent) were relatively more common in Zimbabwe, where a greater proportion of maize is processed mechanically in hammer mills located throughout the country (Eicher and Kupfuma, 1997). Averaged across the three countries, there were more dent and semi- dent landraces versus flint and semi flint types (Figure 2.3).

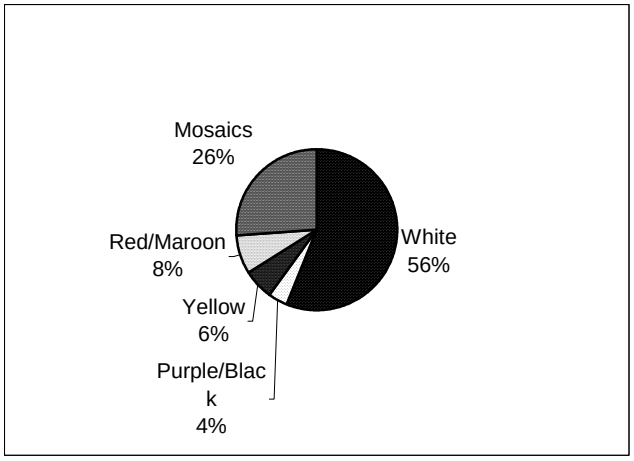

Fig. 2.2. Percentage of grain color types for the different landraces collected in Zimbabwe, Zambia and Malawi.

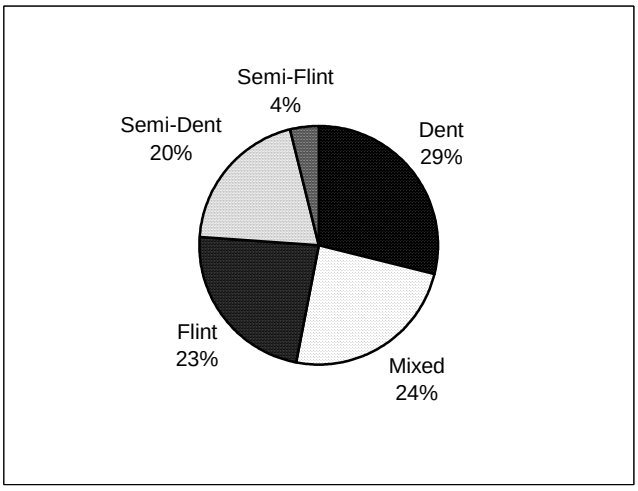

Fig. 2.3. Percentage of grain texture types for the different landraces collected in Zimbabwe, Zambia and Malawi.

Originally in the three countries, local varieties on small-scale farms tended to be flinty while improved varieties grown mostly by commercial farmers tended to be dent (McCann, 2005). However over time, small-scale farmers' fields became cross-pollinated with improved dent varieties such as Hickory King (the improved version), SR52, Southern Cross and Salisbury White from neighboring commercial farms thus introducing the dent texture into the landraces. For the samples collected as ears, the

number of kernel rows on the ears ranged from eight to twenty-two representing a wide diversity for this characteristic.

The maize landraces collected from this survey typically exhibit traits that are not found in most of the improved varieties grown in the three countries such as eight-rowed ears with large grains, red cobs, small flint grains, irregular kernel arrangement on cobs, black, yellow, orange, red, maroon, and red-stripped kernel colors. Comparing with a description of the main characteristics of maize hybrids developed in and released in southern Africa from 1996 to 1997 (Hassan et al., 2001), it was clear that landraces collected in this survey have different morphological characteristics to those of improved varieties. Most of these unique characteristics of landraces trace back to early maize introductions into Africa, such as yellow-orange colored flint "*cateto*" maize introduced in coastal zones of southern Africa by the Portuguese (Miracle, 1966), large grains and eight-rowed Hickory King introduced from the USA (Weinamann, 1972), and maize ears with irregularly arranged large kernels, probably introduced as "Horsetooth" from the USA. This clearly shows that distinct traditional maize varieties still form a major component of the smallholder farming sector in the three countries despite the development and release of modern improved varieties.

In some cases, especially in Zimbabwe and Zambia, an individual farmer was growing more than one landrace. Up to six landraces, each with different morphology were collected from a single farm in Zimbabwe (Table 2.2). It is worth noting that in most of these cases, there was a deliberate effort made to grow and store the different landraces separately. However, isolation distances were not usually adequate due to the small nature of the farmers' fields and small distances between neighboring farms. A few farmers reported planting the landraces in mixture and only selected based on ear characteristics after harvest. These facts result in cross-pollination among the landraces or cross-pollination with improved varieties in neighboring fields resulting in a gradual evolution of the landraces. This situation is inevitable except in very isolated localities. In many cases, smallholder farmers buy new improved maize seeds and plant them alongside other saved landrace varieties. Through promoting the hybridization of among the landraces or landraces with improved varieties, either by design or by accident, then exposing the resultant crosses to their conditions and management, and continually

selecting seed of these varieties for replanting, farmers continually change the genetic composition of traditional varieties. In México, the centre of origin and greatest diversity of maize, smallholder farmers have, in this way, created varieties have been termed "*creolized*" varieties (Bellon, 1991).

Besides the preparation of the main maize staple dish - a thick porridge made by mixing boiling water with ground maize meal and stirring continuously until a thick paste is formed, maize landraces are also used in (i) traditional beer brewing, (ii) brewing of "*maheu*"- a non-alcoholic beverage favored by most rural folk, (iii) preparation of boiled or roasted green mealies, (iv) preparation of other local dishes such as '*samp*' or mealie-rice, (v) making popcorn, (vi) porridge, and (vii) baking mealie-bread. These products are used as snacks mostly, but in some cases they are used as main meals especially during periods of hunger.

Most landraces are maintained by the farmers because of their adaptation to marginal areas and yield stability under both biotic and abiotic stress conditions. The main factors mentioned by the farmers were tolerance to drought, early maturity, low input requirements, and resistance to storage weevils. The underlying factor is obtaining a sufficient harvest in an uncertain environment. Data presented by Hassan et al. (2001) shows that the majority of hybrids available in Zimbabwe, Zambia and Malawi were intermediate to late maturing and thus not popular with smallholder farmers in the three countries.

Maize landraces are also maintained in the three countries because of their unique or better taste over commercial varieties. Better ear and grain appearance and good post-harvest processing qualities were mentioned by the farmers as some of the reasons why they continue to grow landraces despite the advent of improved varieties. Non-the-less a consistent part of the landraces is maintained because of seed security issues. Most of the farmers who grow maize landraces stated that improved varieties, especially hybrids, could not be successfully used as saved seed in comparison to the landraces. Most of the farmers used saved landrace seed in periods of great distress e.g. drought years, or as a security precaution.

All the 267 seed samples derived from the collection will be stored in the National Genebanks of the three countries. These genebanks are part of the SADC Plant Genetic

Resources Network and the data and seeds will be internationally available after agronomic and molecular characterization and seed multiplication. Information about specific variety characteristics that the farmers provided will be entered in the database, alongside passport information for each of the collected landraces. The characterization and evaluation of the material will be carried out by CIMMYT and the respective National Genebank of the three countries. The 100 landraces samples collected from Zimbabwe constitute the first comprehensive maize genetic resources ever collected and conserved in the country, while the 167 samples collected from Zambia and Malawi form a significant addition to the already stored maize germplasm in the genebanks for the two countries.

The importance of maize germplasm conservation has been highlighted by many authors especially for Central America, the area where maize was domesticated (Manglesdorp, 1974). The necessity to preserve genetic resources appeared after the introduction of the first commercial hybrids starting in the USA and led to the birth of many national maize collections worldwide. Even though Zimbabwe, Zambia and Malawi are not centers of origin of maize, that fact that diverse types of maize were introduced in this area from different parts of the world and underwent both natural and human selection for local adaptation and consumer preference for over 100 years probably resulted in a set of maize landraces that represent a unique source of genetic diversity that merits collection, characterization, conservation and utilization in crop improvement programs. In the USA, thousands of accessions of maize that formed the foundation of the present maize varieties are maintained in germplasm banks across the country. Elsewhere, in 1996 seven European countries, France, Germany, Greece, Italy, Portugal, Spain and the Netherlands, started a common program of preservation, evaluation and use of maize landrace genetic resources and currently over 2,900 accessions exist in the collection (Gauthier et al., 2002). In Asia, large efforts have been made to conserve maize landraces with China leading the region with more than 17,000 accessions in its genebanks (Li et al., 2004). All these effort indicate that unique and useful maize genetic resources not only occur in the main centers of the origin of a crop, but diversity also results from both farmer and natural selection for specific adaptation and consumer use in different agro-ecologies and societies outside central America.

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Aggregation of the collected samples by country and mega-environment showed that maize landraces are still cultivated in all of the MEs found in the three countries (Table 2.2 and Figure 2.4).

Table 2.2. Landraces collected in different agro-ecologies in Zimbabwe, Zambia and Malawi classified according to the Mega-environment of collection.

Country	Mega-environment					
	A	B	C	E	F	Total
Zimbabwe	22	28	13	37	0	100
Zambia	67	35	0	9	0	111
Malawi	26	17	0	10	3	56
Total	115	80	13	56	3	267

The collection expedition covered a wide range of microclimates, farming areas, altitudes, agro-ecological zones, soil types, and farmer management conditions throughout Zimbabwe, Zambia and Malawi. Information on the geographic distribution of genetic variation of a crop species is important for planning future germplasm collection missions and for efficient utilization of collected germplasm in crop improvement programs (Steiner and Greene, 1996). Therefore, an effort was made in the present survey to assess patterns of diversity distribution in relation to mega-environment in the three countries. The fact that maize landraces are presently cultivated across the predominant agro-ecological regions of the three countries shows how widely the crop has adapted since the introduction of a set of OPVs at the turn of the century (Weinamann, 1972; McCann, 2005).

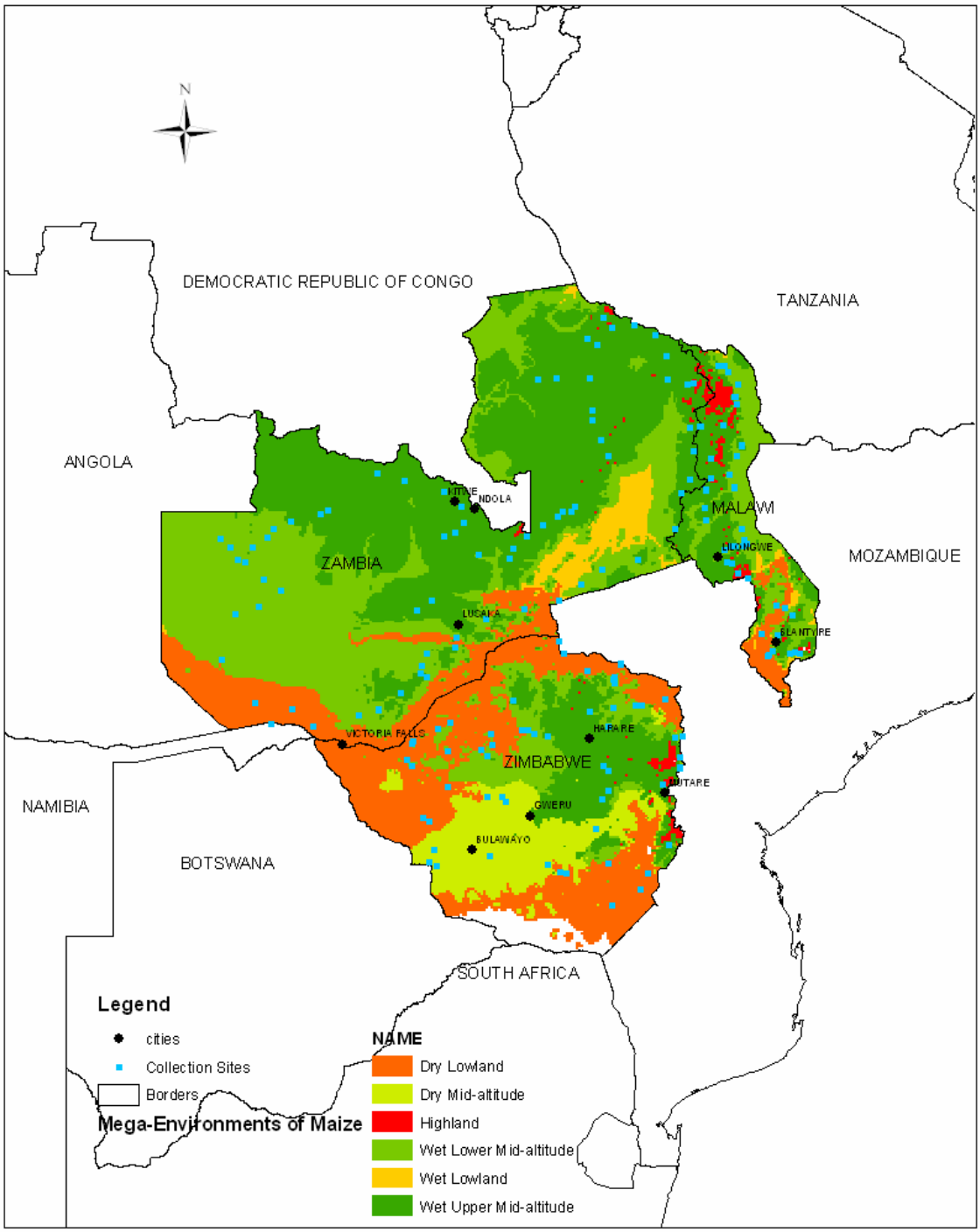

Fig. 2.4. Areas where maize landraces where collected.
The map was constructed using ESRI software Arc/Info version 7 (http://www.esri.com/base/products/arcinfo/arcinfo.html) and ArcView version 3a http://www.esri.com/base/products/arcview/arcview.html).

The current wide diversity of traditional maize probably resulted from both farmer and natural selection acting on the introduced varieties, and intercrossing through trading of grain and exchange of seeds. An example of an introduced OPV is the variety Hickory King (HK). Various versions of HK were collected from this study, dent types, semi-dent types, semi-flint types, six-rowed types, eight-rowed types, ten-rowed types, maroon and black kernelled types. An example of an obsolete OPV that is still grown by farmers is Matopo Topcross which was released in 1956 by the Zimbabwean government (Eicher and Kupfuma, 1997). Results from this survey show that some farmers in Gokwe district in Zimbabwe still cultivate this variety (Table 2.3). Yet another example of an obsolete OPV is Kalahari Early Pearl (KEP), introduced into Zimbabwe from Botswana to address the requirements of farmers in marginal areas of Zimbabwe after 1980 (Bourdillon et al., 2002). This OPV was grown only for short period due to government restrictions in the early 1980s, but farmers have continued to maintain different versions of this OPV as shown in Table 2.3.

The elevation of collection sites for the landraces collected in this survey ranged from 93 to 1822 meters above sea level (Table 2.2). Considering this wide range in variation and to give proper representation of accessions from each elevation zone, collection sites were classified as lowland tropical (<400masl), mid-altitude (400–1500masl), and highlands (>1500masl). This classification resulted in 56 lowland tropical, 208 mid-altitude, and 3 highland accessions (Table 2.2). These groups are roughly comparable to the region's three agro-ecological zones: lowveld, middleveld to highveld, and highlands (Vincent and Thomas, 1960). The lowveld is characterized by an elevation ranging from 0–400masl, mean annual temperature of 25–31ºC and mean annual rainfall of 80–400mm. Similarly, the middleveld to highveld ranges in elevation from 400–1600masl with a mean annual temperature of 22ºC and mean annual rainfall of 400–1200 mm and the highlands with elevations above 1600masl and mean annual temperature of 16ºC and mean annual rainfall of 1000–1200 mm (Vincent and Thomas, 1960).

The results of the present study imply that environmental factors such as soil, elevation, temperature and rainfall are the important determinants of variation patterns of maize landrace diversity in Zimbabwe, Zambia and Malawi. These factors are useful in

predicting the genetic characteristics of the collected samples. Natural selection pressure for adaptation to different elevations coupled with farmers' selection for cultivation under a wide range of growing conditions could account for observed diversity patterns. These results can also be used when planning future collecting missions so that particular areas can be targeted for their habitat or soil type. Gap analysis can also be conducted to determine areas of importance to collect material for conservation. Also, if a particular landrace is required, its specific eco-geographic requirements can be targeted.

The results from this survey lead to the hypothesis that variability in abiotic stress tolerances exist among the landraces and suggest that these are related to geographic origin. Furthermore, the use of a representative sample of the collection can help to identify segments of the collection that are especially promising as sources of desirable traits for abiotic stress tolerance.

CONCLUSIONS

A total of 267 distinct landraces and traditional varieties of maize were collected from farmers in the three countries. Both field and molecular characterization of populations will be conducted to quantify the levels of diversity and determine genetic relationships among them. Seed samples of the populations will be made available by each country to bona fide research workers and plant breeders after the completion of field, greenhouse and laboratory trials. The objective of conserving this collection is to preserve the diversity in the maize landraces before much loss as farmers are shifting to planting modern hybrids. This study presents the first report of the range of variability of maize landraces and traditional varieties in Zimbabwe, Zambia and Malawi and provides important baseline data for future diversity assessments in the three countries.

This collection survey also showed that a diverse array of maize landraces continues to have private value for smallholder farmers in the three countries. The main factors that favor continued cultivation of the landraces within farming systems in the country include the heterogeneity in the physical, economic, and cultural contexts of local smallholder agriculture. Cultivation of traditional maize landraces also takes place in order to ensure a continuous seed supply for the future. The reasons why landraces are

conserved on-farm as outlined in this study, reflects their specific adaptation to drought and low soil fertility that frequently occur in the collection sites, and this warrants their continued collection, characterization, preservation and use in crop improvement programs. This is particularly crucial considering the rapid replacement of traditional landraces by modern hybrids from large seed companies now operating in the three countries.

The results from this survey also confirm that a diverse set of maize landraces continue to be cultivated and maintained by farmers in all the principal agro-ecological zones of the three countries, and that this diversity is related to geographic origin of the landrace. Furthermore, this landrace diversity could be associated with diversity for abiotic stress tolerance since the geographic areas where the landraces were collected are frequently subjected to different types and intensities of drought, and low soil fertility – the major abiotic stresses occurring in the three countries.

Table 2.3. Maize landraces collected during the survey and geographic data for the collection sites in Zimbabwe, Zambia and Malawi.

Acc	Code	Landrace Name	Country	District	ME	Megaenvironment	Long	Lat	Alt	Rain Start	Ann Precip	Temperature Ave	Temperature Min	Temperature Max	Soil Type
1	MMM-001	Garaba	ZIM	Musana	B	Wet-Lower-Mid-altitude	31.46	-17.56	1186	11	787	22.6	17.0	28.2	Luvisols
2	MMM-003	Hickory-King	ZIM	Bushu	B	Wet-Lower-Mid-altitude	31.57	-17.15	1005	11	748	23.1	17.5	28.8	Luvisols
3	MMM-012	Bharabhara	ZIM	Mt.-Darwin	B	Wet-Lower-Mid-altitude	31.59	-16.64	944	11	695	23.6	18.0	29.2	Luvisols
4	MMM-016	Hickory-King	ZIM	Mt.-Darwin	B	Wet-Lower-Mid-altitude	31.56	-16.57	1079	11	741	22.9	17.4	28.4	Luvisols
5	MMM-019	Hickory-King	ZIM	Mt.-Darwin	B	Wet-Lower-Mid-altitude	31.55	-16.57	1054	11	741	22.9	17.4	28.4	Luvisols
6	MMM-020	Kenya	ZIM	Mt.-Darwin	B	Wet-Lower-Mid-altitude	31.57	-16.56	1033	11	741	22.9	17.4	28.4	Luvisols
7	MMM-021	Kamoza	ZIM	Mt.-Darwin	B	Wet-Lower-Mid-altitude	31.57	-16.56	1033	11	741	22.9	17.4	28.4	Luvisols
8	MMM-022	Red-Colored-Local	ZIM	Mt.-Darwin	B	Wet-Lower-Mid-altitude	31.57	-16.56	1033	11	741	22.9	17.4	28.4	Luvisols
9	MMM-023	Small-Yellow-Flint-Local	ZIM	Mt.-Darwin	B	Wet-Lower-Mid-altitude	31.57	-16.52	1043	11	757	22.7	17.2	28.1	Luvisols
10	MMM-026	Mukadziusaende	ZIM	Mt.-Darwin	E	Dry-Lowland	31.72	-16.24	464	11	630	26.6	20.5	32.6	Lithosols
11	MMM-027	Mbanga	ZIM	Centenary	E	Dry-Lowland	31.00	-16.39	415	11	638	26.8	21.1	32.6	Luvisols
12	MMM-028-	Red-Local	ZIM	Centenary	E	Dry-Lowland	31.00	-16.39	415	11	638	26.8	21.1	32.6	Luvisols
13	MMM-029	Local-(Wine-Colored)	ZIM	Centenary	E	Dry-Lowland	31.00	-16.39	415	11	638	26.8	21.1	32.6	Luvisols
14	MMM-030	Kenya	ZIM	Centenary	E	Dry-Lowland	31.00	-16.39	415	11	638	26.8	21.1	32.6	Luvisols
15	MMM-031	Local-(Black)	ZIM	Centenary	E	Dry-Lowland	31.00	-16.39	415	11	638	26.8	21.1	32.6	Luvisols
16	MMM-033	Kanongo	ZIM	Guruve	E	Dry-Lowland	30.51	-16.03	395	11	653	27.3	21.7	32.9	Luvisols
17	MMM-044	Kanongo	ZIM	Guruve	E	Dry-Lowland	30.41	-15.74	383	11	669	27.5	21.9	33.2	Luvisols
18	MMM-045	Local	ZIM	Guruve	E	Dry-Lowland	30.41	-15.74	383	11	669	27.5	21.9	33.2	Luvisols
19	MMM-047	Hickory-King	ZIM	Hurungwe	B	Wet-Lower-Mid-altitude	29.45	-17.01	1235	11	759	23.3	18.4	28.2	Lithosols
20	MMM-050	White-Flint	ZIM	Mt.-Darwin	B	Wet-Lower-Mid-altitude	31.57	-16.56	1033	11	741	22.9	17.4	28.4	Luvisols
21	MMM-051	Local-(Wine-Colored)	ZIM	Mt.-Darwin	B	Wet-Lower-Mid-altitude	31.57	-16.56	1033	11	741	22.9	17.4	28.4	Luvisols
22	MMM-052	16-Line	ZIM	Mt.-Darwin	B	Wet-Lower-Mid-altitude	31.57	-16.56	1033	11	741	22.9	17.4	28.4	Luvisols
23	MMM-053	Local-(Maroon-w/-white-tips)	ZIM	Mt.-Darwin	B	Wet-Lower-Mid-altitude	31.57	-16.56	1033	11	741	22.9	17.4	28.4	Luvisols
24	MMM-054	Hickory-King	ZIM	Mt.-Darwin	B	Wet-Lower-Mid-altitude	31.57	-16.56	1033	11	741	22.9	17.4	28.4	Luvisols
25	MMM-055	Mozambique	ZIM	Mt.-Darwin	E	Dry-Lowland	31.70	-16.22	460	11	641	26.5	20.4	32.5	Lithosols
26	MMM-057	Mabahaudhi/Garaba/Hickory-King	ZIM	Zvimba	A	Wet-Upper-Mid-altitude	30.16	-17.77	1288	11	740	21.5	16.0	27.1	Luvisols
27	MMM-058	Chemavara	ZIM	Zvimba	A	Wet-Upper-Mid-altitude	30.17	-17.77	1288	11	740	21.5	16.0	27.1	Luvisols
28	MMM-059-	Kenya	ZIM	Zvimba	A	Wet-Upper-Mid-altitude	30.17	-17.77	1288	11	740	21.5	16.0	27.1	Luvisols
29	MMM-070	Garaba	ZIM	Makonde	A	Wet-Upper-Mid-altitude	29.88	-17.70	1039	11	735	22.5	17.0	28.0	Lithosols
30	MMM-072	Local-(Small-White-Flint)	ZIM	Makonde	A	Wet-Upper-Mid-altitude	29.88	-17.70	1039	11	735	22.5	17.0	28.0	Lithosols
31	MMM-073	Kalahari-Kenya	ZIM	Makonde	B	Wet-Lower-Mid-altitude	29.75	-17.63	986	11	725	23.7	18.3	29.2	Lithosols
32	MMM-074	Kalahari-8-Line	ZIM	Gokwe-North	E	Dry-Lowland	28.99	-17.72	885	11	721	24.8	19.6	30.0	Lithosols
33	MMM-075	3-Months	ZIM	Gokwe-North	E	Dry-Lowland	28.99	-17.72	885	11	721	24.8	19.6	30.0	Lithosols
34	MMM-078	Kenya	ZIM	Makonde	B	Wet-Lower-Mid-altitude	29.64	-17.63	904	11	721	24.3	18.9	29.8	Lithosols
35	MMM-079	Local-(Purple)	ZIM	Makonde	B	Wet-Lower-Mid-altitude	29.64	-17.63	904	11	721	24.3	18.9	29.8	Lithosols

Table 2.3 Continued

Acc	Code	Landrace Name	Country	District	ME	Megaenvironment	Long	Lat	Alt	Rain Start	Ann Precip	Ave	Temperature Min	Temperature Max	Soil Type
36	MMM-080	Botoma-8-Line	ZIM	Gokwe-North	B	Wet-Lower-Mid-altitude	28.99	-17.95	1120	11	703	24.6	19.2	30.0	Lithosols
37	MMM-081	Local-(Mixed-Black-and-White)	ZIM	Gokwe-North	B	Wet-Lower-Mid-altitude	28.99	-17.95	1120	11	703	24.6	19.2	30.0	Lithosols
38	MMM-088	Matobo	ZIM	Gokwe-North	B	Wet-Lower-Mid-altitude	28.99	-17.96	1127	11	703	24.6	19.2	30.0	Lithosols
39	MMM-089	Matopo-8-Line	ZIM	Gokwe-North	B	Wet-Lower-Mid-altitude	28.99	-17.96	1127	11	703	24.6	19.2	30.0	Lithosols
40	MMM-100	Bogwe-8-Line	ZIM	Gokwe-South	B	Wet-Lower-Mid-altitude	28.89	-18.19	1192	11	695	23.0	17.6	28.5	Arenosols
41	MMM-105	Local-(Mixed-Black-and-White)	ZIM	Gokwe-South	B	Wet-Lower-Mid-altitude	28.89	-18.19	1192	11	695	23.0	17.6	28.5	Arenosols
42	MMM-106	Bhunu-8-Line	ZIM	Gokwe-South	E	Dry-Lowland	28.77	-18.06	889	11	674	24.8	19.4	30.3	Lithosols
43	MMM-111	Kabhalebhale/Kaile/Katata	ZIM	Binga	E	Dry-Lowland	28.10	-17.47	664	11	672	25.8	21.0	30.5	Lithosols
44	MMM-118	8-Line	ZIM	Binga	E	Dry-Lowland	28.11	-17.47	664	11	672	25.8	21.0	30.5	Lithosols
45	MMM-122	Kaile	ZIM	Binga	E	Dry-Lowland	27.86	-17.61	666	11	653	26.6	21.8	31.4	Lithosols
46	MMM-129	Kaile	ZIM	Binga	E	Dry-Lowland	27.34	-17.87	618	11	616	26.5	21.4	31.7	Luvisols
47	MMM-130	Katonga	ZIM	Binga	E	Dry-Lowland	27.29	-17.91	568	11	606	26.8	21.5	32.0	Luvisols
48	MMM-134	Bhabadhla	ZIM	Binga	E	Dry-Lowland	27.19	-18.27	913	11	630	24.8	19.2	30.4	Luvisols
49	MMM-138	Chisalala-(Red)	ZIM	Binga	B	Wet-Lower-Mid-altitude	27.30	-18.40	1051	11	641	23.7	17.9	29.4	Luvisols
50	MMM-139	Kaile	ZIM	Binga	B	Wet-Lower-Mid-altitude	27.30	-18.40	1051	11	641	23.7	17.9	29.4	Luvisols
51	MMM-144	Bhabadhla---Red-Cob	ZIM	Lupane	E	Dry-Lowland	28.07	-18.84	992	11	546	25.4	19.1	31.8	Arenosols
52	MMM-145	Bhabadhla---White-Cob	ZIM	Lupane	E	Dry-Lowland	28.07	-18.09	904	11	671	24.5	19.0	30.0	Luvisols
53	MMM-153	Siquesibovu-(Red-Cob)	ZIM	Nkayi	C	Dry-Mid-altitude	28.90	-19.04	1173	11	583	22.9	16.9	28.9	Arenosols
54	MMM-154	Karifonia/Mbudhlwana	ZIM	Nkayi	C	Dry-Mid-altitude	28.90	-19.04	1173	11	583	22.9	16.9	28.9	Arenosols
55	MMM-155	Bogwe	ZIM	Kwekwe	C	Dry-Mid-altitude	29.21	-19.06	1272	11	575	22.4	16.5	28.3	Luvisols
56	MMM-168	Matopo/Karifonia/3-Months	ZIM	Kwekwe	C	Dry-Mid-altitude	29.29	-19.14	1302	11	561	22.3	16.4	28.2	Arenosols
57	MMM-182	Bhabadhla	ZIM	Tsholotsho	E	Dry-Lowland	27.67	-19.57	1049	11	513	24.0	17.2	30.7	Arenosols
58	MMM-185	Bhabadhla/Ihwanqa	ZIM	Tsholotsho	E	Dry-Lowland	27.54	-19.49	1032	11	509	24.1	17.3	30.8	Arenosols
59	MMM-189	Umumbu-we-Sikalanga	ZIM	Bulilimamangwe	C	Dry-Mid-altitude	27.84	-20.51	1366	11	503	21.8	15.9	27.6	Arenosols
60	MMM-193	Malaba/Kalanga	ZIM	Bulilimamangwe	C	Dry-Mid-altitude	27.66	-20.43	1314	11	494	22.6	16.6	28.6	Arenosols
61	MMM-205	Gushe	ZIM	Umzingwane	C	Dry-Mid-altitude	28.94	-20.31	1173	11	553	21.5	15.6	27.5	Luvisols
62	MMM-206	Hwaqa	ZIM	Umzingwane	C	Dry-Mid-altitude	28.94	-20.31	1173	11	553	21.5	15.6	27.5	Luvisols
63	MMM-207	Red-Cork	ZIM	Zvishavane	C	Dry-Mid-altitude	30.18	-20.47	893	11	494	23.6	17.9	29.4	Luvisols
64	MMM-214	Red-Cob	ZIM	Mberengwa	E	Dry-Lowland	30.44	-20.63	792	11	512	24.7	18.8	30.6	Lithosols
65	MMM-217	Kalahari-8-Line	ZIM	Chivi-South	C	Dry-Mid-altitude	30.56	-20.67	693	11	535	24.1	18.3	30.0	Luvisols
66	MMM-223	Red-Local	ZIM	Makonde	B	Wet-Lower-Mid-altitude	29.75	-17.63	986	11	725	23.7	18.3	29.2	Lithosols
67	MMM-224	Chibage-Chitsvuku	ZIM	Marondera	A	Wet-Upper-Mid-altitude	31.36	-18.38	1528	11	684	20.4	15.0	25.8	Luvisols
68	MMM-225	Red-Cob	ZIM	Marondera	A	Wet-Upper-Mid-altitude	31.36	-18.38	1528	11	684	20.4	15.0	25.8	Luvisols
69	MMM-226	Hickory-King	ZIM	Marondera	A	Wet-Upper-Mid-altitude	31.36	-18.38	1528	11	684	20.4	15.0	25.8	Luvisols
70	MMM-229-	Hickory-King	ZIM	Marondera	A	Wet-Upper-Mid-altitude	31.46	-18.49	1444	11	692	21.1	15.6	26.6	Luvisols
71	MMM-231	Chitsvuku	ZIM	Chikomba	A	Wet-Upper-Mid-altitude	31.46	-18.91	1357	11	692	20.8	15.3	26.3	Luvisols

Table 2.3 Continued

Acc	Code	Landrace Name	Country	District	ME	Megaenvironment	Long	Lat	Alt	Rain Start	Ann Precip	Temperature Ave	Temperature Min	Temperature Max	Soil Type
72	MMM-232	Local-(Wine-Color)	ZIM	Chikomba	A	Wet-Upper-Mid-altitude	31.46	-18.91	1357	11	692	20.8	15.3	26.3	Luvisols
73	MMM-233	Hickory-King	ZIM	Chikomba	A	Wet-Upper-Mid-altitude	31.46	-18.91	1357	11	692	20.8	15.3	26.3	Luvisols
74	MMM-234	Chindawu	ZIM	Chikomba	A	Wet-Upper-Mid-altitude	31.46	-18.91	1357	11	692	20.8	15.3	26.3	Luvisols
75	MMM-239	Hickory-King	ZIM	Chikomba	A	Wet-Upper-Mid-altitude	31.32	-19.09	1319	11	688	21.1	15.6	26.7	Luvisols
76	MMM-240	Kenya	ZIM	Gutu-North	C	Dry-Mid-altitude	31.19	-19.72	1347	11	583	20.5	15.2	25.9	Luvisols
77	MMM-241	Hickory-King	ZIM	Gutu-North	C	Dry-Mid-altitude	31.19	-19.71	1365	11	583	20.5	15.2	25.9	Luvisols
78	MMM-244	Spotted-Local	ZIM	Zaka	E	Dry-Lowland	31.46	-20.44	721	11	670	24.6	18.7	30.5	Luvisols
79	MMM-245	Hickory-King	ZIM	Zaka	E	Dry-Lowland	31.46	-20.44	721	11	670	24.6	18.7	30.5	Luvisols
80	MMM-246	Abatonga	ZIM	Chiredzi	E	Dry-Lowland	31.46	-20.44	721	11	670	24.6	18.7	30.5	Luvisols
81	MMM-247	Matuba	ZIM	Chiredzi	E	Dry-Lowland	31.46	-20.44	721	11	670	24.6	18.7	30.5	Luvisols
82	MMM-248	Chibhubhane/Mahloatiwa	ZIM	Chiredzi	E	Dry-Lowland	31.52	-21.33	393	11	443	25.7	19.6	31.8	Vertisols
83	MMM-255	Chibage-che-Chivanhu	ZIM	Chiredzi	E	Dry-Lowland	32.12	-21.01	384	11	433	25.7	19.7	31.6	Vertisols
84	MMM-266	Chindawu	ZIM	Chipinge	E	Dry-Lowland	32.28	-20.66	402	11	457	25.3	19.4	31.2	Luvisols
85	MMM-273	Nyaguru	ZIM	Mutasa	A	Wet-Upper-Mid-altitude	32.60	-18.79	1141	11	799	19.9	15.2	24.5	Luvisols
86	MMM-277	Chimanyika	ZIM	Mutasa	A	Wet-Upper-Mid-altitude	32.97	-18.43	704	11	1067	20.4	16.0	24.9	Ferralsols
87	MMM-280	Chindawu	ZIM	Chipinge	A	Wet-Upper-Mid-altitude	32.73	-20.06	963	11	803	22.4	17.1	27.7	Ferralsols
88	MMM-281	Samanyika	ZIM	Mutasa	A	Wet-Upper-Mid-altitude	32.97	-18.43	865	11	1067	20.4	16.0	24.9	Ferralsols
89	MMM-284	Chimanyika	ZIM	Nyanga	A	Wet-Upper-Mid-altitude	32.96	-18.20	1525	11	987	21.0	16.4	25.6	Ferralsols
90	MMM-286	Njeke	ZIM	Nyanga-North	B	Wet-Lower-Mid-altitude	33.01	-17.78	832	11	927	23.6	18.2	29.0	Luvisols
91	MMM-290	Njeke/Hickory-King	ZIM	Nyanga	B	Wet-Lower-Mid-altitude	32.87	-17.79	905	11	811	22.8	17.6	28.0	Luvisols
92	MMM-298	Mbuyaingafe	ZIM	Mudzi	E	Dry-Lowland	32.65	-16.97	721	11	595	24.9	19.0	30.9	Luvisols
93	MMM-299	Kanjerenjere	ZIM	Mudzi	E	Dry-Lowland	32.65	-16.97	721	11	595	24.9	19.0	30.9	Luvisols
94	MMM-300	Mbuyamusafe	ZIM	Mudzi	E	Dry-Lowland	32.65	-16.97	721	11	595	24.9	19.0	30.9	Luvisols
95	MMM-305	Hickory-King	ZIM	Mazowe	A	Wet-Upper-Mid-altitude	31.06	-17.24	1145	11	792	22.1	16.6	27.6	Luvisols
96	MMM-306	Mbanga	ZIM	Centenary	-	-	-	-	-	-	-	-	-	-	-
97	MMM-315	Mbuyaingafe	ZIM	Mutoko	E	Dry-Lowland	32.16	-17.12	1211	11	594	24.4	18.6	30.1	Luvisols
98	MMM-316	Hickory-King	ZIM	Mutoko	B	Wet-Lower-Mid-altitude	32.03	-17.11	-	11	621	23.9	18.2	29.6	Luvisols
99	MMM188	UMUMBU-WESIKALANGA	ZIM	Bulilimamangwe	C	Dry-Mid-altitude	27.76	-20.17	-	11	519	22.8	16.6	29.0	Arenosols
100	MMM272	CHINDAWU	ZIM	Chipinge	-	-	-	-	-	-	-	-	-	-	-
101	W-001	Kambiri	MW	Kasungu	A	Wet-Upper-Mid-altitude	33.50	-13.21	1015	11	813	22.5	17.6	27.5	Ferralsols
102	W-002	Local	MW	Kasungu	A	Wet-Upper-Mid-altitude	33.50	-13.21	1172	11	813	22.5	17.6	27.5	Ferralsols
103	W-003	Local	MW	Kasungu	A	Wet-Upper-Mid-altitude	33.49	-12.94	1219	11	805	23.0	18.1	27.9	Ferralsols
104	W-004	Local	MW	Kasungu	A	Wet-Upper-Mid-altitude	33.52	-12.57	1524	12	807	21.2	16.4	26.0	Ferralsols
105	W-005	Local	MW	Kasungu	A	Wet-Upper-Mid-altitude	33.52	-12.54	1524	12	807	21.2	16.4	26.0	Ferralsols
106	W-006	Local	MW	Mzimba	A	Wet-Upper-Mid-altitude	33.62	-12.30	1513	12	826	21.0	16.3	25.8	Ferralsols
107	W-007	Masika	MW	Mzimba	A	Wet-Upper-Mid-altitude	33.62	-12.30	1386	12	826	21.0	16.3	25.8	Ferralsols
108	W-008	Local	MW	Mzimba	A	Wet-Upper-Mid-altitude	33.63	-12.31	1379	12	837	21.1	16.5	25.8	Ferralsols
109	W-009	Local	MW	Mzimba	A	Wet-Upper-Mid-altitude	33.62	-11.90	499	11	815	20.9	16.1	25.8	Lithosols

Table 2.3 Continued

Acc	Code	Landrace Name	Country	District	ME	Megaenvironment	Long	Lat	Alt	Rain Start	Ann Precip	Temperature Ave	Temperature Min	Temperature Max	Soil Type
110	W-010	Local	MW	Mzimba	A	Wet-Upper-Mid-altitude	33.61	-11.89	594	11	815	20.9	16.1	25.8	Lithosols
111	W-011	Local	MW	Mzimba	B	Wet-Lower-Mid-altitude	33.91	-9.94	579	12	996	25.4	21.3	29.4	Fluvisols
112	W-012	Local	MW	Mzimba	B	Wet-Lower-Mid-altitude	33.77	-9.95	514	12	1031	24.5	20.3	28.7	Lithosols
113	W-013	Local	MW	Karonga	B	Wet-Lower-Mid-altitude	33.78	-9.94	506	12	1012	25.1	21.0	29.3	Lithosols
114	W-014	Local	MW	Karonga	B	Wet-Lower-Mid-altitude	33.97	-10.06	920	12	984	24.6	20.5	28.7	Fluvisols
115	W-015	Local	MW	Karonga	B	Wet-Lower-Mid-altitude	34.18	-10.34	920	12	921	25.0	20.9	29.1	Fluvisols
116	W-016	Local	MW	Rumphi	A	Wet-Upper-Mid-altitude	34.13	-10.59	1036	12	876	23.4	19.3	27.5	Lithosols
117	W-017	Local	MW	Rumphi	A	Wet-Upper-Mid-altitude	34.13	-10.59	1036	12	876	23.4	19.3	27.5	Lithosols
118	W-018	Local	MW	Rumphi	B	Wet-Lower-Mid-altitude	34.17	-10.74	1036	12	908	24.9	20.9	28.9	Nitosols
119	W-019	Bingo	MW	Rumphi	B	Wet-Lower-Mid-altitude	34.17	-10.74	1113	12	908	24.9	20.9	28.9	Nitosols
120	W-020	Local	MW	Rumphi	B	Wet-Lower-Mid-altitude	34.17	-10.74	555	12	908	24.9	20.9	28.9	Nitosols
121	W-021	Local	MW	Mzimba	A	Wet-Upper-Mid-altitude	33.89	-11.19	642	12	764	22.0	17.8	26.1	Ferralsols
122	W-022	Local	MW	Nkhata-Bay	A	Wet-Upper-Mid-altitude	34.27	-11.62	483	12	1052	22.4	18.3	26.5	Lithosols
123	W-023	Local	MW	Nkhata-Bay	A	Wet-Upper-Mid-altitude	34.28	-11.64	510	12	1068	22.2	18.2	26.3	Lithosols
124	W-024	Local	MW	Chinteche	B	Wet-Lower-Mid-altitude	34.17	-11.84	510	12	1162	25.0	21.1	29.0	Nitosols
125	W-025	Local	MW	Nkhotakota	B	Wet-Lower-Mid-altitude	34.00	-12.24	509	12	1113	24.7	20.5	28.8	Water
126	W-026	Bantam	MW	Nkhotakota	B	Wet-Lower-Mid-altitude	34.12	-12.52	491	12	1180	25.2	21.0	29.3	Ferralsols
127	W-027	Local	MW	Nkhotakota	B	Wet-Lower-Mid-altitude	34.12	-12.50	533	12	1180	25.2	21.0	29.3	Ferralsols
128	W-028	Local	MW	Nkhotakota	B	Wet-Lower-Mid-altitude	34.27	-13.37	523	12	1033	24.0	19.4	28.6	Water
129	W-029	Local	MW	Salima	B	Wet-Lower-Mid-altitude	34.32	-13.62	609	12	953	24.6	19.9	29.3	Fluvisols
130	W-030	Local	MW	Salima	B	Wet-Lower-Mid-altitude	34.37	-13.70	914	12	929	24.9	20.2	29.7	Fluvisols
131	W-031	Local	MW	Salima	B	Wet-Lower-Mid-altitude	34.30	-13.75	1233	12	907	24.5	19.8	29.3	Fluvisols
132	W-032	Local	MW	Dowa	A	Wet-Upper-Mid-altitude	34.15	-13.74	1218	12	856	22.9	18.1	27.6	Luvisols
133	W-033	Local	MW	Dowa	A	Wet-Upper-Mid-altitude	34.03	-13.75	1139	12	812	21.0	16.4	25.7	Luvisols
134	W-034	Local	MW	Lilongwe	A	Wet-Upper-Mid-altitude	33.90	-13.82	1280	11	795	22.2	17.3	27.1	Luvisols
135	W-035	Local	MW	Lilongwe	A	Wet-Upper-Mid-altitude	33.95	-14.09	1431	12	782	21.0	16.3	25.8	Luvisols
136	W-036	Monsanto	MW	Lilongwe	A	Wet-Upper-Mid-altitude	34.05	-14.12	1431	12	789	21.8	17.0	26.6	Luvisols
137	W-037	Local	MW	Dedza	A	Wet-Upper-Mid-altitude	34.19	-14.32	1524	12	848	19.7	15.3	24.1	Luvisols
138	W-038	Hybrid	MW	Dedza	A	Wet-Upper-Mid-altitude	34.19	-14.32	1525	12	848	19.7	15.3	24.1	Luvisols
139	W-039	Local	MW	Villa-Ulongwe	F	Highland	34.40	-14.42	590	12	882	19.5	15.2	23.8	Luvisols
140	W-040	Local	MW	Dedza	F	Highland	34.41	-14.42	508	12	882	19.5	15.2	23.8	Luvisols
141	W-041	Local	MW	Balaka	E	Dry-Lowland	35.00	-15.01	771	11	767	25.4	20.6	30.2	Fluvisols
142	W-042	MH18	MW	Machinga	E	Dry-Lowland	35.18	-15.05	616	11	792	26.0	21.1	30.9	Fluvisols
143	W-043	Local	MW	Zomba	A	Wet-Upper-Mid-altitude	35.35	-15.20	726	11	1018	23.1	18.5	27.7	Lithosols
144	W-044	Local	MW	Mlanje	F	Highland	35.49	-16.01	712	12	1525	16.4	12.1	20.6	Lithosols
145	W-045	Local	MW	Mlanje	A	Wet-Upper-Mid-altitude	35.40	-15.99	93	12	1058	21.9	17.4	26.5	Nitosols
146	W-046	Local	MW	Thyolo	B	Wet-Lower-Mid-altitude	35.29	-16.02	93	12	947	24.8	19.8	29.8	Nitosols

Table 2.3 Continued

Acc	Code	Landrace Name	Country	District	ME	Megaenvironment	Long	Lat	Alt	Rain Start	Ann Precip	Temperature Ave	Temperature Min	Temperature Max	Soil Type
147	W-047	Hybrid	MW	Chikwava	E	Dry-Lowland	34.85	-16.04	93	11	681	28.0	22.3	33.7	Fluvisols
148	W-048	Local	MW	Chikwava	E	Dry-Lowland	34.85	-16.04	94	11	681	28.0	22.3	33.7	Fluvisols
149	W-049	Local	MW	Chikwava	E	Dry-Lowland	34.85	-16.04	441	11	681	28.0	22.3	33.7	Fluvisols
150	W-050	Local	MW	Chikwava	E	Dry-Lowland	34.82	-16.07	441	11	677	28.0	22.2	33.7	Fluvisols
151	W-051	Local	MW	Blantyre	E	Dry-Lowland	34.92	-15.98	742	12	713	26.4	21.2	31.7	Luvisols
152	W-052	DK8031	MW	Blantyre	E	Dry-Lowland	34.92	-15.98	742	12	713	26.4	21.2	31.7	Luvisols
153	W-053	Local	MW	Blantyre	A	Wet-Upper-Mid-altitude	35.02	-15.63	423	11	919	23.3	18.8	27.8	Luvisols
154	W-054	Hybrid??	MW	Blantyre	A	Wet-Upper-Mid-altitude	35.02	-15.63	874	11	919	23.3	18.8	27.8	Luvisols
155	W-055	Local	MW	Mwanza	E	Dry-Lowland	34.69	-15.61	897	11	717	26.5	21.2	31.8	Luvisols
156	Z-003	Kafwamba	ZAM	Kafue	B	Wet-Lower-Mid-altitude	28.25	-15.66	1091	11	712	24.4	19.2	29.7	-
157	Z-004	Gankata-1	ZAM	Mazabuka	B	Wet-Lower-Mid-altitude	28.21	-15.88	1008	11	737	22.9	17.8	28.1	-
158	Z-005	Gankata-2	ZAM	Mazabuka	B	Wet-Lower-Mid-altitude	28.21	-15.88	1008	11	737	22.9	17.8	28.1	-
159	Z-006	Gankata-3	ZAM	Mazabuka	B	Wet-Lower-Mid-altitude	28.21	-15.88	1008	11	737	22.9	17.8	28.1	-
160	Z-007	Gankata-4	ZAM	Mazabuka	B	Wet-Lower-Mid-altitude	28.21	-15.88	1008	11	737	22.9	17.8	28.1	-
161	Z-008	Siampungani	ZAM	Monze	B	Wet-Lower-Mid-altitude	27.52	-16.28	1107	11	752	22.8	17.6	28.0	-
162	Z-009	Local-(Eastern-Province)	ZAM	Monze	B	Wet-Lower-Mid-altitude	27.52	-16.28	1107	11	752	22.8	17.6	28.0	-
163	Z-010	Mapongwe-a-Chitonga	ZAM	Monze	B	Wet-Lower-Mid-altitude	27.49	-16.44	1154	11	755	23.0	17.8	28.1	-
164	Z-011	Hickory-King	ZAM	Gwembe	A	Wet-Upper-Mid-altitude	27.61	-16.51	1221	11	773	22.0	16.9	27.2	-
165	Z-012	8-Line	ZAM	Choma	A	Wet-Upper-Mid-altitude	27.08	-16.84	1286	11	756	22.0	16.7	27.4	-
166	Z-013	Chibahwe	ZAM	Choma	A	Wet-Upper-Mid-altitude	27.08	-16.84	1286	11	756	22.0	16.7	27.4	-
167	Z-014	Local	ZAM	Choma	A	Wet-Upper-Mid-altitude	27.08	-16.84	1286	11	756	22.0	16.7	27.4	-
168	Z-015	Red-Cob	ZAM	Choma	A	Wet-Upper-Mid-altitude	27.08	-16.84	1286	11	756	22.0	16.7	27.4	-
169	Z-016	Local	ZAM	Choma	A	Wet-Upper-Mid-altitude	27.08	-16.84	1286	11	756	22.0	16.7	27.4	-
170	Z-017	Panda	ZAM	Kalomo	B	Wet-Lower-Mid-altitude	26.60	-17.20	1227	11	693	22.7	17.1	28.3	-
171	Z-018	Silintuba	ZAM	Kalomo	B	Wet-Lower-Mid-altitude	26.60	-17.19	1219	11	693	22.7	17.1	28.3	-
172	Z-019	Gankata	ZAM	Kalomo	B	Wet-Lower-Mid-altitude	26.19	-17.32	1219	11	685	22.9	17.1	28.7	-
173	Z-020	Kazungula	ZAM	Kazungula	E	Dry-Lowland	25.21	-17.56	887	11	638	24.6	18.4	30.8	-
174	Z-021	Sesheke	ZAM	Sesheke	E	Dry-Lowland	24.94	-18.99	966	11	510	24.8	18.3	31.3	-
175	Z-022	Silozi	ZAM	Sesheke	E	Dry-Lowland	24.77	-17.18	750	11	664	24.3	18.2	30.5	-
176	Z-023	Mboni-ya-Sintu	ZAM	Sesheke	E	Dry-Lowland	24.32	-17.49	962	11	642	24.4	18.1	30.6	-
177	Z-024	Kangalingali	ZAM	Sesheke	E	Dry-Lowland	24.00	-17.06	978	11	665	24.4	18.3	30.4	-
178	Z-025	Mboni-ya-Sintu	ZAM	Shangombo	-	-	-	-	1076	-	-	-	-	-	-
179	Z-026	Mboni-ya-Silozi	ZAM	Senanga	B	Wet-Lower-Mid-altitude	23.29	-16.15	1087	11	730	24.0	18.3	29.7	-
180	Z-027	Mboni-ya-Sintu	ZAM	Kaoma	B	Wet-Lower-Mid-altitude	23.56	-15.17	1117	11	850	23.4	18.0	28.8	-
181	Z-028	Katiko	ZAM	Kaoma	B	Wet-Lower-Mid-altitude	24.06	-15.04	1120	11	855	23.2	17.7	28.7	-
182	Z-029	Mboni-ya-Sintu	ZAM	Kaoma	B	Wet-Lower-Mid-altitude	24.53	-14.67	1120	11	876	23.3	17.8	28.8	-
183	Z-030	Nyamavhunga	ZAM	Lukulu	B	Wet-Lower-Mid-altitude	24.16	-14.44	1084	11	887	23.5	18.0	28.9	-

Table 2.3 Continued

Acc	Code	Landrace Name	Country	District	ME	Megaenvironment	Long	Lat	Alt	Rain Start	Ann Precip	Temperature Ave	Temperature Min	Temperature Max	Soil Type
184	Z-031	Mundele-wa-Chintu	ZAM	Lukulu	B	Wet-Lower-Mid-altitude	24.16	-14.44	1058	11	887	23.5	18.0	28.9	-
185	Z-032	Local	ZAM	Lukulu	B	Wet-Lower-Mid-altitude	23.79	-14.08	1094	11	914	23.7	18.2	29.1	-
186	Z-033	Mboni-ya-Sintu	ZAM	Kabompo	B	Wet-Lower-Mid-altitude	23.63	-14.04	1064	11	915	23.7	18.3	29.2	-
187	Z-034	Mun'indo	ZAM	Zambezi	B	Wet-Lower-Mid-altitude	23.25	-13.59	1128	11	949	23.6	18.0	29.2	-
188	Z-035	Local	ZAM	Zambezi	B	Wet-Lower-Mid-altitude	23.46	-13.78	1034	11	936	23.6	18.1	29.1	-
189	Z-036	Mundele-wa-Chintu	ZAM	Kabompo	B	Wet-Lower-Mid-altitude	23.93	-13.77	1034	11	941	23.4	18.0	28.9	-
190	Z-037	90-Days	ZAM	Kabompo	B	Wet-Lower-Mid-altitude	24.25	-13.58	1047	11	968	22.9	17.5	28.4	-
191	Z-038	Yellow-Maize	ZAM	Kabompo	B	Wet-Lower-Mid-altitude	24.25	-13.58	1047	11	968	22.9	17.5	28.4	-
192	Z-039	Kahilahila	ZAM	Kabompo	B	Wet-Lower-Mid-altitude	24.39	-13.38	1126	11	985	23.0	17.6	28.4	-
193	Z-040	Kahilahila-2	ZAM	Kabompo	B	Wet-Lower-Mid-altitude	24.39	-13.38	1126	11	985	23.0	17.6	28.4	-
194	Z-041	Kabaka-1	ZAM	Mufumbwe	A	Wet-Upper-Mid-altitude	24.75	-13.23	1126	11	1020	22.5	17.2	27.9	-
195	Z-042	Kabaka-2	ZAM	Mufumbwe	A	Wet-Upper-Mid-altitude	24.75	-13.23	1272	11	1020	22.5	17.2	27.9	-
196	Z-043	Kabaka-3	ZAM	Mufumbwe	A	Wet-Upper-Mid-altitude	24.75	-13.23	1272	11	1020	22.5	17.2	27.9	-
197	Z-044	Local	ZAM	Mufumbwe	A	Wet-Upper-Mid-altitude	25.38	-13.06	1309	11	1062	22.3	16.9	27.6	-
198	Z-045	Mbonyi-ya-Chikonde	ZAM	Mufumbwe	A	Wet-Upper-Mid-altitude	25.38	-13.06	1404	11	1062	22.3	16.9	27.6	-
199	Z-046	Local	ZAM	Mufumbwe	A	Wet-Upper-Mid-altitude	25.54	-12.98	1404	11	1081	21.9	16.6	27.3	-
200	Z-047	Kapira-1	ZAM	Solwezi	A	Wet-Upper-Mid-altitude	26.57	-12.21	1404	11	1171	21.7	16.3	27.2	-
201	Z-048	Kapira-2	ZAM	Solwezi	A	Wet-Upper-Mid-altitude	26.57	-12.21	1319	11	1171	21.7	16.3	27.2	-
202	Z-049	Kapira-3	ZAM	Solwezi	A	Wet-Upper-Mid-altitude	26.57	-12.21	1373	11	1171	21.7	16.3	27.2	-
203	Z-050	Local	ZAM	Solwezi	A	Wet-Upper-Mid-altitu	27.16	-12.36	1241	11	1167	22.1	16.5	27.6	-
204	Z-051	Local	ZAM	Chingola	A	Wet-Upper-Mid-altitu	27.97	-12.61	1241	11	1184	21.3	15.8	26.8	-
205	Z-052	Kanjilimane-1	ZAM	Kitwe	A	Wet-Upper-Mid-altitu	28.36	-12.92	1169	11	1136	22.1	16.9	27.3	-
206	Z-053	Kanjilimane-2	ZAM	Kitwe	A	Wet-Upper-Mid-altitu	28.36	-12.92	1189	11	1136	22.1	16.9	27.3	-
207	Z-054	kanjilimane-3	ZAM	Masaiti	A	Wet-Upper-Mid-altitu	28.41	-13.24	1209	11	1094	22.2	17.1	27.3	-
208	Z-055	Local	ZAM	Mpongwe	A	Wet-Upper-Mid-altitu	28.22	-13.46	1291	11	1069	22.1	16.9	27.3	-
209	Z-056	Local	ZAM	Masaiti	A	Wet-Upper-Mid-altitu	27.98	-13.53	1283	11	1061	22.0	16.7	27.2	-
210	Z-057	Local	ZAM	Kapiri-Mposhi	A	Wet-Upper-Mid-altitu	28.71	-13.92	1283	11	1023	20.9	15.9	26.0	-
211	Z-058	Gankata	ZAM	Mkushi	A	Wet-Upper-Mid-altitu	29.36	-14.02	1315	11	977	21.9	16.8	27.0	-
212	Z-059	Gankata-Red	ZAM	Mkushi	A	Wet-Upper-Mid-altitu	29.36	-14.02	1497	11	977	21.9	16.8	27.0	-
213	Z-060	Chilala	ZAM	Mkushi	A	Wet-Upper-Mid-altitu	29.45	-13.81	1372	11	1029	20.8	15.8	25.8	-
214	Z-061	Chilala	ZAM	Mkushi	A	Wet-Upper-Mid-altitu	29.73	-13.54	1529	11	1057	20.6	15.5	25.6	-
215	Z-062	Chilala-8-Row	ZAM	Serenje	A	Wet-Upper-Mid-altitu	30.06	-13.30	1490	11	1081	20.0	14.9	25.0	-
216	Z-063	Chilala	ZAM	Serenje	A	Wet-Upper-Mid-altitu	30.38	-13.12	1567	11	1058	20.2	15.1	25.3	-
217	Z-064	Chilala	ZAM	Serenje	A	Wet-Upper-Mid-altitu	30.66	-13.02	1528	11	1037	20.2	15.1	25.2	-
218	Z-065	Akansalika	ZAM	Serenje	A	Wet-Upper-Mid-altitu	30.49	-13.02	1504	11	1050	20.3	15.2	25.4	-
219	Z-066	Kanjele	ZAM	Serenje	A	Wet-Upper-Mid-altitu	30.76	-12.91	1326	11	1036	20.3	15.2	25.4	-
220	Z-067	Chibempa	ZAM	Mpika	A	Wet-Upper-Mid-altitu	31.45	-11.84	1240	11	1066	21.6	16.6	26.6	-

Table 2.3 Continued

Acc	Code	Landrace Name	Country	District	ME	Megaenvironment	Long	Lat	Alt	Rain Start	Ann Precip	Temperature Ave	Temperature Min	Temperature Max	Soil Type
221	Z-068	Karimwa	ZAM	Mpika	A	Wet-Upper-Mid-altitu	31.28	-11.51	1212	11	1114	21.3	16.4	26.3	-
222	Z-069	Pandama	ZAM	Mpika	A	Wet-Upper-Mid-altitu	31.12	-11.09	1204	11	1150	22.2	17.3	27.1	-
223	Z-070	Karimina	ZAM	Kasama	A	Wet-Upper-Mid-altitu	31.11	-10.89	1450	11	1158	22.1	17.3	27.0	-
224	Z-071	Kalimwa	ZAM	Kasama	A	Wet-Upper-Mid-altitu	31.07	-10.21	1507	11	1173	21.6	16.7	26.5	-
225	Z-072	Kalimwa	ZAM	Mporokoso	A	Wet-Upper-Mid-altitu	30.36	-10.20	1507	11	1200	20.8	16.0	25.6	-
226	Z-073	Kalimwa-Yellow	ZAM	Luwingo	A	Wet-Upper-Mid-altitu	29.97	-10.22	1507	11	1211	20.4	15.6	25.2	-
227	Z-074	Kalimwa-Red-Stripped	ZAM	Luwingo	A	Wet-Upper-Mid-altitu	29.97	-10.22	1507	11	1211	20.4	15.6	25.2	-
228	Z-075	Kalimwa-(HK)	ZAM	Luwingo	A	Wet-Upper-Mid-altitu	29.97	-10.22	1507	11	1211	20.4	15.6	25.2	-
229	Z-076	Kalimwa-(Red)	ZAM	Luwingo	A	Wet-Upper-Mid-altitu	29.97	-10.22	1613	11	1211	20.4	15.6	25.2	-
230	Z-077	Kalimwa-(Spotted-Mixture)	ZAM	Luwingo	A	Wet-Upper-Mid-altitu	29.97	-10.22	1337	11	1211	20.4	15.6	25.2	-
231	Z-078	Karimwa	ZAM	Mbala	A	Wet-Upper-Mid-altitu	31.22	-9.50	1722	11	1123	20.1	15.1	25.2	-
232	Z-079	Kandimwa	ZAM	Mpulunga	A	Wet-Upper-Mid-altitu	31.00	-9.36	1822	11	1088	21.5	16.4	26.5	-
233	Z-080	Chimambwe	ZAM	Mbala	A	Wet-Upper-Mid-altitu	31.36	-8.92	1604	11	987	20.5	15.3	25.7	-
234	Z-081	Chimambwe	ZAM	Mbala	F	Highland	31.54	-9.12	1317	12	997	18.6	13.5	23.7	-
235	Z-082	Chimambwe/Kalimwa	ZAM	Mbala	A	Wet-Upper-Mid-altitu	32.00	-9.09	1305	12	920	20.8	15.5	26.1	-
236	Z-083	Mofati	ZAM	Nakonde	A	Wet-Upper-Mid-altitu	32.45	-9.29	1391	12	922	21.8	16.3	27.2	-
237	Z-084	Avxansi	ZAM	Isoka	A	Wet-Upper-Mid-altitu	32.67	-9.73	1252	12	918	22.8	18.0	27.5	-
238	Z-085	Mofati	ZAM	Isoka	A	Wet-Upper-Mid-altitu	32.71	-10.21	1500	12	914	21.4	17.3	25.5	-
239	Z-086	Pandawe	ZAM	Isoka	A	Wet-Upper-Mid-altitu	33.12	-10.34	1187	12	844	22.1	17.9	26.3	-
240	Z-087	Pandawe	ZAM	Isoka	A	Wet-Upper-Mid-altitu	33.25	-10.30	1192	12	891	20.0	15.6	24.4	-
241	Z-088	Masika	ZAM	Mzimba	A	Wet-Upper-Mid-altitu	33.40	-11.21	993	11	765	22.4	17.7	27.0	Ferralsols
242	Z-089	Kafula	ZAM	Chama	A	Wet-Upper-Mid-altitu	33.39	-11.22	993	11	765	22.4	17.7	27.0	Ferralsols
243	Z-090	Kanjerenjere	ZAM	Chama	A	Wet-Upper-Mid-altitu	33.18	-11.23	993	11	804	22.9	18.2	27.6	-
244	Z-091	Pool-16	ZAM	Chama	A	Wet-Upper-Mid-altitu	33.18	-11.23	1110	11	804	22.9	18.2	27.6	-
245	Z-092	Local	ZAM	Chama	A	Wet-Upper-Mid-altitu	33.18	-11.23	1119	11	804	22.9	18.2	27.6	-
246	Z-093	Local	ZAM	Lundazi	A	Wet-Upper-Mid-altitu	33.25	-11.74	1119	11	804	20.6	15.7	25.5	-
247	Z-094	Local	ZAM	Lundazi	A	Wet-Upper-Mid-altitude	33.18	-12.35	1196	11	817	22.1	17.1	27.1	-
248	Z-095	Kenya	ZAM	Lundazi	A	Wet-Upper-Mid-altitude	33.18	-12.35	1196	11	817	22.1	17.1	27.1	-
249	Z-096	Kanjere	ZAM	Lundazi	A	Wet-Upper-Mid-altitude	32.98	-12.63	1074	11	826	21.9	16.8	26.9	-
250	Z-097	Local	ZAM	Lundazi	A	Wet-Upper-Mid-altitude	32.98	-12.63	1161	11	826	21.9	16.8	26.9	-
251	Z-098	Chamakolo	ZAM	Chipata	B	Wet-Lower-Mid-altitude	32.86	-13.39	935	11	887	23.1	17.9	28.3	-
252	Z-099	Local	ZAM	Katete	A	Wet-Upper-Mid-altitude	32.07	-14.04	935	11	1001	21.7	16.4	26.9	-
253	Z-100	Kenya	ZAM	Petauke	B	Wet-Lower-Mid-altitude	31.33	-14.25	935	11	907	23.9	18.8	29.1	-
254	Z-101	Chibahwe	ZAM	Petauke	B	Wet-Lower-Mid-altitude	31.33	-14.25	719	11	907	23.9	18.8	29.1	-
255	Z-102	Vinchewele	ZAM	Petauke	B	Wet-Lower-Mid-altitude	31.33	-14.25	502	11	907	23.9	18.8	29.1	-
256	Z-103	Senga	ZAM	Nyimba	B	Wet-Lower-Mid-altitude	30.89	-14.55	747	11	877	24.4	19.2	29.6	-
257	Z-104	Senga	ZAM	Nyimba	E	Dry-Lowland	30.42	-14.90	1093	11	782	26.0	20.6	31.3	-

Table 2.3 Continued

Acc	Code	Landrace Name	Country	District	ME	Megaenvironment	Long	Lat	Alt	Rain Start	Ann Precip	Ave	Temperature Min	Temperature Max	Soil Type
258	Z-105	Yachishi	ZAM	Chongwe	E	Dry-Lowland	29.67	-15.09	1129	11	720	25.6	20.3	30.9	-
259	Z-106	Gankata	ZAM	Chongwe	B	Wet-Lower-Mid-altitude	28.87	-15.29	1129	11	754	23.6	18.4	28.7	-
260	Z-107	Gankata-8-Lines	ZAM	Mumbwa	A	Wet-Upper-Mid-altitude	27.41	-15.11	1162	11	865	21.8	16.5	27.2	-
261	Z-108	Gankata-Flint	ZAM	Mumbwa	A	Wet-Upper-Mid-altitude	27.41	-15.11	1030	11	865	21.8	16.5	27.2	-
262	Z-109	Kafuamba	ZAM	Chiomo	A	Wet-Upper-Mid-altitude	27.72	-14.91	1030	11	878	22.3	17.0	27.5	-
263	Z-110	Gankata	ZAM	Mazabuka	B	Wet-Lower-Mid-altitude	27.62	-16.00	1030	11	722	23.5	18.2	28.9	-
264	Z-111	Gankata-10-Lines	ZAM	Mazabuka	B	Wet-Lower-Mid-altitude	27.62	-16.00	1030	11	722	23.5	18.2	28.9	-
265	Z-112	Kafwamba	ZAM	Mazabuka	B	Wet-Lower-Mid-altitude	27.62	-16.00	1199	11	722	23.5	18.2	28.9	-
266	Z-113	Kafwamba	ZAM	Mazabuka	B	Wet-Lower-Mid-altitude	27.62	-16.00	1199	11	722	23.5	18.2	28.9	-
267	Z-118	Bingu	ZAM	Chama	-	-	-	-	-	-	-	-	-	-	-

Acc = Accession number, Code = Collection identification number for each country, ZIM = Zimbabwe, ZAM = Zambia, MW = Malawi, Long = Longitude, Lat= Latitude, Alt= Altitude, Rain Start = Month of the year when the rain season begins, Ann Precip = Annual Precipitation in mm

CHAPTER III

MORPHO-PHENOLOGICAL DIVERSITY OF MAIZE ACCESSIONS

INTRODUCTION

The phenotypic diversity present in maize today is the product of a long tradition of plant breeding practiced by native Americans who played a major role in the development and adaptation of this crop to virtually every habitable environment in the Americas including deserts, tropical rainforest, and high mountains (Manglesdorp, 1974). The study of phenotypic and genetic diversity to identify groups with similar genetic backgrounds is important for conserving, evaluating and utilizing genetic resources, for studying the diversity of pre-breeding and breeding germplasm, and for determining the uniqueness and distinctness of the phenotypic and genetic constitution of genotypes with the purpose of protecting the breeder's intellectual property rights (Franco et al., 2001).

Previous diversity studies in maize have focused on genetic diversity in the Americas and Europe using morphological and agronomical characters, isozymes and molecular techniques (Smith et al., 1997). Comparison of different marker types to assess genetic diversity among accessions or specific groups of genotypes has also been carried out by some authors (Franco et al. 2001; Pejic, et al. 1998). Morphological descriptions of some European maize collections were carried out in Spain, Italy, Yugoslavia and Romania (Brandolini, 1970). Thereafter, other authors reported morphological descriptions of maize populations from Portugal, France and northern Spain (Gouesnard et al., 1997; Llaurado and Moreno-Gonzalez, 1993). Several authors were also interested in morphological classification of maize populations on a European scale (Gauthier et al., 2002). To date, little or no information is available on the phenotypic and genetic diversity of maize landraces and varieties available in Africa in general, and particularly in southern Africa even though some small collections have carried out in several countries (IPGRI, 2003). It is important to characterize the diversity of these genetic resources in order to optimize conservation and facilitate their use. Furthermore, this characterization is necessary for the historical understanding of the introduction of maize in southern Africa. The genetic diversity among and within landraces makes them a

valuable resource as potential donors of genes for the development and maintenance of modern crop varieties, and for direct use by farmers (IPGRI, 2003).

The concept of a core collection was introduced by Frankel and Brown (1984) with the intent of using the core collection to minimize the cost of germplasm conservation whilst ensuring maximum genetic diversity. Many approaches to constructing core collections have been developed through the years. Several sampling methods to select entries for the core collections have been suggested ranging from random sampling (Brown, 1989) to stratified sampling (Johnson and Hodgkin, 1999). In recent years, various researchers have established core collections of maize germplasm for specific regions of the world (Taba et al., 1998; Malosetti and Abadie, 2001; Li et al., 2004). The maize landrace collection mission carried out in Zimbabwe, Zambia and Malawi (reported in Chapter II) resulted in a total of 267 accessions. A further six maize landraces from the USA and 28 obsolete and current commercial varieties from Zimbabwe, Zambia and Malawi were added to the set of landraces and included in the sample for study. In-order to facilitate the agronomic evaluation of these maize varieties there is need to develop a core subset to minimize the cost of evaluation and further characterization while ensuring maximum genetic diversity. The core subset thus formed can be evaluated extensively under different biotic and abiotic stresses and the information derived could be used to guide more efficient utilization of the entire collection.

The objectives of this section of the study were (i) to assess the morphological and phenological diversity of the full set of maize varieties collected, and (ii) to develop a core collection representing the diversity of the whole collection for further genotypic and phenotypic evaluation under different abiotic stresses.

LITERATURE REVIEW

Morphological Diversity in Maize

Maize has been described as one of the most diverse plants on earth and this diversity occurs at both the phenotypic and molecular levels. There are about 65 000 accessions of maize in major germplasm banks of the world, of which more than 90% are

Z. mays. The International Center for Wheat and Maize Improvement (Centro Internacional de Mejoramiento de Maiz y Trigo, CIMMYT), the largest maize collection in the world, includes over 25,000 entries. Most of the diversity in maize remains undescribed, poorly understood and under utilized in modern crop improvement programs largely because of the difficulty of identifying useful genetic variants hidden in the background of low yielding local varieties and landraces (Tanksley and McCouch, 1997). Thus, there is a need to continue to study the genetic diversity to sift through the variation in the maize gene pool and understand how they impact phenotypes of agronomic importance especially for marginal production environments.

The variability of maize worldwide has been the focus of several studies describing morphological, agronomic and racial relationships. Such assessments are especially important to plant genetic resources management and to studies of the evolutionary potential of a species, history of crop domestication, and introduction of crops in areas where it has not been grown before. Ruiz de Galaretta and Alvarez (2001) evaluated 100 landraces of maize from Northern Spain and came up with seven different groups based on twenty-two morphological traits and seventeen ecological variables (climatic, edaphic and topographic) associated with the collection site. From this study, seven populations with promising breeding value were detected. Lucchin et al. (2003) found low genetic differentiation within 22 populations of one maize landrace grown widely in Spain, and attributed this low variation to gene flow and seed exchange among farmers. In a study to investigate past and present evolutionary processes that have shaped quantitative trait variation in maize landraces in Mexico, Pressoir and Berthaund (2004) observed high levels of population differentiation in maize landraces in Mexico and concluded that that farmers select for genes of major and pleiotropic effects, and that farmers' decisions and selection strategies have a great impact on phenotypic diversification in maize landraces.

In yet another study, Ilarslan et al. (2002) found considerable genetic variation for morphological and agronomic traits in a collection of Turkish maize landraces. In a study to determine the relationships and genetic diversity among Mexican races of maize, Sanchez et al. (1993) also observed a very high level of variation among and within the Mexican races while in another study, Doebley et al. (1985) found that the races of maize

in Mexico rank among the most variable species that have ever been studied. In Canada, Azar et al. (1997) evaluated 35 landraces, one experimental population and one control hybrid in order to characterize and classify the landraces and found that most quantitative traits examined exhibited considerable variation among the landraces. Based on seven key traits, the populations were grouped into 10 clusters by centroid clustering analysis.

Appropriate Morphological Characters for Diversity Analysis

Traditionally maize taxonomists and geneticists classify maize populations by the race or combinations of the races to which they belong. A maize race is defined as a group of related maize plants with enough characteristics in common to permit their recognition as a group (Anderson and Cutler, 1942). From a genetics point of view, a race is a group of plants with a significant number of alleles in common, major races having a smaller number of alleles in common than do sub-races (Anderson and Cutler, 1942). The racial taxonomic classification originally developed by Welhausen et al. (1952) and used by earlier authors is based exclusively almost on ear characteristics. However, analysis of other morphological characters can reveal significant variation among races.

Various authors have attempted to classify maize based on given sets of morphological characters and came up with varying recommendations. Anderson and Cutler (1942) considered number of tassel branches, size of glumes on the male spikelet, number of ear husks, kernel row number, and kernel size as the most stable characters across environments for classifying varieties. Sanchez et al. (1993) studied eleven characters in 30 races of maize from Mexico and concluded that the branched part of the tassel, number of tassel branches, and plant and ear height were the most appropriate for classification. From a more detailed study, Ortiz (1985) recommended plant height, ear height, number of leaves, leaf length, leaf width, number of tassel branches, length of branching space, ear length, ear diameter, number of kernel rows, kernel length, kernel width, pith diameter, cob diameter, and capule width as the most appropriate characters for racial classification of 96 maize varieties from Peru. Based on ratios of estimates of components of variance from multi-environment trials for maize varieties from Mexico and the USA, Sanchez et al. (1993) concluded that the most discriminant characters for maize classification were number of leaves per plant, number of branched part

length/tassel length, central spike internode length, male glume length, kernel width, rachis segment length, pith diameter, ear diameter/length, and kernel width/length. In a study to characterize and choose the plant traits that best explain genetic variation in maize landraces collected from northern Spain, Ruiz de Galarreta and Alvarez (2001) found leaf area, ear shape, tassel branches, kernel rows, plant height, cob weight, and ear length as the most important traits for taxonomic classification. From these studies, it can be concluded that the key considerations is to choose those characters that are least subject to environmental biases. In addition, for routine characterization those characters that can be quickly and cheaply measured in the field should be prioritized.

Statistical Analysis of Genetic Diversity

In genetic resources conservation and plant breeding research, it is useful to compute measurements which can indicate the amount of genetic variability of a set of individuals in a given situation. Assessments of genetic diversity has been applied in (i) cultivar identification, (ii) choosing parents for crosses, (iii), assigning germplasm to heterotic groups, (iv) gene introgression, and (v) studying historical aspects of maize introduction and diffusion in given areas of the world. In analysis of genetic diversity, various kinds of data have been used including pedigrees, eco-geographic data, morphological, biochemical and molecular data (Sanchez et al. 1993, Azar et al. 1997, Taba et al. 1998, Santacruz-Valera et al. 2004, Kresovich and Lamkey, 2005).

The basic measure of genetic diversity is genetic distance which has been defined by Beaumont et al. (1998) as “any quantitative measure of genetic difference, be it at the sequence level, or the allele frequency level, that is calculated between individuals, populations or species”. Genetic distances can be calculated in various ways depending on the kind of data. Thus, genetic variability within a population can be measured as (i) the number (and percentage) of genes in the population that are polymorphic, (ii) the number of alleles for each polymorphic gene, and (iii) the number (and percentage) of genes per individual that are polymorphic. Recently developments in molecular biology, such as quantitative genetic analysis, protein electrophoresis, DNA markers, and DNA sequencing have been applied to assay genetic diversity in plant populations. Those methods differ in scopes of studying objectives and analysis costs. Thus, before carrying

out research on genetic diversity, careful planning is needed for choosing a suitable method.

The genetic and mathematical properties of various genetic distance measures have been reviewed extensively by Reif et al. (2005). In this review, a Procrustes analysis of a published data set consisting of seven CIMMYT maize populations demonstrated close affinity between one group of distance measures on one hand, and another group of dissimilarity measures on the other hand. This review showed that genetical and mathematical properties of dissimilarity measures are of crucial importance when choosing a genetic dissimilarity coefficient for analyzing diversity data. The effective population size (N_e) (Wright, 1931), is another important measurement which can indicate the amount of genetic variability of a set of individuals in a given situation. N_e is the size of an ideal population that has the same amount of drift in allele frequency, or the same rate of decrease in heterozygosity, as the actual population (Vencovsky and Crossa, 2003). It is a basic parameter that largely determines allelic retention, preservation, and conservation over generations and is particularly useful when studying genetic diversity of landraces. In production systems at the farmer's level, landrace and local population are normally derived from a limited number of genotypes or closely related genotypes.

In another review, Mohammadi and Prassana (2003) presented some salient statistical tools and considerations that need to be taken into consideration when analyzing genetic diversity in crop plants. For reasonably accurate and unbiased estimates of genetic diversity, adequate attention has to be devoted to (i) sampling strategies; (ii) utilization of various data sets on the basis of the understanding of their strengths and constraints; (iii) choice of genetic distance measure(s), clustering procedures, and other multivariate methods in analyses of data; and (iv) objective determination of genetic relationships. A judicious combination and utilization of statistical tools and techniques, such as bootstrapping, is vital for addressing complex issues related to data analysis and interpretation of results from different types of data sets, particularly through clustering procedures.

For studying the genetic diversity, classification methods that group entries into clusters according to plant characters are used. A multivariate data set consisting of measurements of several variables for each entry can be conceptualized as containing

entries that are placed in a multi-dimensional space in which there is one dimension for each character. Those entries with similar values for each character would be close to each other in this multi-dimensional space (Franco et al., 1998). Clustering methods can be either hierarchical or non-hierarchical. In hierarchical methods such as the Ward method (Ward, 1963), entries are organized into a tree or hierarchy where entries or groups are fused one at a time to entries or groups with the most similar patterns for all characters. In nonhierarchical methods such as the Gaussian Mixed model or Normix model (Wolfe, 1970), initial groups must be defined a priori, and then the method improves the initial groups by an iterative process that results in a solution that corresponds to a maximum (global or local) of the likelihood function.

Most of the time in research, a great number of variables are evaluated in the populations involved in a breeding program. One of the problems that appear when several characteristics are considered is the understanding of the relative importance of each of them on treatment discrimination, to allow the elimination of those characteristics of lesser importance due to their non-variance, redundancy, and/or correlation with other characteristics present in the analysis. The multivariate analysis, through the canonical variables, enables the determination of the relative importance of those characters, allowing the simplification of data, summarizing the information originally contained in a small number of variables, which present the property of retaining the maximum of the variation originally available and independence from each other. This analysis can be used on studies of genetic divergence between individuals or parents and in the determination of the relative importance of the characteristics. According to Mardia et al. (1979), the elimination of characters is accomplished by choosing those variables associated with the largest elements of the last canonical variable. After the elimination of characters, the first canonical variable may still involve most of the estimated variance. The coefficients associated with the first canonical variable of the analysis involving only the remnant characteristics may be used to generate a function that serves as a multivariate index to represent the group of the evaluated characters in the experiment (Mardia et al., 1979).

Franco et al. (1997) proposed a two stage clustering strategy, in which initial groups are defined by a hierarchical method like that of Ward using Gower's distance

(1971). In this method, continuous and categorical variables are included. After the initial group definition, the Gaussian Mixture model (GM) (Franco et al., 1998), using only the continuous variables, is applied to the Ward groups in an attempt to improve their structure. Thus, the information contained in the categorical variables is not used by the GM model to improve the initial groups. In attempt to use both categorical and continuous variables in final improving the final classification of accessions, Lawrence and Krzanowski (1996) developed the location model (LM), but their model did not take into consideration empty cells that frequently occur in large data sets, and thus their method could not be applied to most practical situations where empty cells frequently occur in the data matrix. Recently a modified location model (MLM) that uses both categorical and continuous variables in improving initial groups has been developed by Franco et al. (1998). When applied o a sets of maize and wheat accessions, this method has been found to produce compact and well-separated groups with respect to all the variables (categorical and continuous) compared with classifications obtained with only categorical variables, with only continuous variables, and with the standard Location model (Franco et al. 1998). In a study comparing the MLM strategy with a racial classification of Uruguayan maize landraces, the MLM method generated more homogeneous groups than those corresponding to a previous preliminary racial classification (Gutierrez et al. 2003).

Core Collections of Germplasm

Germplasm core collections have been suggested to improve the efficiency of germplasm utilization. The core collection concept was suggested by Frankel and Brown (1984), who defined it as a representative sample of a collection where as much as possible of the diversity of the collection is retained with minimum of redundancy. The accessions not included in the core are retained as the reserve collection, composing the collection's backup. Although, a core collection should contain as much diversity as possible, in most of the cases high priority is given to certain types of material, reducing the amount of diversity but increasing the utility of the core (van Hintum, 1999). This can be seen as an evolution of the core collection concept, now being defined as a germplasm collection optimally representing specific genetic diversity.

The advantages of a core collection include (i) putting a high priority for conservation activities, such as germination tests and regeneration, on the entries of the core, (ii) decisions about the increase of the collection can be guided by the core recollection, allowing the identification of gaps and/or duplicates in the current germplasm, (iii) the reduced size of the core and the increased seed availability of those accessions, (iv) the core collection is a logical first step in the screening for desirable alleles, but the search can be continued, if needed, in the reserve collection, (v) reducing the overall cost of evaluation of accessions, (vi) the economy of size makes it possible to increase the number of characteristics evaluated, and (vii) promote the use of other techniques for screening (e.g. molecular markers).

The assembly of a core collection of any crop is basically a sampling exercise that tries to assure maximum sampling of the alleles present in the base collection (Malosetti and Abadie, 2001). Different sampling strategies have been proposed for formation of core collections in plants. In general, sampling strategies should include the more frequent alleles which have been shown to be related with general adaptation (high frequencies and wide distribution) and alleles related to specific adaptation (intermediate to high frequencies, but localized) (Allard, 1992). Considering that common widespread alleles will almost certainly be included in any subset, the sampling process that will lead to the core collection normally attends to the conservation of less frequent but wide spread alleles, and common localized alleles. Two key issues related to trying to attain conservation of these two classes of alleles are sample size and sample type. Brown (1989) concluded that when considering an infinite number of multi-allelic neutral loci, a sample size of about 10% retained at least 70% of the alleles present in the whole collection with 95% certainty. Practical issues such as limited resources also have to be considered when forming the core collection. Stratified (e.g. based on geographic origin, other traits, etc) and random samples have been used by different authors with most favoring stratified methods. The proportion of entries going into each strata has been determined based on constant, proportional, or logarithmic strategies (Brown, 1989). Once the proportion of each stratum has been established, the selection of the members of the core from each group can be done at random, or following some criteria of representativeness from the group (Malosetti and Abadie, 2001).

Core subsets of maize germplasm collections have been established by a few authors. Malosetti and Abadie (2001) compared various methods of core collection and concluded that the relative diversity method combined with the logarithmic strategy produced the most diverse core sample from a collection on 852 Uruguayan maize landraces based on morphological characters. From this study, eight core collections, each with 90 accessions was formed. Using geographical distribution and characterization data, a core collection comprising of 951 landraces and 242 inbred lines was formed from 13,521 landraces and 3,258 inbred lines currently preserved in the China National Genebank (Yu et al., 2004). Taba et al. (1998) used a selection index based on key agronomic traits to form a core collection of 100 accessions from 498 Caribbean maize landraces stored at the CIMMYT Germplasm bank in México. Based on the above, it is intuitively clear that, when resources are limited, smaller working collections are more conducive to the efficient evaluation and utilization of crop germplasm.

MATERIALS AND METHODS

Germplasm

A set of maize varieties (Table 3.1) comprising 6 original open pollinated varieties (OPVs) introduced into Zimbabwe, Zambia and Malawi from the USA, 267 local landraces collected from smallholder farmers in the three southern African countries, 5 historically important OPVs, and 22 improved varieties developed in the region was evaluated in a field trial during the 2003/04 season at Harare to determine the pattern of phenotypic diversity and classify the varieties in groups, for further evaluation of representative sets of landraces originating from different environments under different abiotic stresses.

Experimental Design and Plot Management

A single characterization trial was planted on 26 November 2003 as an alpha-lattice (0,1) design with two replications and incomplete blocks of seven plots. Plot size was 3.375 m^2 and each plot consisted of 17 planting stations in one row 75 cm apart and

4 m long, resulting in a density of 5.3 plants per m^2. Fertilizer equivalent to 170-56-24 kg ha^{-1} of N-P_2O_5-K_2O was applied as per standard local practices. Weeds were controlled by application of Atrazine (4.5 l/ha) and Dual (1.8 l/ha, 96% Metalachlor) pre-emergence herbicides. Escaped weeds were controlled by hand hoeing and application of Basagran (3 l/ha, 48% Bentazon). For protection against maize streak virus vectors, Furadan (100 kg/ha, carbofuran) was applied at planting, while fungal diseases, (*Cercospora zeamaydis*, *Excerohilum turcicum* and *Puccinia sorghi*) were controlled using Tilt 250EC (0.5 l/ha). Maize stalk borers (*Busseola fusca* and *Chilo partellus*) were controlled using Thiodan 1G (4 kg/ha) and Thionex (230 g/ha, endosulfan). Cutworms were controlled with Karate (5 g/ha, Lambda-cyhalothrin) applied at emergence.

Data Collection

During the growing season, data was collected as follows:

(a) on a plot basis: number of days from planting to 50% of the plants shedding pollen (AD); number of days from plating to 50% of the plants having silks at least 1 cm long (SD); silk coloration (SC) recorded as red or white (%); number of root lodged (RL) plants (%); number of stalk lodged (SL) plants (%), number of plants with ears that are not completely covered by the husks (HC).

(b) On five plants taken at random within each row: tassel length (TL) (cm); number tassel branches (NTB); ear leaf length (ELL) (cm); ear leaf width (ELW) (cm).

(c) On five random plants at milk stage: plant height (PLHT)(cm) to the flag leaf insertion, ear height (EHT) (cm) at the upper ear insertion node, stalk diameter (SD) (cm) at the second internode, stay green (SG) (1=10% dead leaf area 10=100% dead leaf area).

(d) On five random plants at harvest: kernel row arrangement (KA) (1=regular, 2=irregular, 3=straight, and 4=spiral), ear length (EL) (cm); ear diameter (ED) (mm), rachis diameter (RD), cob color (PCC) (0=red, 5=white), number of rows per ear (NKR), 100 kernel weight (HKWT).

(e) On plot basis after harvest: number of harvested plants (NP), number of ears (EN), grain texture (KTEX) (1=flint, 5=dent), grain color (0=white, 1=other colors), kernel length (KL) (mm), kernel width (KW) (mm), kernel thickness (KT) (mm), ear weight (EWT) (kg), shelled grain weight (GWT) (kg), and grain moisture (MOIST)(%).

Additional variables calculated from direct measurements were: grain yield (YLD) calculated as shelled grain weight per plot adjusted to 125 g kg^{-1} moisture and converted to Mg ha^{-1}, anthesis to silking interval (ASI) (days) calculated as SD-AD, number of ears per plant calculated as number of ears (NE) with at least one fully developed grain divided by NP, percent husk cover (HUSK) calculated as HC divided by NE, percent root lodging (RLODG) calculated as RL divided by NP, percent stalk lodging (SLODG) calculated as SL divided by NP, ear position calculated as EHT divided by PLHT, and cob diameter (CD) calculated as ED-RD (mm).

Statistical Analysis

For each trait, an individual analysis of variance (ANOVA) was conducted using the ASREML procedure (Burgueño et al., 2000) considering the maize accessions as random effects and reps and blocks within reps as random effects. Lattice-adjusted means for each trait were calculated for the different maize accessions which were included in the study using the ASREML procedure.

A data matrix was constructed from the mean values, after standardizing the means to avoid the influence of different units for the different traits. Genotypic and phenotypic correlations were calculated between traits by considering the maize accessions as random effects using PROC MIXED of SAS (SAS Institute, 2005).

Heritability (H^2) for each of the key quantitative traits assuming accessions random was calculated as:

$$\mathbf{H^2 = [\sigma^2 g/(\sigma^2 g+(\sigma^2 e/r)]}$$

where $\sigma^2 g$ is the genotypic variance, $\sigma^2 e$ is the error variance and r is the number of replications. Genotypic and phenotypic correlations and repeatability were calculated using SAS macros (Holland et al., 2003).

Genetic distance (GD) estimates between the maize populations were calculated in all possible pair-wise comparisons using the Euclidean coefficient for quantitative traits (AD, NKR, HKWT, KL, KW, PLHT, EHT, EL, ED, RD, CD and SC) using the Ward MLM method of Franco et al. (1997). The mean genetic distances of each population from the landrace as a whole were obtained by averaging between-population

estimates using the whole set of maize. The SAS procedure PROC CLUSTER (SAS Institute, 2005) was used for the classification of the accessions. Groups of populations with similar characteristics were built using a hierarchical cluster analysis. Key agronomic traits were used to describe the characteristics of each of the groups formed from the cluster analysis.

To better visualize the relationship between populations and traits, a variety x trait two-way table was first standardized then subjected to PCA to obtain information on the traits most effective in discriminating the maize populations. The standardized table was then decomposed into principal components (PC) via singular value decomposition (SVD). In this analysis the biplot was generated using an excel add-in. Biplot v1.1 (Smith, 2004; http://www.stat.vt.edu/facstaff/epsmith.html).

The choice of proportion of accessions to be included in the core is arbitrary, usually in the 5–20% range, and will depend on the purpose of the core collection (Marshall and Brown, 1975). From each cluster formed, about 25% of the landraces (excluding improved varieties) with the highest average genetic distance were chosen to form the core subset for further evaluation.

RESULTS AND DISCUSSION

Analysis of Variance

For grain yield and most of its components (EPP, NKR, HKWT, KL, KW), there was significant variation among the maize varieties (Table 3.1). In general landraces produced much less grain than improved varieties from NARS, private seed companies

Table 3.1. Range, mean, standard error, standard deviation and mean squares for the 34 phenotypic traits measured in 294 maize varieties at grown at Harare in 2004 main season.

Trait	Minimum	Maximum	Mean	Std. Error	Std. Deviation	Signi ($_{VARIETY}$)
GY (Mg ha^{-1})	1.072	8.797	4.367	0.078	1.339	***
EPP (no.)	0.452	1.355	0.809	0.009	0.158	***
NKR (no.)	7.364	17.494	11.508	0.100	1.714	***
HKWT (g)	22.370	67.330	43.421	0.449	7.702	***
KL (mm)	10.117	15.087	12.671	0.042	0.727	***
KW (mm)	8.528	14.592	10.921	0.065	1.113	***
KT (mm)	4.202	5.680	4.836	0.015	0.264	ns
ANTHESIS (days)	56.488	82.156	73.887	0.208	3.570	***
SILK (days)	57.758	83.769	75.242	0.222	3.800	***
ASI (days)	-2.468	4.503	1.352	0.060	1.030	ns
PLHT (cm)	178.477	333.699	269.527	1.448	24.823	***
EHT (cm)	82.694	209.110	149.977	1.269	21.754	***
EPOS (%)	0.400	0.697	0.555	0.003	0.044	***
EL (mm)	126.387	193.104	161.012	0.793	13.596	***
ED (mm)	36.643	50.748	44.313	0.136	2.337	***
RD (mm)	11.400	19.200	15.369	0.094	1.609	**
CD (mm)	23.216	36.075	28.942	0.115	1.970	***
ELL (cm)	97.136	133.763	113.901	0.335	5.735	***
ELW (cm)	8.699	12.751	10.773	0.039	0.663	***
SD (mm)	23.044	34.150	29.144	0.109	1.873	**
TL (cm)	32.160	47.204	40.635	0.169	2.897	**
NTB (no.)	10.226	18.626	14.643	0.097	1.666	***
LAE	4.596	6.642	5.565	0.020	0.349	***
CIRCUM (cm)	7.001	11.026	9.172	0.035	0.605	***
RL (count)	0.000	3.700	1.195	0.040	0.678	ns
SL (count)	-0.300	23.300	4.314	0.272	4.664	ns
HC (count)	0.000	6.500	1.518	0.060	1.026	ns
KA (score)	-0.600	33.600	10.410	0.407	6.970	ns
KTEX (score)	1.000	2.900	1.864	0.019	0.318	ns
PKC (score)	2.500	6.000	3.977	0.037	0.638	***
PES (score)	1.000	9.000	1.662	0.072	1.230	***
PCC (score)	1.000	3.000	1.585	0.030	0.512	***
SC (score)	1.000	2.000	1.053	0.012	0.207	***
SG (%)	0.400	13.100	3.670	0.098	1.673	***

GY = Grain Yield (Mg ha^{-1}), EPP = number of ear per plant, NKR = number of kernel rows per ear, HKWT = weight of 100 kernels (g at 12.5% moisture), KL = kernel length (mm), KW = kernel weight (mm), KT =kernel thickness (mm); AN = number of day from planting to 50% of the plants shedding pollen (days); SILK =number of days from planting to 50% of the plants having silks 1cm long or more(days); ASI = interval anthesis to silking interval(days); PLHT= plant height (cm); EHT = ear height (cm); EPOS = ear position (%); EL = ear length (mm); ED = ear diameter (mm); RD = rachis diameter (mm); CD = cod diameter (mm); ELL = ear leaf length (cm); ELW = ear leaf width (cm); SD = stalk diameter (mm); TL = tassel length (cm); NTB = number of primary tassel branches; LAE = leaf angle; CIRCUM =stalk circumference (cm); RL = number of root lodged plants; SL = number of stalk lodged plants; HC number of plants with exposed ear tips; KA =kernel arrangement on the ear (score); KTEX = kernel texture (score); PKC = principal kernel color (score); PES = principal ear shape (score); PCC = principal cob color (score); SC = silk color (score); SG = stay green (%).

and CIMMYT, but more than ancestral varieties from the USA and early OPVs developed in southern Africa (Table 3.1). Based on the mega-environment of the collection site for landraces, those from mega-environment the highlands zones (mega-environment F (Table 2.1) were outyielded by landraces from zones the mid-altitude and lowland tropical (zones A, B, C and E). Kernel dimensions (KL, KW and KT) varied moderately while EPP, NKR and HKWT varied considerably among the varieties.

Highly significant differences were also observed for phenological traits AD and SILK in the whole collection (Table 3.1). In general, late-maturing varieties tend to be grown in favorable production environments (Zone A and B) characterized by relatively low levels of climatic variability and assured water supplies (Table 3.2). Landraces collected from the high potential mega-environments A and B in general had higher flowering dates compared to landraces from the drier zones C and E indicating their adaptation to different to respective season growing lengths in each mega-environment. Early maturing varieties tend to be grown in marginal production environments subject to high levels of climatic variability, including frequent water stress (drought or low soil fertility) (Zones C and E). Farmers in marginal environments choose to grow early-maturing varieties precisely because these varieties' reduced growth cycle minimizes their exposure to stresses. Early-maturing varieties tend to be popular also in highly intensified cropping systems, since the earlier the maize crop can be harvested, the sooner the field can be prepared for the following crop. In this study the earlier flowering of highland accessions (Zone F) was probably accentuated by the lack of adaptation to the trials site Harare, which is located in the mid-altitude zone B. There was a 26-day period between the anthesis of the earliest and the latest accessions in the trial (Table 3.1). Most of the landraces flowered later than the commercial varieties (Table 3.2). This is consistent with the fact that all landraces are normally late maturing while commercial varieties have been bred for earlier maturity (Taba et al., 1998). There were no significant differences for ASI indicating that the trial was not subjected abiotic stress during the season. Significant differences for ASI would indicate differential responses of the varieties to abiotic stresses, mainly drought, low N and low soil pH (Edmeades et al., 1999).

Table 3.2.Grain yield, days to 50% anthesis, number of ears per plant, 100-kernel weight and plant height for 294 maize varieties grouped into country of origin (COUNTRY), mega-environment (ME) of the collection site for landraces, and germplasm type (GERMPLASM).

COUNTRY	GY	AD	EPP	HKWT	PLHT
Zimbabwe	4.502a	72.7b	0.83a	44.11a	260.9b
Zambia	4.429a	75.0a	0.77a	44.87a	276.5a
Malawi	3.953a	74.6a	0.84a	40.27b	275.1a
USA	1.898b	67.9c	0.62b	29.37c	244.6b

ME	GY	AD	EPP	HKWT	PLHT
Commercial Cvs	4.733a	71.0d	0.81a	39.82c	253.4a
A	4.162ab	75.2a	0.77a	45.38ab	276.3a
B	4.442a	74.1ab	0.82a	44.19abc	275.8a
C	4.451ab	71.8cd	0.82a	46.48a	260.4bc
E	4.239ab	73.4bc	0.84a	40.84c	259.3bc
F	3.491b	72.2d	0.73a	42.19bc	265.6ab

GERMPLASM	GY	AD	EPP	HKWT	PLHT
Landraces	4.258b	74.3a	0.8ab	44.03a	271.6a
Seed Co.s	5.98a	70.2b	0.91a	38.11b	243.1b
Early Zim OPVs	4.111b	74.3a	0.67bc	49.00a	274.4a
CIMMYT OPVs	5.947a	69.0bc	0.92a	37.40b	238.7b
Ancestors (USA)	1.892c	67.8c	0.62c	29.20c	243.8b

GY=Grain Yield (Mg ha^{-1}); AD=number of days from planting to 50% pollen shed; EPP=number of ears per plant; HKWT=weight of 100 kernels (g at 12.5% moisture); PLHT=plant height (cm); Commercial Cvs=commercial cultivars; A=Wet Upper Mid-Altitude, B=Wet Lower Mid-Altitude, C=Dry Mid-altitude, E=Dry Lowland Tropical, F=Highlands; means followed by the same letter are not significantly and means followed different letters are significantly different.

There were highly significant differences among the varieties for most of the agro-morphological traits except KT, HC, RL, SL, KA and KTEX (Table 3.1). Populations displayed dramatic variation for plant architecture traits (PLHT, EHT, EPOS, ELL, LAE, ELW), tassel traits (TL, NTB), ear traits (NKR, EL, ED, RD, CD, PES, PCC) and kernel characteristics (HKWT, KL, KW, PKC) (Table 3.1). In summary, results from the ANOVA reveal that local maize landraces are tall, late maturing and low yield when compared to commercial varieties. Landraces collected from favorable growing environments (mega-environments A and B) tend to be tall, higher yielding and later flowering than those collected from semi-marginal to marginal environments (mega-

environments C and E). Landraces from high elevations (mega-environment F) may be a good source for early maturity, medium plant height and large seeds. In breeders view, this type of material is very useful in plant breeding programs (Vivek and Bänziger, 2005). However, these types were poorly represented in the collection, and there is need for further collections.

Cluster Analysis

According to the plot of the first two canonical variables (Figure 3.1), three non-overlapping groups of accessions were obtained by cluster analysis of the 294 varieties tested. Lattice-adjusted means for AD, NKR, HKWT, KL, KW, PLHT, EHT, EL, ED, RD, CD, and CIRCUM were used in classifying the varieties. These characters were reported to be among the most heritable and discriminatory morphological and agronomic variables for racial classification of maize (Sanchez et al., 1993). The results of the cluster analysis also suggest that the traits used for the classification (AD, NKR, HKWT, KL, KW, PLHT, EHT, EL, ED, RD, CD, and CIRCUM) can be considered appropriate for classification of the southern African maize landraces.

Different members within a cluster were assumed to be more closely related in terms of the traits under consideration with each other than those members in different clusters. Similarly, members in clusters with non-significant distance were assumed to have more close relationships with each other than they are with those in significantly distant clusters. Cluster C1 was the smallest with 68 varieties or constituting close to 23% of the total populations while clusters C2 and C3 each had 113 varieties or almost 38% of the total

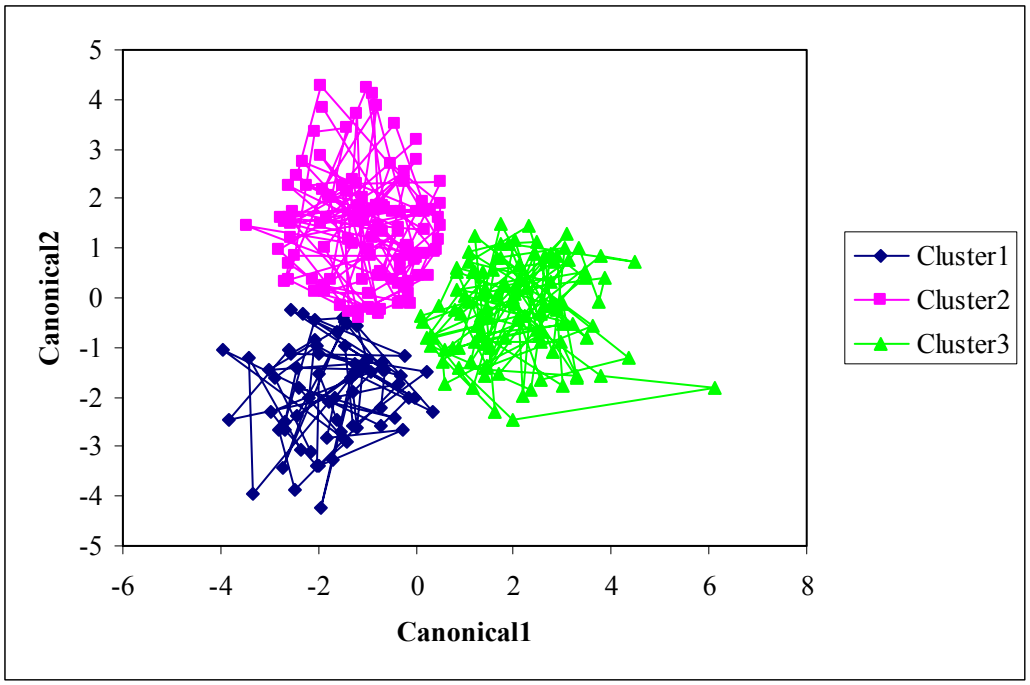

Fig. 3.1. Plots of the first and second canonical variables of the 294 maize varieties grouped in 3 clusters based on key morphological and agronomic traits.

population for each of the two groups. Cluster C1 constituted mainly of local landraces with the least yield potential and prolificacy (EPP) among the three clusters, intermediate seed size (HKWT) and number of kernel rows per ear, and late flowering (Table 3.3). Cluster C2 was composed mostly of local landraces that have phenotypic characteristics similar to the OPV Hickory King, historically important OPVs from Zimbabwe (i.e. Salisbury White and Southern Cross), and Hickory King from the US. This group was characterized by intermediate grain yields, late flowering, least number of kernel rows per ear and the largest seed size. Cluster C3 was composed mainly of improved varieties from southern Africa, five of the six ancestral OPVs from the US, and some landraces, including some HK types. This cluster constituted the best yielding varieties with the highest prolificacy, highest number of kernel rows per ear, small seed sizes and early flowering. The fact that most landraces clustered separately from improved varieties indicates that maize breeders in the region have been selecting for different

Table 3.3. Key characteristics of the three groups formed from cluster analysis of the 294 African varieties evaluated for agro-morphological diversity in Zimbabwe in 2004.

Characteristic	Cluster1	Cluster2	Cluster3
n	68	113	113
50 % Anthesis (dap*)	75	75	72
Grain Yield (Mg ha^{-1})	3.395	4.388	4.932
Ears per plant	0.76	0.77	0.88
100 Kernel weight (g)	41.4	50.9	38.2
Kernel row number	11	10	13
Group Composition	Mostly local landraces	Historic OPVs from Southern Africa, HK type landraces, USA HK	Improved Cvs, USA OPVs, a few landraces

dap = days after planting

morpho-agronomic traits as compared to smallholder farmers. Thus within the landraces exists a vast amount of variation, much of which is not present in advanced breeding lines and improved varieties available in the three countries. Even though some degree of selection is practiced by farmers, there is no strict isolation of such maize fields from the neighboring farms so gene flow between maize plants within the same farm as well as maize between different farms is likely to occur. From this study, it can also be hypothesized that that the landraces that grouped together with improved and introduced varieties (C3) may actually be "creolized" varieties, i.e. hybridizations between traditional landraces from groups 1 and 2 with improved varieties available in the three countries. From the three clusters identified from this study, the upper 25% of the landraces representing those with the highest average diversity of the clusters were used to form a subset for further field evaluation under drought, low soil fertility and acid soils. In general, landraces collected from different mega-environments were distributed over all phenotypic clusters, reflecting the wide variation within a particular mega-environment.

Some accessions like HK collected from different mega-environments and countries were distributed over all three phenotypic clusters reflecting the differential impact of farmer and natural selection. This fact is consistent with the hypothesis that different versions of HK have resulted from about 100 years of cultivation of the original

HK introduction from the USA in different agro-ecologies and under different cultural systems. On the other hand most of the HK types were quite similar and could be grouped together in C2. The joint clustering of different HK types from different mega-environments and countries in this cluster (C2) is consistent with previous knowledge that the local HK originated from the US HK (McCann, 2005) and that these different versions are still similar despite 100 years of population differentiation. This result reflects the exchange of maize seeds among farmers and across different breeding programs in the three southern African countries particularly after the introduction of the large white grained trait into the farming systems (Weinamann, 1972). For example, HK and its descendants (SW and SC) have been used to introduce large white grained into many different varieties in different breeding programs, especially in Zimbabwe and Zambia.

The clustering of some of the first OPVs developed in Zimbabwe (Salisbury White and Southern Cross) in C2 together with most of the HK types confirms the knowledge that these two varieties either originated from an improvement of the HK introduced in Zimbabwe, or were derived from a cross of the US HK with other germplasm (Weinamann, 1972). These two varieties together with HK went on to dominate maize production in southern Africa before the development of hybrids and up to today these varieties are still found in some farmers fields (Chapter II). In addition, it is from SW and SC that the parents of the world's first commercially successful single-cross hybrid SR52 were developed (Mashingaidze, 1994). SR52 also went on to dominate maize production in many southern, central and east African countries from about 1970 to the mid-1990s (McCann, 2005).

Biplot Analysis

A biplot analysis was run to further evaluate the relationships among the maize accessions and all the traits measured. The biplot was generated by singular value decomposition on two way data table for varieties and traits. The importance of various variables in distance computation was balanced by standardizing the variables. The biplot is presented in Figure 3.2. In this biplot accessions are represented as points and traits as vectors. An acute angle between any two vectors indicates a strong positive correlation

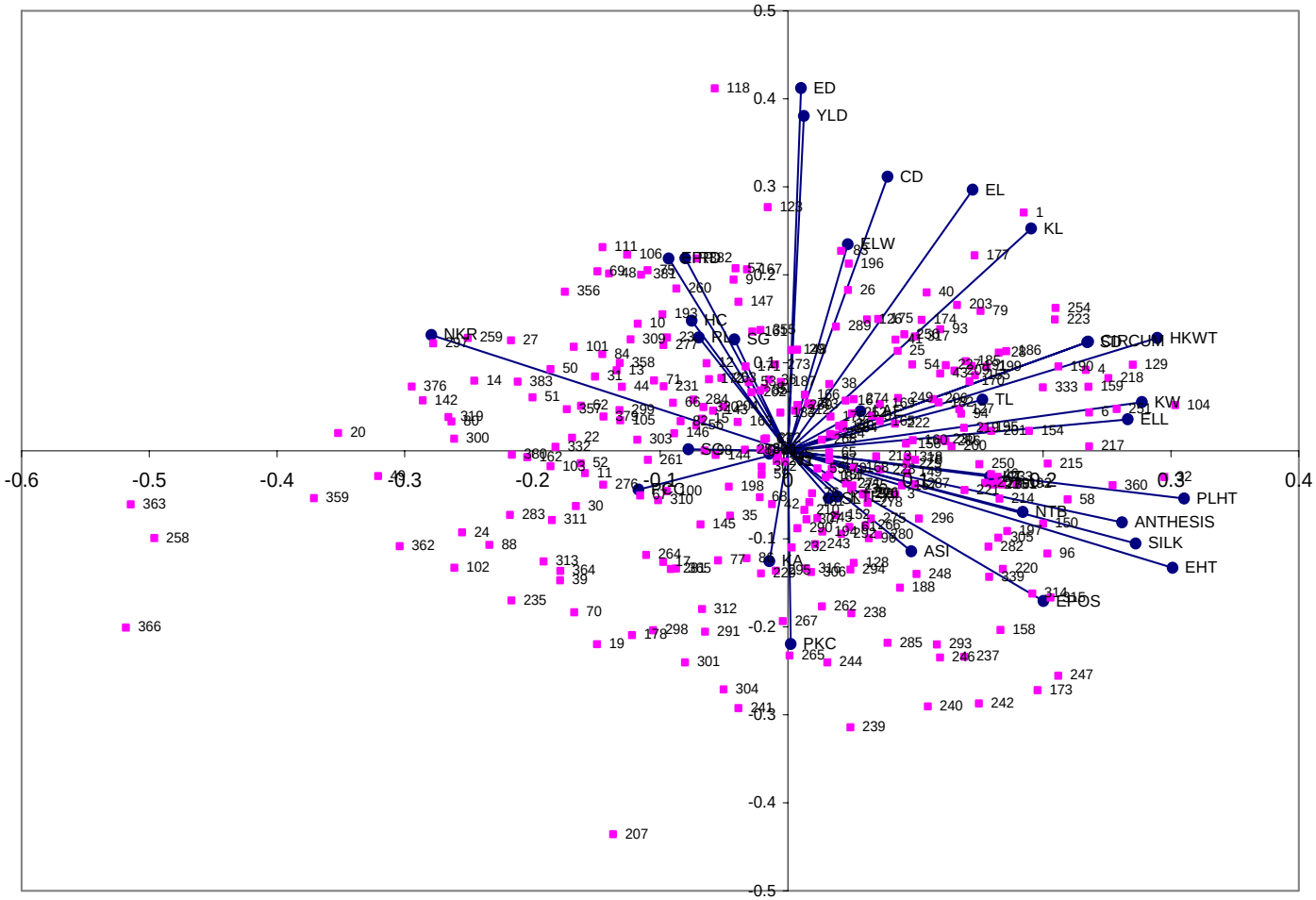

Fig. 3.2. Singular value decomposition biplot showing the relationships among traits and accessions for the 294 maize varieties grown at Harare during the 2004 season.

among the traits. Such traits would then discriminate the accessions in a similar way. Trait vectors at 90° or greater indicate negative correlation amount the traits and different discrimination among accessions for these traits.

Grain yield (GY) showed a very tight angle with most of the yield component traits (EPP, ED, CD, El, KL and EPP) indicating a strong positive correlation but was moderately correlated with NKR, HKWT, KW. Most of the yield component traits were also highly correlated with each other as shown by the small angle between them in the biplot. The phenological traits AD and SILK were closely correlated but were moderately correlated with GY as indicated by the respective angles between the vectors. Anthesis to silking interval (ASI) had a large angle with grain yield and most of the yield component traits, thus showing a negative correlation between ASI and grain yield and its components even though ASI was non-significant in this trial. There was intermediate to large angles between most morphological traits and GY indicating moderate to week associations between the morphological traits and GY. However, most of the morphological traits exhibited acute angles between them indicating strong positive correlations. The first two principal components comprised of 17.6% and 12.6% respectively explaining a total of 30.2% of the variation. It is normally assumed that characters with larger absolute values closer to unity within the first principal component influence the clustering more than those with lower absolute values closer to zero. The biplot also visually revealed the highest and lowest performers for each trait, e.g. the highest yielding accessions were 118 and 123 while the worst yielding accessions were 207 and 241.

The results of cluster analysis, the biplot and PCA may be used to design a strategy to maintain or enhance the genetic diversity of future varieties, for example, by crossing some improved varieties with some specifically adapted local landraces. Another approach may be to cross high yielding landraces that possess many random genetic differences with the expectation that this will increase the number of transgressive recombinants. For example, the high yielding varieties such as HK and Leaming are distinctly separated in different clusters and in the biplot. Crossing these varieties may result in transgressive recombinant progenies to select for in a breeding program. In addition, if the separation of this collection of vaieties truly reflects genetic

divergence, then crosses among clusters may have more heterotic potential than crosses among landraces within the same cluster.

All the improved varieties were grouped into C3. This low differentiation among most of the improved varieties that were developed in southern Africa may encourage the breeders to include local landraces in their breeding programs. This may broaden the genetic base and maximize genetic gains from selection, as favorable alleles may be accumulated. In tomato, for example, despite their apparent inferior phenotypes, exotic germplasm and wild species contain desirable alleles for some economically important characters (de Vicente and Tanksley, 1993). A greater effort to introgress diverse germplasm into locally adapted cultivars that do not carry a yield penalty may offer greater rewards in crop improvement and reduced genetic vulnerability. The use of exotic germplasm in many crops may be limited to traits, such as disease resistance, that are controlled by few genes.

Genetic and Phenotypic Correlations

The matrix of genetic and phenotypic correlations (Table 3.4) between traits shows that there was mostly weak and non-significant correlations between the three sets of traits, i.e., those related to yield and productivity; those related to plant morphology; and those related to plant phenology. This result indicates that there was a very low genetic relation between those three kinds of traits under non-stressed conditions in this evaluation. This could result from different genetic and environmental mechanisms involved in the expression of three sets of traits. A similar result has been observed in other studies in tropical maize and in Europe and the Americas (Rebourg et al., 2001).

Table 3.4. Genotypic (upper diagonal) and phenotypic (lower diagonal) correlations among traits measured for 294 maize accessions grown at Harare during the 2004 growing season.

	GY	EPP	NKR	HKWT	KL	KW	AD	SILK	PLHT	EHT	ELL	ELW	TL	EL	ED	CD	RD
GY		0.64**	0.35**	0.12	0.50**	-0.10**	-0.11	-0.18	-0.02	-0.13	0.17**	0.39**	0.35**	0.65**	0.73**	0.73**	0.72**
EPP	0.56**		0.60**	-0.33**	0.00	-0.46**	-0.20	-0.25	-0.24	-0.24**	-0.11	0.23	0.04	0.20	0.45**	0.44**	0.46
NKR	0.26**	0.27**		-0.81**	-0.43**	-0.87**	-0.35**	-0.38**	-0.61**	-0.52**	-0.49**	0.02	-0.39**	-0.13	0.26**	0.07	0.51**
HKWT	0.19**	-0.10**	-0.62**		0.80**	0.93**	0.30**	0.32**	0.59**	0.41**	0.54**	0.26**	0.44**	0.46**	0.25**	0.47**	-0.05
KL	0.29**	0.02	-0.19**	0.54**		0.69**	0.38**	0.35**	0.46**	0.24**	0.46**	0.30**	0.07	0.46**	0.69**	0.94**	0.33
KW	0.00	-0.18**	-0.70**	0.75**	0.47**		0.29**	0.30**	0.52**	0.35**	0.50**	0.14	0.43**	0.33**	0.13	0.32**	-0.13
AD	-0.08	-0.11	-0.25**	0.19**	0.09**	0.19**		0.99**	0.77**	0.70**	0.59**	0.02	0.30	0.12	-0.07	0.20	-0.43**
SILK	-0.13**	-0.15	-0.27**	0.18**	0.08	0.21**	0.95**		0.81**	0.73**	0.60**	0.01	0.31	0.08	-0.12	0.22	-0.58**
PLHT	0.07	-0.08	-0.34**	0.32**	0.18**	0.31**	0.37**	0.38**		1.01**	0.87**	0.03	0.54**	0.28**	-0.15	0.01	-0.37**
EHT	-0.02	-0.10**	-0.37**	0.26**	0.10**	0.22**	0.42**	0.43**	0.79**		0.72**	-0.06	0.50**	0.08	-0.24**	-0.03	-0.53**
ELL	0.11**	-0.04	-0.28**	0.30**	0.18**	0.27**	0.27**	0.26**	0.34**	0.32**		0.04	0.94**	0.44**	0.04	0.17	-0.13
ELW	0.29**	0.17**	0.06	0.14**	0.14**	0.03	-0.02	-0.06	0.00	-0.05	0.09**		0.01	0.39**	0.54**	0.69**	0.33
TL	0.14**	0.07	-0.06	0.14**	0.06	0.08	0.02	0.01	0.14	0.14**	0.26**	0.08		0.55**	-0.02	0.09	-0.17
EL	0.54**	0.16**	-0.04	0.38**	0.26**	0.2**1	0.06	0.02	0.19	0.07	0.23**	0.29**	0.24**		0.40**	0.49**	0.28
ED	0.53**	0.22**	0.27**	0.24**	0.43**	0.13**	-0.04	-0.07	-0.01	-0.10**	0.07	0.32**	0.04	0.39**		1.00**	0.99**
CD	0.36**	0.15**	0.09**	0.27**	0.38**	0.20**	0.02	-0.01	0.06	0.01	0.10**	0.25**	0.02	0.29**	0.68**		0.97**
RD	0.25**	0.10	0.24**	-0.01	0.08**	-0.08	-0.07	-0.08	-0.08	-0.14**	-0.03	0.11**	0.02	0.15**	0.45**	-0.35**	

GY = Grain Yield (Mg ha^{-1}), EPP = number of ear per plant, NKR = number of kernel rows per ear, HKWT = weight of 100 kernels (g at 12.5% moisture), KL = kernel length (mm), KW = kernel weight (mm), KT =kernel thickness (mm); AD= number of day from planting to 50% of the plants shedding pollen (days); SILK =number of days from planting to 50% of the plants having silks 1cm long or more(days); PLHT= plant height (cm); EHT = ear height (cm); EL = ear length (mm); ED = ear diameter (mm); RD = rachis diameter (mm); CD = cod diameter (mm); ELL = ear leaf length (cm); ELW = ear leaf width (cm); TL = tassel length (cm); ** = significant correlation.

However, concerning the set of grain yield component traits, most of the correlations among the traits were strong (both negative and positive) except for YLD and HKWT, YLD and KW, YLD and EL, YLD and Ed, YLD and CD, YLD and RD suggesting that these traits contribute substantially to final grain yield. There were also strong correlation between KL and ED, KL and CD, ED and RD and CD and RD, and EPP and KL. For this set of traits, the highest values were observed for ED and RD (r = 0.99), CD and RD (r = 0.98), HKWT and KW (r = 0.98), HKWT and KL (r = 0.80), and HKWT and NKR (r = -0.81). As expected, the correlation between the phonological traits AD and SILK was strong and positive (r = 0.95 to 0.99) which is normal under non-stress conditions in maize. For the set of plant morphological traits (PLHT, EHT, CIRCUM, TL, ELL and ELW), the coefficients of correlation between many of them were moderate to high which is the usual result observed in maize, i.e. the taller a plant is the bigger the vegetative material (Hallauer and Miranda-Filho, 1988). In general, the coefficients decrease for the phenotypic correlations versus genotypic correlations suggesting that factors other than genetic causes, probably environmental effect, influence the correlation between traits.

Most of the genotypic correlation coefficients were significant, but in practice only coefficients of ±0.71 have been suggested to be biologically important (Hallauer and Miranda-Filho, 1988) as more than 50% of the variation in one trait is predicted by the other (Snedecor and Cochran, 1980). The genotypic correlations between yield component and morphological traits found in this study suggest the design or breeding of specific crop ideotypes. For example, yield was negatively correlated with plant height and days to anthesis suggesting that high yielding early maturing varieties with short plants could be developed from this collection of accessions.

Broad-sense Heritability

Heritability estimates based on plant and on variety mean basis were significant and ranged from 16% to 82% and 27% to 90% respectively (Table 3.5). For estimates based on variety mean basis, heritability estimates were highest for yield and its component traits, and plant phenology traits, followed by plant morphology traits, and finally ear traits. A summary of expected heritability estimates for maize under non-stress

growing conditions is presented by Hallauer and Miranda-Filho (1998). These range from less that 30% for grain yield and most of its component traits to between 50% and 70% for plant morphological and phenological traits.

Yield component traits, such as 100 kernel weight, and grain yield showed equal or higher heritability than most morphological traits (Hallauer and Miranda-Filho, 1988). Any difference that may be observed may be due to may be due to different origins of the analyzed germplasm. The relatively high heritability estimates for yield and its component traits observed in this study indicates that progress in yield can be obtained by selection for these traits in the environment where the trials was grown. This is particularly important for local maize landraces which could thus be improved for grain yield with a few cycles of recurrent selection with focus on among other traits, number of kernel rows per ear, kernel width, ear length and ear diameter. However these data should be taken with caution since this trial was conducted in only one environment and one season, thus the effect of Genotype x Environment interaction may have inflated the heritability estimates. In this study, heritability estimate for NKR, KW, EL and ED was high at 81%, 90%, 63% and 70% respectively. This implies that simple selection of plants with higher number of rows, higher kernel width, and long thick ears within a maize landrace could result in improving the yield of the landrace. The highest values of heritability found for these ear and kernel traits (Table 3.5) agrees with those reported by Hallauer and Miranda-Filho (1988). In their study, Goodman and Paterniani (1969) also found the highest value for rows of kernels, which agrees with the results from this study.

Phenological and morphological traits, such as days from planting anthesis, and to silking, plant and ear height, also showed high values, which is also in agreement with the results of Hallauer and Miranda-Filho (1988) and Llaurado and Moreno-Gonzalez (1993). Ear leaf length, ear leaf width and tassel length showed intermediate to low heritabilities, a result also found by Geraldi et al. (1985).

Table 3.5. Heritability estimates (H2) and standard errors (SE) on plot and variety mean basis for various traits calculated for 294 maize varieties grown at Harare during the 2004 growing season.

TRAIT	PLOT		VARIETY	
	H^2	SE	H^2	SE
YLD	0.48	± 0.0455	0.65	± 0.0417
EPP	0.28	± 0.0536	0.43	± 0.0658
NKR	0.82	± 0.0195	0.90	± 0.0118
HKWT	0.66	± 0.0329	0.79	± 0.0239
KL	0.38	± 0.0512	0.55	± 0.0541
KW	0.68	± 0.0316	0.81	± 0.0224
AD	0.55	± 0.0426	0.71	± 0.0353
SILK	0.53	± 0.0446	0.69	± 0.0382
PLHT	0.41	± 0.0499	0.58	± 0.0502
EHT	0.54	± 0.0429	0.70	± 0.0363
ELL	0.43	± 0.0488	0.60	± 0.0479
ELW	0.34	± 0.0513	0.51	± 0.0570
TL	0.16	± 0.0583	0.27	± 0.0874
EL	0.46	± 0.0464	0.63	± 0.0436
ED	0.54	± 0.0416	0.70	± 0.0353
CD	0.20	± 0.0552	0.33	± 0.0766
RD	0.16	± 0.0549	0.27	± 0.0821

H^2 = Broad-sense heritability; YLD = Grain Yield; EPP = number of ear per plant; NKR = number of kernel rows per ear, HKWT = weight of 100 kernels;, KL = kernel length; KW = kernel weight; AD = number of day from planting to 50% of the plants shedding pollen; SILK =number of days from planting to 50% of the plants having silks 1cm long or more; PLHT= plant height; EHT = ear height (cm); EL = ear length (mm); ED = ear diameter; RD = rachis diameter (mm); CD = cod diameter (mm); ELL = ear leaf length (cm); ELW = ear leaf width (cm); TL = tassel length (cm);

Formation of the Core Subset

The landrace accessions chosen for the core subset are indicated in boldface in Table 3.1. In this study we decided on a proportion of 25% of the total collection. Thus, a total of 74 landraces from the initial 267 landraces tested were chosen for molecular characterization (Chapter IV) and detailed agronomic evaluation under different abiotic stress levels (Chapter V). The proportion of the core subset is negatively related to the total collection size on a log scale as recommended by van Hintum (1999), who stated that small collections have the largest core size. A total of 34 improved varieties comprising 6 ancestral OPVs originating from the USA, 3 historically important OPVs developed in Zimbabwe during the pre-hybrid era, 8 OPVs from CIMMYT, and 17 improved OPVs and hybrids from seed companies and national maize breeding programs

of Zimbabwe and Zambia were added to the 74 landraces for further studies and to enable a comparison of farmer selection versus formal breeding.

It is also important to emphasize that a core formed by deliberately sampling of entries with the highest average genetic distance presents some disadvantages. For example, as reported by Brown and Spillane (1999), it does not eliminate the possibility of duplication or doubling of entries and, yet, it is equally clear that any particular rare variant, found perhaps in only one entry of the whole collection, is likely to be absent from the core. The total core subset in for the maize landraces included entries from all three countries of origin (Zimbabwe, Zambia and Malawi) and representing most of the mega-environments where maize is grown in the region (Table 3.6).

Table 3.6. Composition of the core subset chosen based on phenotypic characteristics of 267 maize landraces evaluated at Harare during the 2002 growing season.

ME	ENTIRE	CORE	%	COUNTRY	ENTIRE	CORE	%
A	115	33	29	ZIMBABWE	100	23	31
B	80	26	33	ZAMBIA	111	33	45
C	13	3	23	MALAWI	56	18	24
E	56	11	20				
F	3	0	0				
TOTAL	267	73	104	TOTAL	267	74	100

A=Wet Upper Mid-Altitude, B=Wet Lower Mid-Altitude, C=Dry Mid-altitude, E=Dry Lowland Tropical, F=Highlands

Only landraces from the highlands (mega-environment F) were absent in the core subset. The number of entries from each mega-environment included in the core subset was directly proportional to the size of landraces collected from each respective mega-environment and ranged from 3 to 33. Zambia contributed about 45% to the total core subset while Zimbabwe and Malawi contributed 31% and 24%, respectively. Means, ranges, and standard errors for the whole and core subsets of the maize accessions collected and tested were close to those of the whole subsets. In other words, the indices for the core subsets were not significantly different from the respective indices for the

whole subsets for all descriptors. Thus, in terms of diversity, the core subset was not significantly different from the whole subset according to the traits used in the clustering and core formation for the accessions.

CONCLUSIONS

The primary goal of the study was to examine the amount of genetic diversity among 294 maize varieties originating from farmers and different breeding programs in three countries together with selected accessions from related OPVs from the USA. In general the ANOVA revealed highly significant differences among the accessions for most of the characters evaluated indicating that there was adequate morphological, phonological and agronomical variability among the accessions for most of the traits. Considerable variation was found between the landraces for most of the quantitative traits examined. This study first confirmed that traditional maize populations and improved varieties from southern Africa display a large range of phenotypic variation for morphological, agronomic and phonological traits. Groups with different agro-morphological traits could be identified. In particular, three morphological types were clearly distinct: (i) local landraces characterized by low yields, late flowering and intermediate seed size, (ii) Hickory King types consisting of tall and late flowering plants, with few kernel rows per ear and large seed size, and (iii) improved varieties and some landraces including “creolized” types consisting of short and early flowering plants, with more kernel rows per ear and higher yields. This clustering pattern showed that farmers’ adaptive selection occurring in areas where the landraces grown in different agro-ecologies of the three countries facilitated the accessions to maintain their distinct identities.

The fact that most landraces clustered separately from improved varieties indicates that maize breeders in the region have been selecting for different morpho-agronomic traits as compared to smallholder farmers. Thus, within the landraces exists a vast amount of variation, much of which is not present in advanced breeding lines and improved varieties available in the three countries. The differences observed among the landraces and improved varieties could provide a basis for choosing germplasm for development of improved cultivars. If this separation reflects genetic divergence, then

crosses among clusters may have more heterotic potential than crosses among landraces within the same cluster.

A wide range in genotypic and phenotypic correlations was observed in the accessions for the quantitative plant traits studied. In general correlations were weak between the three sets of traits studied; yield component traits, phonological traits, and morphological traits. This result indicates that there is a very low genetic relation between those three kinds of traits under non-stressed conditions in maize. However correlations were generally strong among traits within a set. Higher values for broad-sense heritability were associated with ear traits, such as length, diameter, row number, and plant traits such as days to flowering, plant height, ear height and to some extent grain yield.

From the three clusters identified from this study, the upper 25% of the landraces representing those with the highest average diversity of the clusters were used to form a subset for further field evaluation under drought, low soil fertility and acid soils alongside improved and ancestral varieties. The core collection described here was developed to improve the evaluation of the wide diversity of accessions from the collection. It is also useful for conservation of our maize genetic resources in southern Africa, and to increase knowledge of local materials and to stimulate its use. The procedure adopted to set up the core reflected our knowledge of the material and was based on a combination of practical experience and passport data. Entry selection based on the highest average genetic distances between accessions resulted in a core subset comprising of entries from each country and predominant mega-environments in southern Africa. Results showed that most of the phenotypic variation in the original data sets is represented in the core subset defined by this sampling method. This suggests that the selected core subset from the landrace collection represents nearly all of the phenotypic variations in the whole collection.

CHAPTER IV

SSR DIVERSITY OF MAIZE ACCESSIONS

INTRODUCTION

Maize is one of the most diverse crop species in the world with the diversity manifested at both the phenotypic and molecular levels. Most maize diversity remains undescribed, poorly understood and under utilized in modern plant improvement largely because of the difficult of identifying useful genetic variants hidden in the background of low yielding local varieties or lines (Tanksley and McCouch, 1997). Classical methods of estimating diversity among groups of plants have relied chiefly upon morphological characters, which still play a central role in the ANOVA in crop species and their relatives (reviewed in Chapter III). However, because of the strong environmental influence on morphological traits, mainly on those of a quantitative nature, new techniques which analyze diversity at biochemical or molecular level have been developed and successfully applied in diversity studies of different crops (Tanksley and McCouch, 1997). Molecular techniques are more expensive than most morphological approaches to the study of genetic or species diversity and consequently they should be used only where other techniques are less powerful or not feasible.

Literature abounds with genetic diversity studies at the molecular level in maize with a focus on genetic diversity of temperate American and European populations (Lu and Bernado, 2001; Barcaccia et al. 2003; Lucchin et al. 2003; Carvalho et al. 2004; Kresovich et al. 2005). Different marker types have been used to study genetic diversity (Tanksley and McCouch, 1997). Of recent, several diversity studies have also focused on tropical maize populations from the Americas and Asia (Franco et al. 2001; Warburton et al. 2002). However, no reports were found in literature for molecular diversity studies for Africa, particularly southern Africa. Even though maize is a relatively new crop in southern Africa as compared to the sorghums and millets, it still important to characterize the diversity that has resulted from over a 100 years of farmer and natural selection. Since genomic approaches to assessing diversity are more powerful than morphological and biochemical approaches, there is a compelling opportunity to apply molecular tools to sift through the countless allelic variants in the maize gene pool and understand how they

impact phenotypes of agronomic and evolutionary importance. Using molecular markers, the landraces containing unique alleles at given loci can be identified and further characterized for markers associated with abiotic stress tolerance, as these are the populations most likely to contain new favorable alleles for enhanced performance under stress and unstressed conditions. The genetic characterization data will provide useful information for utilizing these populations in maize breeding programs to create abiotic stress tolerant maize. Furthermore, this characterization is important to optimize conservation and facilitate the use of maize landraces maintained by smallholder farmers. In addition, this characterization is necessary for the historical understanding of the introduction of maize in southern Africa.

The objectives of this section of the study was to characterize the extent and distribution of genetic variation in ancestral maize populations introduced into southern Africa from the USA about 100 years ago, historically important open-pollinated maize populations of southern Africa, improved varieties and local farmer landraces originating from diverse agro-ecological zones of Zimbabwe, Zambia and Malawi.

LITERATURE REVIEW

Molecular Diversity in Maize

With the advent of molecular methods of assessing genetic variation, many studies have reported genetic diversity and relatedness of maize inbred lines, hybrids and open pollinated populations. Molecular diversity in maize has been studied for various purposes ranging from (i) baselines surveys to assess diversity in a given context, (ii) historical understanding of maize in a given area, (iii) analyzing the structure of diversity, (v) investigating phylogenic and evolutionary relationships of maize and its relatives, (vi) studying heterosis and hybrid performance prediction, (vii) analyzing diversity trends over time, (viii) formulating germplasm maintenance and conservation strategies, (ix) varietal identification and maintenance, and (x) relating diversity to agronomic performance. In earlier studies, restriction fragment length polymorphic (RFLP) markers were used more than any other type of markers, while PCR-based markers are now the

markers of choice for most maize diversity studies, even though RFLP markers are still used to some extent.

Mumm and Dudley (1994) were able to identity major heterotic groups and subgroups in a set of 148 U. S. maize inbred lines using 46 RFPL markers. In contrast, Warburton et al. (2005) did not find discrete clusters corresponding to heterotic groups in a set of 218 phenotypically diverse inbred lines developed at CIMMYT. They concluded that supplementing molecular marker results to cross performance information may be beneficial in refining heterotic groups and select representative testers for use in a hybrid breeding program. In China, Xia et al. (2004) demonstrated that SSR could be used to assess relationships between inbred lines of maize, but it was difficult to predict heterosis and hybrid performance. Using amplified fragment length polymorphism (AFLP) markers to investigate genetic relationships among 96 tropical maize inbred lines from Brazil, Oliveira et al. (2004) were not able to separate the inbred lines into well defined groups and had to use other procedures to separate the lines into more accurate groups.

Using 255 random amplified polymorphic DNA (RAPD) markers on 81 maize accessions from Brazil, Valdemar et al. (2004) found genetic similarity among the landraces ranging from 0.78 to 0.91 and clustered the populations into two main groups that correlated according to kernel color. Their results showed that the field isolation practiced by smallholder farmers helped maintain genetic variability and identity preservation of the landraces. In a study involving 130 European maize populations, Rebourg et al. (2001) found high levels of genetic diversity and differentiations within the maize populations using 29 RFLP markers. In addition, a clear relationship between the genetic diversity of the populations and agronomic performance was found. Also using RFLP markers on maize inbred lines, Gauthier et al. (2002) used 23 RFLP markers and distinguished three main clusters within 488 European maize populations. In this study genetic diversity was appeared higher in those geographic regions where the first maize populations were thought to have been introduced from the Americas.

Based on molecular analysis using 83 SSR markers, Lu and Bernado (2001) concluded that genetic diversity among current US maize inbreds had been reduced at the gene level but not at the population level when compared with historically important inbreds. Thus hybrid maize breeding in the USA has maintained, rather than decreased,

genetic diversity over time. George et al. (2004) assessed genetic diversity for downy mildew disease in 102 Asian maize inbred lines using 76 SSR markers and concluded that maize breeding activities in Asia had not caused a decline in the overall amount of diversity in the region for that particular trait.

Xia et al. (2005) were able to separate temperate versus non-temperate inbred lines using 75 simple sequence repeat (SSR) markers in a study to investigate the genetic diversity and relationships among CIMMYT's subtropical, tropical midaltitude, and highland maize lines and elite US and European maize lines. However, there was no clear clustering within the subtropical, tropical midaltitude, and highland maize lines, indicating a mixture of CIMMYT's populations and pools and that large amounts of genetic variation have been incorporated into CIMMYT's germplasm by breeders.

In Italy, a comparative characterization of 10 maize populations of one popular maize landrace based on Inter-SSR (ISSR) and SSR markers, Barcaccia et al. (2003) showed that most of the variation (83%) was within population rather than between populations. These results demonstrated that, although a high variability could be found among plants, most of the plant genotypes belong to same landrace. After analyzing genetic diversity and investigating relationships between 155 CIMMYT lowland tropical maize inbreds using 79 SSR markers, Xia et al. (2004a) revealed a lack of structure within the lines, which could be explained by the mixed origin of the populations used to extract the lines and the specific choice of testers for CIMMYT's reciprocal recurrent selection breeding method. In sets of important U.S.A maize lines (B73, CM105, Mo17, Oh43, W153R, and Wf9) originating from different seed sources, small but statistically significant levels of variation existed based on SSR characterization (Gethi et al., 2002). It was predicted that these differences could have arisen through differences in seed maintenance and the variations could raise concerns in germplasm conservation, mapping studies, marker development, and long-term recombinant line development.

In summary, the above review shows the wide application of molecular markers in maize diversity studies.

Molecular Markers for Diversity Analysis

There are three major types of genetic markers: (i) morphological markers which are phenotypic traits or characters; (ii) biochemical markers, which include allelic variants of enzymes called isozymes; and (iii) DNA (or molecular) markers, which reveal sites of variation in DNA (Tanksley and McCouch, 1997). Morphological markers are usually visually characterized phenotypic characters such as flower color, seed shape, growth habits or pigmentation. Isozyme markers are differences in enzymes that are detected by electrophoresis and specific staining. The major disadvantages of morphological and biochemical markers are that they may be limited in number and are influenced by environmental factors or the developmental stage of the plant (Tanksley and McCouch, 1997).

DNA markers are the most widely used type of marker predominantly due to their abundance. They arise from different classes of DNA mutations such as substitution mutations (point mutations), rearrangements (insertions or deletions) or errors in replication of tandemly repeated DNA (Matsuoka et al., 2002). These markers are selectively neutral because they are usually located in non-coding regions of DNA. Unlike morphological and biochemical markers, DNA markers are practically unlimited in number and are not affected by environmental factors and/or the developmental stage of the plant (Tanksley and McCouch, 1997). Apart from the use of DNA markers in the construction of linkage maps, they have numerous applications in plant breeding such as assessing the level of genetic diversity within germplasm (Warburton et al. 2002) and cultivar identity (Gethi et al., 2002).

In recent years, the number of molecular assays available for crop diversity studies has increased dramatically, with each method differing in principles, applications, type and amount of polymorphism detected, as well as cost and time requirements (Tanksley and McCouch, 1997). DNA markers may be broadly divided into three classes based on the method of their detection: (i) hybridization-based e.g. RFLPs; (ii) polymerase chain reaction (PCR)-based e.g. AFLPs, RAPDS, SSRs, and (iii) DNA sequence-based e.g. SNPs (Tanksley and McCouch, 1997).

The greatest advantage of RFLP for genetic diversity analysis is the large number of polymorphic loci found in breeding materials (Messmer et al. 1992). Studies with elite

lines from the U.S. Corn Belt and also with some European maize inbred lines showed that RFLPs are suitable to (i) define heterotic groups, (ii) assign inbred lines to such groups, (iii) reveal genetic relationships among lines, and (iv) identify diverse germplasm sources. However, RFLPs have several disadvantages, which stimulated the development of other markers based on PCR such as the AFLPs (Vos et al., 1995). AFLPs, genomic fragments detected after selective PCR amplification, in addition to being highly reproducible, have the generation of multiple bands in a single assay as a principal advantage. The use of AFLP to estimate genetic diversity was demonstrated at first in 58 temperate maize inbred lines (Smith et al., 1993). More recently, other markers based on polymerase chain reaction (PCR), such as RAPDs have been used in analysis of genetic distance in several plant species. RAPD markers are commonly used because they are quick and simple to obtain, enabling genetic diversity analysis in several types of plant materials, such as natural populations, populations in breeding programs and germplasm collections (Ferreira and Grattapaglia, 1996). When compared with RFLPs, RAPDs are equivalent in determining intraspecific genetic diversity among genotypes but RAPDs are superior when simplicity and cost were considered (Dos Santos et al. 1994). In maize, RAPD markers have been used in the analysis of genetic distance among segregant lines to predict the best crosses among lines for hybrid development, and to assess genetic diversity among collections of native maize (Tanksley and McCouch, 1997).

Microsatellites, also called, SSRs are becoming the markers of choice for fingerprinting and genetic diversity studies in plants (Warburton et al., 2002). SSRs represent an ideal marker system due to their codominant inheritance, locus specificity, and multi-allelic character. PCR-based molecular markers such as SSRs can generate large datasets in a short period of time and, thus, facilitate the evaluation of large numbers of germplasm accessions in seed banks (Rebourg et al. 2001). In a study of 33 maize inbred lines, SSRs produced twice as much information as AFLPs and RAPDs, and 40% more than RFLPs in terms of numbers of alleles per locus (Pejic et al. 1998).

Statistical Analysis for Molecular Diversity Data

A range of statistics and multivariate methods are widely used for pattern analyses of DNA genotypes in plant diversity studies. Typically analyses of DNA genotypes are

performed on genetic similarity or distance matrices among entities rather than on raw multivariate data matrices. The appropriate choice of a genetic distance measure is an important component in the analysis of genetic diversity among a set of genotypes.

For molecular marker data where the amplification products may be equated to alleles, as for example in SSRs and RFLPs, allele frequencies can be calculated and the data employed to generate a binary matrix for statistical analysis. For co-dominant markers, simple matching coefficients (Sokal and Michener, 1958), Jaccard's (1908) coefficient, Nei and Li's (1979) coefficient, and Modified Rogers' (Rogers, 1972) distance are commonly used genetic similarity measures where the data is in binary form (present-absent). The simple matching coefficient is the ratio of the sum of matches to the sum of matches and mismatches while Jaccard's coefficient is the ratio of positive matches to the sum of positive matches and mismatches. George et al. (2004) calculated matrices of genetic similarities among pair-wise comparisons of maize inbred lines in relation to Downey Mildew resistance using the Simple Matching and Jaccard coefficients. In a study on Brazilian maize inbred lines, Oliveria et al. (2004) found Jaccard's similarity coefficients ranging from 0.345 to 0.891 and determined the genetic relationships of some pairs of lines based on this co-efficient. Modified Roger's Distance has been used in determining the genetic distance between maize inbred lines among lowland tropical maize inbred lines (Xia et al., 2004b), and CIMMYT inbred lines and open pollinated populations (Warburton et al., 2002). The Nei and Li (1979) genetic distance estimator was developed for the analysis of restriction site polymorphisms, and is the estimator proposed by Dice (1945) in the pre-molecular era. Barcaccia et al. (2003) have used Nei's unbiased genetic distance measure in calculating distances between populations from one Italian maize landrace called Nostrano I.

Gower (1971) proposed a similarity measure for cases where mixed variable types are measured (e.g., mixtures of binary, ordinal, categorical, and continuous variables). This coefficient can be used, for example, to combine dominant (binary) and multi-allelic, co-dominant (categorical) DNA markers or discrete genotypic and continuous phenotypic variables and is one of several similarity measures used in genetic pattern analysis. The genetic and mathematical properties of various similarity and dissimilarity coefficients used in genetic diversity studies and plant breeding studies have been

reviewed in detail by Reif et al. (2005). Other similarity/dissimilarity statistics have been reported in literature, but the above measures have been used more widely than others.

Cluster analysis (CA), Principal Component Analysis (PCA), Principal Coordinate Analysis (PCoA) and Multidimensional Scaling (MDS) are among some of the common multivariate methods widely used for genetic diversity studies. These methods seek to uncover hidden patterns among objects on which two or more independent variables have been measured. The salient statistical tools and considerations in the analysis of genetic diversity in plants have been reviewed by Mohammadi and Prasanna (2003).

Cluster analysis aims to group items, in this case genotypes based on the characteristics that they posses so that individuals with similar descriptions are mathematically gathered into the same cluster. Clustering methods usual lead to a graphical representation such as tree or dendrogram in which clusters may be visually identified (Mohammadi and Prasanna (2003). In a study to assess the phylogenetic relationships among North American populations, Santacruz-Valera et al. (2004) calculated pair-wise Gower distances between popcorn populations and performed cluster analysis on the populations and came up with three groups of popcorn with distinct morphological characteristics and genetic profiles. Furthermore, a phylogenetic tree was produced using the Neighbor-Joining method (Santacruz-Valera et al., 2004)). Cluster analysis has also been used in studying SSR variation in important U.S. maize inbred lines (Gethi et al., 2002). Oliveira et al. (2004) used cluster analysis in an evaluation of the relationships between tropical maize inbred lines from Brazil, but failed to separate the line into clear well-defined groups. Other examples of the use of CA in maize were reported in literature by Barcaccia et al. (2003), Lu and Bernardo (2001), and Warburton et al. (2005).

PCA and PCoA are multivariate techniques used to produce two or three dimensional scatter plots of items so that the geometrical distances among items in the plot reflect the genetic distances among them with little distortion. Grouping of items in scatter plots will reveal sets of genetically similar individuals. In order to better visualize differences in Downey Mildew resistance among a set of Asian maize inbred lines, George et al. (2004) used PCoA while Warburton et al. (2005) used PCA on a data set

generated from SSR and RFLP analyses. Xia et al. (2005) utilized PCoA of a set of subtropical, mid-altitude and highland maize inbred lines based on SSR data. MDS is a procedure that represents a set of individuals or genotypes in a few dimensions using a similarity/distance matrix between them such that the inter-individual proximities in the map nearly match the original similarity/distances (Johnson and Wichern, 2002). Warburton et al. (2005) has utilized MDS in genetic characterization of 218 elite CIMMYT maize inbred lines based on RFLP data.

There are no formal statistical rules for deciding how many genetic markers are needed to accurately classify accessions, describe genetic patterns, or accurately estimate genetic distances and phenograms. Smith et al. (1991) used 200 RFLP markers dispersed across the maize genome to fingerprint 11 inbred lines (the genetic distance matrix was comprised of 55 elements). They estimated distance matrices by sampling 5 to 200 RFLP markers in increments of five (e.g., 5, 10, 15, ..., 200). They concluded that accuracy was sufficient with 100 or more markers. Bernardo (1993) concluded that 250 or more marker loci were needed to produce precise estimates of coefficients of coancestry in maize. The number of genetic markers used in an analysis may be dictated by nonstatistical factors. The outcome of the analysis might be one of the criteria used to select genetic markers for future analyses. The genetic similarity between two entities is affected by the number and characteristics of the genetic markers sampled; however, as with most sampling problems, increasing the number of markers produces a diminishing return. Ideally, genetic markers for protecting intellectual property and classifying unknown genetic materials should be highly polymorphic and dispersed across the genome.

Diversity and Geographical Origin

Understanding the extent and geographic patterns of genetic diversity within a plant species is essential for effective future collection, the development of conservation strategies, and efficient use of genetic resources for improvement of crop varieties. In most crops different landraces appear to be adapted to specific agro-ecologies, but farmers select what to grow for socio-economic reasons.

Various studies have attempted to study the relationship between genetic diversity and geographic origin of some important crops. In a study to determine RFLP diversity and relationships among traditional European maize populations, Gauthie et al. (2002) observed a correlation between allelic frequencies at some loci and latitude and/or longitude. In another study to characterize geographical patterns of genetic variation in wild annual *Cicer* germplasm, phylogenetic analysis of 146 accessions revealed four distinct groups corresponding well to primary, secondary and tertiary gene pools of chickpea (Iruela et al., 2002). Long-term evolution and adaptation to climatic conditions in these genepool centers makes these wild annual species rich in resistance genes for a range of biotic and abiotic stresses experienced in *Cicer* production. Patterns of RAPD markers also have been shown to be associated with geographical origin in barley (Fernandez et al., 2002), common bean (Beebe et al., 1995), wild emmer-wheat (Fahima et al., 1999), and durum wheat (Spagnoletti Zeuli and Qualset, 1993).

In contrast to the above studies, a specific pattern of geographic distribution of genetic diversity based on molecular data was not observed for common bean landraces in Nicaragua (Gomez et al., 2004). In this study most of the variation of the landraces at the molecular level was explained by differences within or among landraces but not among agro-ecological zones. The authors therefore suggested that molecular differentiation of the landraces was due to founder effect and not the effect of adaptation. Brown-Guedira et al. (2000) did not find an association between origin and RAPD markers among soybean lines of more modern origin in the USA. It is highly likely that these genotypes have been dispersed by human intervention from the areas of actual origin. In another study, RAPD marker variation was not correlated to geographical origin in the cultivated races of sorghum (Menkir et al., 1997), which may be the result of high levels of gene flow among the regions.

MATERIALS AND METHODS

Genetic Material

The most diverse 25% of the landraces selected based on morphological characters (Chapter III), six OPVs obtained from the USA, and 19 commercially-bred

varieties from seed companies in southern Africa and CIMMYT formed the core set of germplasm for molecular characterization (Table 4.1). The landraces had been selected based on highest average genetic distances using morphological characters, while the six OPVs from the USA are among some of the original OPVs introduced into Zimbabwe, Zambia and Malawi from the USA during the late 19th century. Commercially-bred varieties included historically important OPVs from Zimbabwe and improved varieties developed in Zambia, Zimbabwe and Malawi by national agriculture research programs, seed companies and CIMMYT. Thirty seeds of each of the 108 maize accessions were sown in small pots at El Batán, México. The pots were watered for 20 days, until the length of the seedlings was approximately 10 cm. A total length of approximately 10 cm of leaf tissue from 15 seedlings of each accession was pooled for DNA isolation on the 21st day after planting.

Molecular Analysis

DNA from 15 plants of each of the 108 populations sampled was used for the amplification of 24 SSR loci (microsatellites) distributed throughout all 10 chromosomes of maize. We used the set of SSR markers described by Warburton et al. (2002), which provides uniform coverage of the entire maize genome. DNA was extracted using a modified CTAB procedure according to the CIMMYT Applied Biotechnology Center (ABC)'s Manual of Laboratory Procedures available on the internet at http://www.cimyt.org/ABC/Protocols/manualABC.html (verified July 2006). For quantification of DNA concentration, readings of the absorbance at 260 and 280 nm were performed with a Power WaveXmicroplate scanning spectrophotometer (Bio-Tek Instruments, Winoosi, VT). The DNA quality of each sample was evaluated by running

Table 4.1. Description of the 108 maize accession analyzed for SSR diversity in this study.

Entry	Accession	Origin	Type
1	Hickory King (USA)	USA	Ancestral
2	Iowa Silver Mine	USA	Ancestral
3	Leaming	USA	Ancestral
4	Boone County White	USA	Ancestral
5	Eureka	USA	Ancestral
6	Golden King	USA	Ancestral
7	Kanongo-2	Zimbabwe	Landrace
8	Mapongwe a Chitonga	Zambia	Landrace
9	Hickory King-14	Zambia	Landrace
10	Local-49	Zambia	Landrace
11	Kahilahila-2	Zambia	Landrace
12	Local-60	Zambia	Landrace
13	Kenya-6	Zambia	Landrace
14	Kanjere-2	Zambia	Landrace
15	Local-62	Zambia	Landrace
16	Senga-1	Zambia	Landrace
17	Senga-2	Zambia	Landrace
18	Local-3	Malawi	Landrace
19	Masika	Malawi	Landrace
20	Local-13	Malawi	Landrace
21	Local-16	Malawi	Landrace
22	Bantam	Malawi	Landrace
23	Local-26	Malawi	Landrace
24	Local-29	Malawi	Landrace
25	Local-33	Malawi	Landrace
26	Local-37	Malawi	Landrace
27	Local-39	Malawi	Landrace
28	Local-44	Malawi	Landrace
29	Hybrid??	Malawi	Landrace
30	Local-46	Malawi	Landrace
31	Bharabhara	Zimbabwe	Landrace
32	Local (Maroon w/ white tips)	Zimbabwe	Landrace
33	Botoma 8-Line	Zimbabwe	Landrace
34	Local (Mixed Black and White)	Zimbabwe	Landrace
35	Bhabadhla - White Cob	Zimbabwe	Landrace
36	Bogwe	Zimbabwe	Landrace
37	Malaba/Kalanga	Zimbabwe	Landrace
38	Red Cob-1	Zimbabwe	Landrace
39	Hickory King-7	Zimbabwe	Landrace
40	Chindawu-1	Zimbabwe	Landrace
41	Hickory King-10	Zimbabwe	Landrace
42	Samanyika	Zimbabwe	Landrace
43	Njeke/Hickory King	Zimbabwe	Landrace
44	Kangalingali	Zambia	Landrace
45	Kabaka-1	Zambia	Landrace
46	Local-54	Zambia	Landrace
47	Karimina	Zambia	Landrace
48	Kalimwa (HK)	Zambia	Landrace
49	Karimwa-2	Zambia	Landrace
50	Mofati-2	Zambia	Landrace
51	Chibahwe-2	Zambia	Landrace
52	Gankata 8-Lines	Zambia	Landrace
53	Gankata 10-Lines	Zambia	Landrace

Table 4.1 Continued

Entry	Accession	Origin	Type
54	Bingo	Zambia	Landrace
55	Red Local-1	Malawi	Landrace
56	White Flint	Malawi	Landrace
57	Local (Wine Colored)-2	Malawi	Landrace
58	Kenya-3	Malawi	Landrace
59	Kaile-1	Malawi	Landrace
60	Kaile-2	Malawi	Landrace
61	Red Local-2	Malawi	Landrace
62	Chitsvuku	Malawi	Landrace
63	Gankata-1	Zambia	Landrace
64	Gankata-3	Zambia	Landrace
65	Mundele wa Chintu-1	Zambia	Landrace
66	Mboni ya Sintu-5	Zambia	Landrace
67	Mun'indo	Zambia	Landrace
68	Local-50	Zambia	Landrace
69	Mundele wa Chintu-2	Zambia	Landrace
70	Local-52	Zambia	Landrace
71	Kanjilimane-1	Zambia	Landrace
72	Chilala-3	Zambia	Landrace
73	Chimambwe/Kalimwa	Zambia	Landrace
74	Local-15	Malawi	Landrace
75	Local-20	Malawi	Landrace
76	Local-21	Malawi	Landrace
77	Monsanto	Malawi	Landrace
78	MH18	Malawi	Landrace
79	Salisbury White	Seedco - Zimbabwe	Early OPVs
80	Hickory King (SC)	Seedco - Zimbabwe	Early OPVs
81	Southern Cross	Seedco - Zimbabwe	Early OPVs
82	DK8031	Monsanto-Zimbabwe	Commercial
83	SC403	Seedco - Zimbabwe	Commercial
84	SC513	Seedco - Zimbabwe	Commercial
85	SC633	Seedco - Zimbabwe	Commercial
86	SC713	Seedco - Zimbabwe	Commercial
87	NPP (SC)	Seedco - Zimbabwe	Commercial
88	MMV400	Mt. Makulu-Zambia	Commercial
89	MMV600	Mt. Makulu-Zambia	Commercial
90	Pool 16	Mt. Makulu-Zambia	Commercial
91	ZUCA-SR-C2	Mt. Makulu-Zambia	Commercial
92	POP 25	Mt. Makulu-Zambia	Commercial
93	POP 10	Mt. Makulu-Zambia	Commercial
94	Kalahari	AREX (Zimbabwe)	Commercial
95	01ZM040035	NTS - Zimbabwe	Commercial
96	ZM521	NTS - Zimbabwe	Commercial
97	ZM421	NTS - Zimbabwe	Commercial
98	0101-M01SP	NTS - Zimbabwe	Commercial
99	ZM305	CIMMYT-Zimbabwe	CIMMYT OPV
100	ZM621	CIMMYT-Zimbabwe	CIMMYT OPV
101	ZM423-#	CIMMYT-Zimbabwe	CIMMYT OPV
102	ZM523-#	CIMMYT-Zimbabwe	CIMMYT OPV
103	ZM623-#	CIMMYT-Zimbabwe	CIMMYT OPV
104	ZM611	CIMMYT-Zimbabwe	CIMMYT OPV
105	SC411	Seedco - Zimbabwe	Commercial
106	SC525	Seedco - Zimbabwe	Commercial
107	SC637	Seedco - Zimbabwe	Commercial
108	SC719	Seedco - Zimbabwe	Commercial

1µl of DNA on a 1% agarose gel. Pre-prepared DNA from two inbred lines, CML051 and CML292, was used as internal controls.

Primers and PCR conditions were described in detail by Warburton et al. (2002). PCR amplifications were performed in a Peltier Thermo Cycler-100 (MJ Research, Watertown, MA) thermocycler. Forward primers were 5- fluorescently labeled with one of the ABI Prism dyes (PE Applied Biosystems, Foster City, CA): HEX, 6-FAM, or NED. In summary, the protocol for the PCR amplification consisted of an initial denaturation at 94°C for 2 min, followed by about 30 cycles of 94°C for 30 seconds (denaturing), *X*°C for 1 min (annealing), and 72°C for 1 min followed by extension at 72°C for 5 min. The *X*°C refers to the annealing temperature which varied with each primer and is described in the CIMMYT Applied Biotechnology Center (ABC)'s Manual of Laboratory Procedures. Each individual PCR amplification reaction consisted of 1xPCR buffer (500 m*M* KCl, 100 m*M* Tris-HCl, pH 9.0 at 25°C), 10 m*M* dNTPs (2.5 m*M* each dNTP), 25 m*M* $MgCl_2$, Taq DNA polymerase (1 unit total), 1.5 µL of template DNA(10 ngµL_1), 4 p*M* of each primer pair (1µL each, forward and reverse), and ddH_2O. Again the quantities of each reagent in the PCR amplification varied depending on the primer used. Each of these primer loci are documented and described on the Internet at http://www.maizegenomedb.org/ssr.php (verified July 2006). A list of the loci evaluated, chromosomal location (Bin number) and associated primers flanking the repeats is shown in Table 4.2. This group of primers is used by the Applied Biotechnology Centre at CIMMYT.

For electrophoresis, 0.4 µl of PCR products plus 8 µl of formamide and ROX internal standard were loaded in groups (multiloading) in 96-welll plates in an ABI3100 Genetic Analyzer (Applied Biosystems, Inc., Foster City, CA). The ABI3100 is an automated capillary electrophoresis system that can separate, detect, and analyze several fluorescently labeled DNA fragments in one run. Multiloading was designed to create combinations in a way that the sizes of the PCR products and the labeling of primers allowed the differentiation of banding patterns without interference among markers. The DNA samples were electrophoresed in 1xTBE buffer (pH 8.3) at a constant voltage (3.00 kV) for 2.5h in the ABI 3100 Analyzer. Fragment sizes were automatically calculated

with GeneMapper 3.5 (Perkin-Elmer Ltd., Bucks, UK; and Applied Biosystems, Inc., Foster City, CA) and fragment peak sizes were converted to alleles by creating categories in Genotyper 2.1 (Perkin-Elmer Ltd., Bucks, UK) , which combines peak sizes within a predetermined range into the same allele. The data was then exported as an Excel file recording peak size for each individual.

Statistical Analyses

Of the 24 SSR markers used in this study, 23 showed high reproducibility, with high consistency in the amplified product between the PCR and ABI runs of the two controls, CML051 and CML292. Therefore, only 23 markers were included in the analysis. Peak size data were transformed to a binary code based on the presence (1) or absence (0) of each allele with columns representing the variety and rows the different SSR markers. The resulting matrix was analyzed with NTSYS-pc version 2.1 software package (Exeter Software, Setauket, NY) to estimate the genetic similarities among all pairs of varieties using Dice's coefficient of similarity as follows:

$$\mathbf{GS_{ij} = 2\ N_{ij}/(N_i + N_j)}$$

where N_{ij} is the number of alleles (scored bands) shared by lines i and j, and N_i and N_j are the total number of scored bands in lines i and j, respectively. Standard statistics for characterizing genetic variability were computed for each locus and for the whole set of varieties: the total number of alleles (allelic richness), the number of rare alleles (those with a frequency of <5%), and the number of unique alleles (those that appear only once). Landrace accessions were then grouped according to the mega-environment where they were collected in order to calculate the allelic richness, number of alleles, PIC, and GS within and between different mega-environments as well as between landraces and commercial varieties. The Dice similarity coefficients were also used to perform cluster analysis on the whole set of varieties using the unweighted pair group method of arithmetic means (UPGMA) of NTSYS-pc version 2.1 software package (Exeter Software, Setauket, NY).

RESULTS AND DISCUSSION

Overall Diversity

Of the 108 varieties selected for this study, only 99 produced good quality DNA that amplified well to enable good genotyping data. Standard statistics are summarized in Table 4.2. Except for chromosomes 3, 4 and 6 which had one SSR marker each, the rest of the chromosomes were represented by at least two SRR markers. The majority of the SSR (70%) loci had 6 or more alleles. A total of 214 alleles were detected from the 23 amplified loci, with an average number of 9.3 alleles per SSR marker and ranging from four alleles for markers phi062, phi076, phi123 and umc2047 to 17 alleles for marker umc1304.

Table 4.2. Summary of SSR markers, bin number, repeat units, and average number of loci of the 99 African maize accessions.

SSR	Bin	Repeat Unit	Alleles/locus
nc133	2.05	GTGTC	5
phi051	7.06	AGG-AAAG	10
phi056	1.01	CCG	11
phi059	10.02	ACC	13
phi062	10.04	ACG	4
phi063	10.02	TATC	16
phi065	9.03	CACTT	8
phi076	4.11	GAGCGG	4
phi085	5.07	AACGC	13
phi112	7.00-7.02	AG	8
phi123	6.07	AAAG	4
phi108411	9.06	AGCT	10
phi109188	5	AAAG	12
phi227562	1.12	ACC	9
phi308707	1.10	AGC	6
umc1161	8.06	GCTGGG	11
umc1196	10.07	CACACG	10
umc1266	3.06	CAG	10
umc1304	8.02	TCGA	17
umc1332	5.04	CTA	15
umc1367	10.03	CGA	9
umc2047	1.09	GACT	4
umc2250	2.04	ACG	5
Total			214
Minimum			4
Maximum			17
Mean			9.30

Both the frequencies of rare alleles (ranging from 49 to 72%) and unique alleles (ranging from 16 to 40%) varied considerably. These results indicate the presence of a relatively large proportion of rare and unique alleles among the maize accessions studied.

This study revealed a higher total molecular allelic richness 9.30 which is comparable to the 8.02 alleles per locus reported by Reif et al. (2005). However, most previous studies of SSR diversity in maize revealed a lower allelic diversity. For example, Lu and Bernardo (2001) reported 40 U.S. maize inbreds averaging 4.9 alleles for 83 SSR loci. Senior et al. (1998) reported an average of 5.0 alleles. A total of 85 SSR loci studied by Warburton et al. (2002) amplified 416 bands in CIMMYT maize inbred lines, with an average number of 4.9 and a range of 2 to 14. However, it should be noted that most of these studies were on inbred lines which are expected to be more homozygous than populations reported in this study. In fact for populations, Warburton et al., 2002 reported higher levels of allelic diversity (6.3) with populations as compared to inbred lines.

The higher number of alleles per locus found in this study is most likely attributable to the increased genetic diversity in the plant material investigated (heterogeneous landraces, OPV and hybrids from diverse countries and environments). Maize landraces and creolized varieties have been broadly and independently cultivated throughout southern Africa and they are of relevant socio-economic importance for the family farming systems. As a result, different accessions are developed and selected for different environments and morphological characteristics (Paterniani et al., 2000). The genetic diversity of landraces is, therefore, the most immediately useful part of this biodiversity research.

The genetic similarity coefficient of Dice ranged from 0.344 to 0.943, with an average of 0.652 for all accessions. The average gene diversity across all populations (0.652) in this study was the same as reported by Matsuoka et al. (2002). The highest genetic similarity (0.9431) was observed between the Kanjere-2 and Kenya-6 landraces. These accessions were collected in different villages in Zambia but in one district, and are probably one and the same landrace but have different local names according to the farmers growing them. However, the next highest genetic similarity (0.901) was observed

between Bantam and Chibahwe-2 landraces. These accessions have been cultivated in distinct regions by unrelated small farmers and are known by different names. Bantam was collected from Malawi while Chibahwe-2 was collected from Zambia.

Regional Variation- Allelic Richness

Allelic diversity parameters were calculated from SSR marker data at the mega-environment level (Figure 4.1). There was considerable allelic diversity between mega-environments. The variation in average number of alleles per locus was fairly large, with accessions from semi-arid and arid environments (C and E) having significantly lower values than those from humid mega-environments (A and B). Commercially-bred varieties had comparable levels of allelic diversity to those landraces from mega-environments A and B, but significantly higher allelic diversity than those landraces collected from mega-environments C and E.

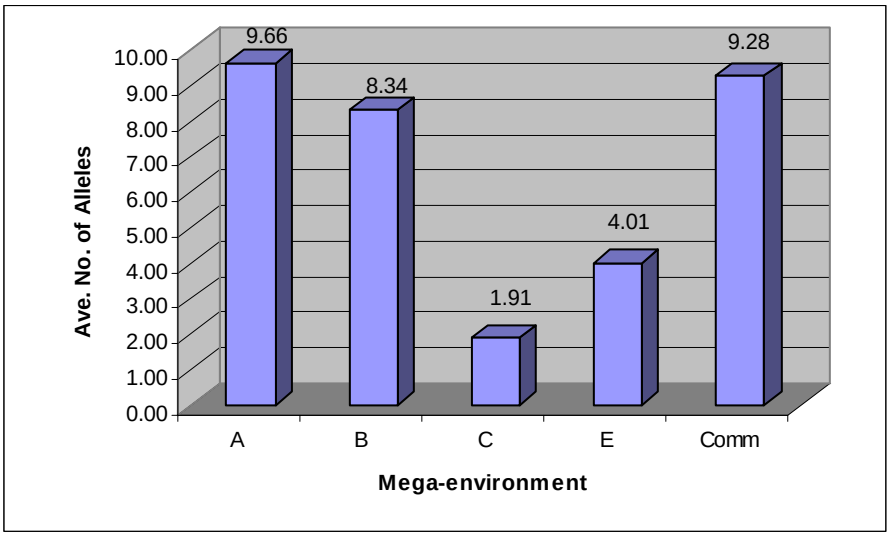

Fig. 4.1. Average number of alleles (allelic richness) per mega-environment and for commercially-bred maize varieties genotypes with 23 SSR markers.

There were considerable numbers of rare alleles detected in the landraces collected from semi-arid and arid mega-environments C and E based on alleles with frequencies of 5% or higher (Figure 4.2). The percentage of unique alleles were

significantly higher for the landraces collected from the semi-arid and arid mega-environments than for populations collected from humid mega-environments A and B (Figure 4.3). Commercially-bred varieties had comparable values with landraces collected form mega-environments A and B for rare and unique alleles (Figures 4.2 and 4.3).

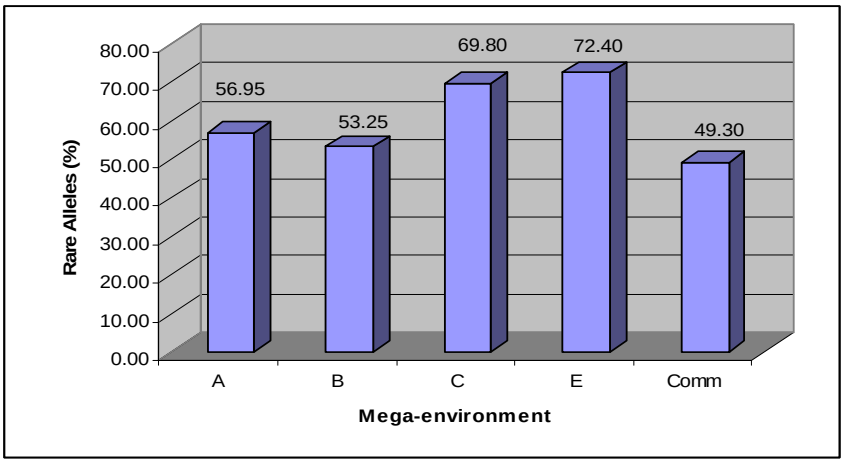

Fig. 4.2. Percentage of rare alleles per mega-environment and for commercially-bred maize varieties genotypes with 23 SSR markers.

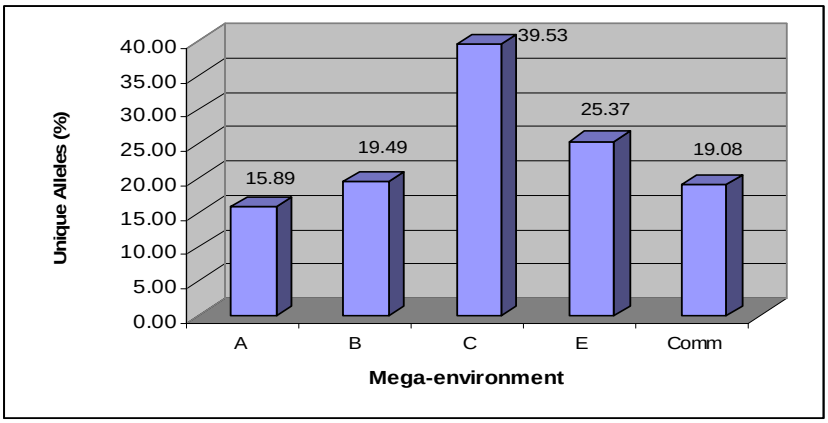

Fig. 4.3. Percentage of unique alleles per mega-environment and for commercially-bred maize varieties genotypes with 23 SSR markers.

The landraces collected from humid mega-environments A and B, and commercial varieties showed the largest variability for SSR allelic patterns and had the highest frequency of rare alleles. These results correspond with the humid areas being the predominant areas of maize production in the three southern African countries. The

relative lower diversity exhibited by maize landraces collected from the dries areas (mega-environments C and E) fits well with the crop production patterns of these areas, i.e. the major staple crops produced in these areas are sorghums and millets which can tolerate droughts which are frequent in these areas. However, the fact that the proportion of unique alleles is higher from these drier environments may indicate the presence of gene related to tolerating drier conditions that exist in these areas.

Regional Variation - Gene Diversity

The average Dice similarity between all pairs of populations within mega-environments (ME) ranged from 0.344 to 0.943 and averaged 0.652. This indicated that there is a fair amount of variation among the accessions. The average similarity between all pairs of populations of different MEs was maximum for the landraces collected from mega-environment C and minimum for commercially-bred populations (Figure 4.4). Mega-environment C marks the transition from high potential areas to low potential area for maize growing in southern Africa, and is thus the most heterogeneous in terms of growing conditions. The high average genetic diversity observed in this mega-environment probably reflects the variable climatic conditions and hence a wider range of maize varieties to fit the variable growing conditions in the zone.

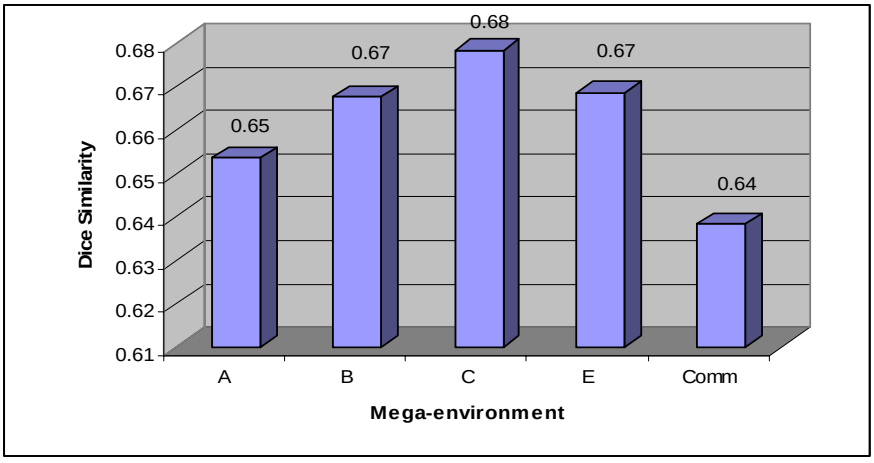

Fig. 4.4. Average Dice similarity coefficients per mega-environment and for commercially-bred maize varieties genotypes with 23 SSR markers.

The total gene diversity of the maize populations in this study (0.652) was similar to the gene diversity of the tropical and the non-Stiff Stalk maize pools (0.68) reported by Liu et al. 2003. Research on genetic diversity in maize with molecular markers has mostly concentrated on temperate inbred lines and their pedigree relationships as well as assign to heterotic groups (Melchinger, 1999). No known report have been found in literature investigating the genetic diversity and structure of traditional maize populations from southern Africa. The landraces reported here have originated from few geographic regions and maintained separately by farmers and early breeders for the past 100 years and thus have a fairly short evolutionary history compared to American temperate and European temperate populations. However, the results suggest that significant amounts of genetic diversity is present in African accessions.

Regional Variation – Common and Shared Alleles

In order to identify qualitative variations in allelic diversity between different mega-environments, the number of shared alleles was also analyzed between mega-environments. In addition, the number of alleles specific to landraces versus commercially-bred varieties and landraces versus original introductions from the USA was also determined. Close to 81% of the alleles in commercially-bred varieties was also found in landraces, indicating a high relationship between the two groups. Two main reasons may have caused this high relationships; common parentage or ancestry of the two groups, and *creolization* between improved varieties and landraces. As explained earlier (Chapters II and III), the majority of landraces grown in the three countries probably descended from a common source of USA developed OPVs (McCann, 2005). Smallholder farmers in many parts of the world consciously or unconsciously cross maize landraces with improved varieties in-order to tap the best characteristics from both groups (Bellon et al., 2006). *Creolization* can be explained by taking into account the gene flow among farmer populations which is likely to have occurred in two ways: through either dispersion of pollen to neighboring cultivated fields, successful fertilization of eggs and final establishment of the resulting seeds within the farmer site or through exchange of seed among farmers who reproduce their own seed stocks, and the successful establishment of exchanged seeds within a different field population.

About 41.75% of the alleles landraces were common across all the four mega-environments studied. About 73.5% of the total number of alleles observed in original introductions was present in landraces, while about 86% of the total number of alleles observed in original introductions from the USA was recovered in commercially-bred varieties. The advent of new alleles in modern cultivars gives evidence of the introduction of new genetic material in breeding programs.

Cluster Analysis

The UPGMA dendrogram (Figure 4.5) grouped the maize varieties into three main clusters. Genetic associations in cluster 1 (C1) revealed high similarity among the accessions. In this cluster, 53 accessions grouped together, most of which showed similar kernel characteristics and flowering time (Chapter III). The original Hickory King from USA (HICKORYKING#), the southern African version of Hickory King (HICKORYKINGSC) and many other local landraces with the name Hickory King and other names were very close in the dendrogram, and with Dice similarity coefficients of above 0.70.

Most of these accessions display eight-row ears and very long kernel width, characteristics that are also observed in the Hickory King race introduced in southern Africa from United States (Weinamann, 1972) around 1890. It is possible that Salisbury White (SW), Natal Potschestroom Pearl (NPP) and Southern Cross (SC) were derived from the Hickory King race as indicated by the proximity of these varieties to the USA Hickory King in C1. Other accessions of cluster 1 that share similar kernel characteristics and flowering time were also very close as revealed by SSRs, and with genetic similarities that ranged from 0.7 to 0.9. The other ancestral OPVs introduced from the USA around the 1900s also grouped in cluster 1.

Cluster 2 (C2) contains mainly local landraces, OPVs from the national program of Zambia, OPVs and OPvs from local seed companies, and CIMMYT developed varieties. There were 38 accessions in this cluster. Most of the accessions are white seeded, late maturing and have flint grain texture. Cluster 3 (C3) contained only a few accessions (8) and were mostly commercially-bred hybrid varieties. This association is consistent with their common origin since the hybrids clustering here are mainly from one seed company, Seedco. These accessions display similar kernel characteristics but varying flowering time and seed color (Chapter III).

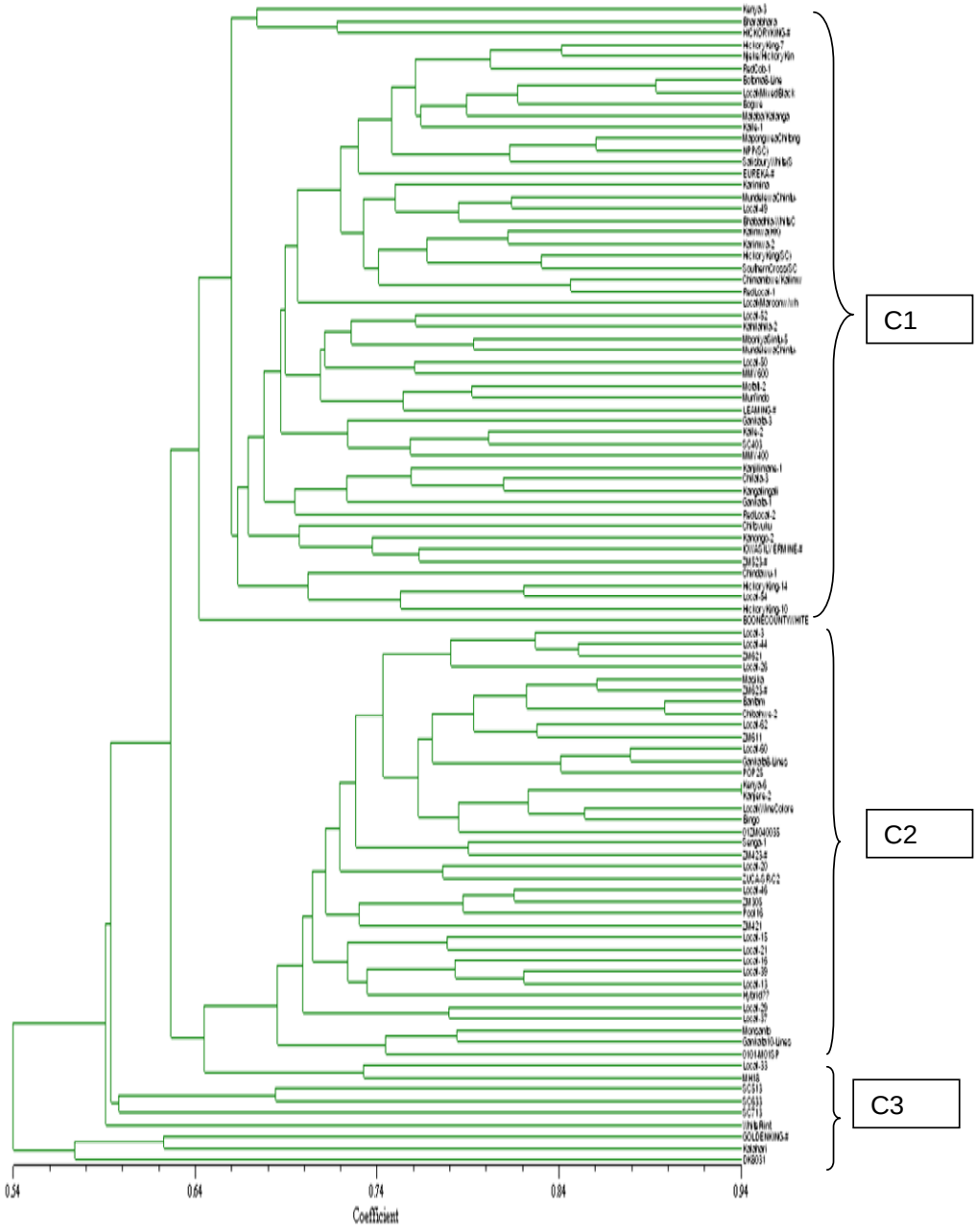

Fig. 4.5. UPGMA dendrogram generated from Dice's similarity coefficients for 99 African maize accession genotypes with 23 SSR markers.

CONCLUSIONS

The main objective of the research reported here was to analyze the pattern of genetic diversity within ancestral maize varieties from the USA, their descendant OPVs from early breeding work, local landraces adapted to different growing environments, and modern varieties. The high allelic richness, frequencies of rare and unique alleles within populations and MEs, gene diversity values, and clustering pattern in this study confirm the broad genetic diversity and the relationships of the accessions expected from historical information, phenotypic diversity (Chapter III) and pedigree data.

Molecular markers also revealed high levels of genetic diversity between landraces originating from different growing environments, and between landraces and commercially-bred varieties. The data also showed that the genetic diversity introduced from the original gene pool from USA about 100 years ago is still found in both the descendant landraces and commercially-bred varieties. The study also shows that the plant material grown for a long time in southern Africa and maintained by local farmers through yearly selection has resulted in many different landraces identifiable by different names and with different traits (Chapter III). These results further agree with the high number of common and shared alleles among populations and occurring in the landraces as a whole. The selection carried out over the years by each farmer according to his own criteria produced some differentiation within the original populations introduced.

CHAPTER V

AGRONOMIC PERFORMANCE OF MAIZE ACCESSIONS

INTRODUCTION

Maize is now the number one crop in the world surpassing other cereals in terms of production and area planted (FAOSTAT, 2005). However, maize is grown under a wider range of environments with major areas of production in the Americas, Asia and Africa. The environmental conditions in Zimbabwe, Zambia and Malawi where maize is produced are extremely diverse ranging from high potential to low potential areas (Banziger et al., 2002). Drought stress, low nitrogen stress and acid soils greatly limit the productivity of maize in these three countries.

Drought, like many other environmental stresses has adverse effects on crop yields. The severity of drought stress is increased on shallow or sandy soils typical of areas into which maize production is currently expanding. One clear way to improve the livelihoods of rural smallholders is to reduce the fluctuations in maize production over the region in the face of varying annual rainfall. Decreasing the susceptibility of maize to drought, while maintaining or increasing yield in good rainfall years, would increase and stabilize rural incomes, reduce chronic food shortages that plague these areas prior to harvest, and lessen the risk of famine.

Nitrogen availability represents another major factor limiting maize yields in the world, requiring the addition of large quantities of N fertilizers to achieve high yields. Nitrogen stress reduces grain yield by delaying plant growth and development and reducing kernel number, leaf area index, leaf area duration, and photosynthetic rate (Uhart and Andrade, 1995). Maize genotypes can differ in their nitrogen use efficiency (NUE), which is defined as the ability of a genotype to realize superior grain yields at low soil N conditions in comparison with other genotypes (Balko and Russell, 1980). Maize cultivars with improved N-use efficiency would be beneficial for low-input production systems.

Soil acidity is an important cause of soil infertility in the tropics (Gaume et al., 2001). Soils are acidic because their parental materials are acidic and initially low in basic cations (Ca, Mg, K, and Na) or because these elements have been removed from the

soil by leaching or by harvested crops. Maize suffers in soils with pH<5.6 mainly due to toxicity from Al and Mn and deficiency of Ca, Mg, P, Mo and Fe. The use of acid tolerant maize cultivars is an important component of a successful production system on tropical acid soils with limited lime and P inputs.

Knowledge of the degree of genetic diversity among local maize landraces for key selection traits will facilitate the development of high yielding, stress tolerant maize varieties. Evaluation of genetic diversity levels for abiotic stresses among adapted local landraces can provide sources of new alleles for these stresses. In addition a better understanding of the genetic basis of this variability will improve the efficiency of maize improvement for abiotic stress tolerance. Farmers' local varieties collected from marginal environments may possess some unique physiological attributes that may not be present in germplasm not exposed to abiotic stress (Blum and Sullivan, 1986).

In the context of in situ conservation as well as identifying sources of new genes for both biotic and abiotic stresses of landraces, both molecular and morphological marker evaluations are useful. Complementing agro-morphological analyses with molecular analyses can enable a comparison of the populations for both measures of diversity which will in turn allow scientists to evaluate the best way to combine the information in a comprehensive approach.

The objectives of this section of the study were to (i) assess the genetic variability in the response of a core set of maize varieties to optimum growing conditions, drought stress, low N stress, acid soil stress, and random stress (combination of drought stress, low N and acid soils) in-order to identify superior sources of tolerance to these abiotic stresses; (ii) to compare the yield potential and abiotic stress tolerance of the core set of maize landraces with improved open-pollinated varieties and hybrids developed in southern Africa by National Agriculture Research Programs, seed companies and CIMMYT; and (iii) to associate agronomic performance with molecular diversity of the core set of maize varieties collected.

LITERATURE REVIEW

Maize Improvement for Drought Tolerance

Drought is the most important factor limiting maize crop productivity in many areas of the world, and large yield losses can occur when maize is exposed to drought conditions around flowering (Bänziger et al., 2002). Edmeades et al. (1999) reported that 34–40% of the inter-annual variability of the yields in the principal maize-growing region of the tropics is explained by variations in rainfall. Drought stress has its most devastating impact when it occurs around flowering (Banziger et al., 2002). These facts have led to an interest in developing drought-tolerant genotypes for water-limited regions (Bruce et al., 2002).

A strategy for developing drought tolerance materials is the conventional breeding approach which relies on multilocation testing of progenies in environments representing a random selection of the variation in drought stress in the target environment with emphasis on high and stable yield across sites (Ludlow and Muchow, 1990). Breeding for high yield in drought prone environments using multilocation testing and selection for grain yield is difficult because of low heritability under these conditions and year-to-year variability in the amount and temporal distribution of available soil water (Banziger et al., 2002). Better progress has been made using drought-stressed nurseries with adequate trial management and appropriate statistical techniques, but even here the results are strongly dependent on the timing of drought (Edmeades et al., 1999). Yield gains for drought close to flowering have been useful, but gain under seedling drought stress and terminal drought stress has been poor (Banziger et al., 2002). The use of secondary traits involving physiological components provides an alternative and complementary approach to this problem. Progress in drought tolerance using this approach depends on being able to identify traits related to improved crop performance under drought and successful transfer of these traits to agronomically interesting genotypes. An ideal secondary trait should be genetically associated with grain yield under drought, carry no yield penalty under favorable conditions, be hereditable, and be easy to measure (Ludlow and Muchow, 1990).

Since many factors contribute to high plant performance under water deficits, efforts are being made to elucidate the nature of drought stress tolerance in an attempt to

improve maize varieties further (Bruce et al., 2002). Such factors include better partitioning of biomass to the developing ear resulting in faster spikelet growth and improved reproductive success. An emphasis on faster spikelet growth may result in a reduction in the number of seed set by reducing water and carbon constrains per spikelet. To understand the molecular mechanisms for drought tolerance in improved maize varieties better, a variety of genomic tools are being used. Newer molecular markers and comprehensive gene expression profiling methods provide opportunities to direct the continued breeding of genotypes that provide stable grain yield under drought stress (Bruce et al., 2002).

Maize Improvement for Low Soil N Stress

N-use efficiency is defined as the ability of a genotype to produce superior grain yields under low soil N conditions in comparison with other genotypes (Uhart and Andrade, 1995). Experiments with the U.S. Corn-Belt (Balko and Russell, 1980), tropical (Banziger et al., 1997), and European maize (Bertin and Gallais, 2000) indicated that genotypes can differ considerably in NUE. It would be desirable to combine the breeding goals of yield improvement for conditions with high input of N fertilizers and yield improvement for low N input conditions. In principle, the following two breeding strategies are possible for improving maize for NUE: (i) indirect improvement: selection at only one N level, whereby performance at the other N level is improved by correlated response, and (ii) combined improvement where selection is based on an index of the weighted performance means at high and low input of N (Banziger et al., 2002).

To decide which of the strategies would be the most appropriate, knowledge of quantitative genetic parameters such as genotypic variance components, heritabilities, coefficients of genotypic correlation, as well as economic weights for yield under high and low N conditions is necessary. So far, quantitative genetic parameters for the adaptation of European maize to low soil N conditions were only provided by Bertin and Gallais (2000). The authors studied the testcross performance of 99 recombinant inbred relines at two N levels. However, comprehensive studies on the N-use efficiency of maize using different sets of materials across a wide range of environments are only

available for tropical maize (Banziger et al., 1997). Hence, breeding for tolerance to low soil N seems feasible.

Maize Improvement for Acid Soil Tolerance

Aluminium toxicity is a major problem for maize production on acid soils in the tropics, affecting about 8 million ha in central/south America and Asia (Gaume et al., 2001). Exchangeable and soluble Al content are nil or negligible for soil pH greater than 5, but they increase exponentially below this pH value. The relationships between pH, exchangeable and soluble Al depend largely upon soil mineralogy, and for a given pH the amount of soluble Al may vary three times depending on clay content (Sierra et al., 2003). As pH generally decreases with depth, the subsoil layers of acid soils are currently more toxic that the topsoil. Liming is sometimes used to reduce Al toxicity, but lime is often too expensive or impractical in many parts of the tropics. In addition, because lime leaching is very small, liming currently affects only the topsoil and does not remove Al toxicity in the subsoil. For this reason, using germplasm improved for Al tolerance is an important step for developing maize-based systems on these acid soils (Gaume et al., 2001).

Many investigations during recent years have shown that Al toxicity primarily affects root elongation and functioning (Gaume et al., 2001). This finding led to the development of several indicators of root Al-tolerance in order to use them in maize breeding programs, and considerable progress has been made in identifying genes and physiological mechanisms involved in root Al-tolerance (Gaume et al., 2001). Among these mechanisms, the exudation of organic acids by maize roots as a response to a high Al concentration has been intensively studied, mainly in relation to the timing of the response and the root region concerned in exudation and detoxification (Gaume et al., 2001). As free Al concentration in acid soils generally increases with depth, Al tolerant cultivars having roots in the more toxic subsoil might be able to obtain soil resources such as water and nutrients from that layer (Gaume et al., 2001).

Another constraint related to soil acidity is P deficiency. Phosphorus is relatively insoluble in acid soils and possesses a low diffusion potential that is associated with several fixation processes. In response to P stress, plants have developed mechanisms for

making soil P more available; e.g. mycorrhizal symbioses and the release of exudates (Strom et al., 2002). In addition, it is known that maize acquires P under P stress in acid soils by changes in root physiology and morphology; e.g. production of root hairs, P accumulation in roots (Gaume et al., 2001).

Most of the experiments dealing with the response of maize to acid soils have been performed in nutrient solution or in greenhouses in short-term experiments (Gaume et al., 2001) with very few field experiments. Although these experiments are helpful for the plant breeder to distinguish tolerant from susceptible genotypes, field experiments carried out throughout the crop cycle are necessary to evaluate the interactions between the below- and aboveground plant organs, and to assess the plant response in stratified acid soils.

Maize Improvement Under Random Stress Conditions

A strategy for developing abiotic stress tolerant materials is the conventional breeding approach which relies on multilocation testing of progenies in environments representing a random selection of the variation in abiotic stress in the target environment with emphasis on high and stable yield across sites (Ludlow and Muchow, 1990). Breeding for high yield in abiotic stress prone environments using multilocation testing and selection for grain yield is difficult because of low heritability under these conditions and year-to-year variability in the amount and temporal distribution of the different stresses (Banziger et al., 2002). Better progress in maize has been made using abiotic-stressed nurseries with adequate trial management and appropriate statistical techniques, but even here the results are strongly dependent on the timing of drought (Edmeades et al., 1999).

Landrace Diversity and Adaptation

Maize local varieties collected from marginal growing environments including drought conditions posses some unique physiological attributes for drought tolerance that may not be present in germplasm which is not exposed to drought (Blum and Sullivan, 1986). Although local varieties have not been extensively used by breeders because of their undesirable agronomic traits, they can serve as sources of new desirable traits, they

can serve as sources of adapted germplasm under drought stress (Beck et al., 1997). In southern Africa, early-maturing and drought tolerant open-pollinated maize varieties were derived from open populations formed by intercrossing farmers' landrace varieties with good performance under drought. Evaluation of late maturing farmers' local varieties under carefully controlled moisture deficit can, therefore, enhance the opportunities to identify germplasm for introgression into adapted breeding populations. Furthermore, the farmers local varieties can provide the basis to assess the level of improvement in grain yield and hybrids and improved open-pollinated varieties developed through testing at multiple locations.

Molecular and Morphological Diversity

Classifying genotypes into clusters based on DNA fingerprinting, and/or agronomic or morphological attributes, for studying genetic and phenotypic diversity has become common in recent as researchers want move on to try and relate genotype with agronomic performance.

In the context of *in situ* conservation of landraces, both molecular and morphological marker evaluations are useful for identifying populations for conservation, optimum sites for germplasm collection, and ongoing changes in the pattern of diversity in the course of conservation practices (Newbury and Ford-Lloyd, 1997). In two maize data sets, the classification strategy of combining the information on molecular data with the available morpho-agronomic attributes produced compact and well-differentiated groups of genotypes in maize (Franco et al., 2001). This study showed that when simultaneously using genetic markers and phenotypic variables to classify genotypes, it is possible to obtain a relevant minimum subset of RFLP marker-fragments that can be used in conjunction with available morpho-agronomic data to better classify genotypes, compared to using only the continuous or only the discrete variables. In a study of European maize populations, the methodology which appeared as the most effective for the analysis and the description of large collections of maize landraces, was a two-phase process: firstly, a molecular study leading to the definition of closely related groups at the DNA level; secondly a morphological study and description of the populations from the various genetic groups (Malosetti and Abadie 2001). From this study the authors defined

six genetic groups for European maize populations each of which could be referred as European races. In garlic, data for morphological characterization based on 16 traits was highly correlated with previous classifications based on RAPD and isozyme analysis. Comparison of molecular to morphological and physiological data in cactus species in Texas produced generally similar conclusions of relatedness among accessions, confirming the utility of either characterization analysis (Wang and Larkins, 2001).

Other workers have reported a disparity between phenotypic and molecular distances, for example in maize (Burstin and Charcoset, 1997; Senoir et al., 1998). In sessile oak, patterns of genetic differentiation of morphological traits did not coincide with microsatellite differentiation (Smith et al., 1997). Molecular analyses in conjunction with morphological, or agronomic evaluation of germplasm is recommended because these provide complementary information and increase the resolving power of genetic diversity analyses (Singh et al., 1991).

MATERIALS AND METHODS

Germplasm

The most diverse 25% of the landraces selected based on morphological characters plus six OPVs obtained from the USA, and 28 commercially-bred varieties from seed companies in southern Africa and CIMMYT formed the core set of germplasm for agronomic evaluation under different environmental conditions (Table 4.1). The landraces had been selected based on highest average genetic distances using morphological characters, while the six OPVs from the USA are among some of the original OPVs introduced into Zimbabwe, Zambia and Malawi from the USA during the late 19th century. Commercially-bred varieties included historically important OPVs from Zimbabwe and improved varieties developed in Zambia, Zimbabwe and Malawi by National Agriculture Research Programs, seed companies and CIMMYT. For each location and environment different varieties were used as internal controls.

Environments and Stress Management

The set of varieties were evaluated at ART Farm and Kadoma Research Station (optimum conditions), CIMMYT Harare and Golden Valley (low N), Marondera and Misampfu (acid soils), Chiredzi Research Station and Nanga (drought stress), and Makoholi Experiment Station and Lucydale Farm (random stress). Golden Valley, Nanga and Msampfu are in Zambia while the rest of the locations are in Zimbabwe. Fertilizer rate and planting densities were adjusted to reflect the agronomic recommendations for each location. At Chiredzi and Nanga, drought stress was achieved by withholding water from 3 weeks before silking to the end of the flowering period. Both locations are largely rain free during the winter season, allowing the control of drought stress intensity by withdrawing or delaying irrigation for varying lengths of time during flowering and grain filling stages (Edmeades et al., 1999). At Harare and Golden Valley, low nitrogen stress conditions were achieved at the sites by continuous cropping of maize without N fertilizer application for several years. For low pH conditions, sites were chosen with inherent low pH based on previous soil analyses. Standard cultural and agronomic practices were followed in trial management at each location.

Experimental Design and Field Measurements

The trials for optimum conditions, low N, acid soils and random stress were grown during the 2004/2005 rainy season, while the drought stress trials were grown during the rain-free winter months of 2005. All experiments were planted in an alpha-lattice (0,1) design (Patterson and Williams, 1976) with two replicates and two-row plots at each location with incomplete block sizes of nine plots. Plots were overplanted and thinned to one plant every 25 cm (33 cm for Makoholi) in two row plots of 4 m at 75 cm apart (90 cm for Makoholi).

During the growing season, data was collected as follows; number of days from planting to 50% of the plants shedding pollen (AD); number of days from plating to 50% of the plants having silks at least 1cm long (SILK); plant height (PLHT)(cm) to the flag leaf insertion, ear height (EHT) (cm) at the upper ear insertion node. After harvest the following traits were measured on a plot basis: number of harvested plants (NP), number of ears (EN), ear weight (EWT) (kg), shelled grain weight (GWT) (kg). For the drought

and low N trials, rate of leaf was also recorded (SEN) on a1-10 scale (1=10% dead leaf area 10=100% dead leaf area). Additional variables calculated from direct measurements were: grain yield (YLD) calculated as shelled grain weight per plot adjusted to 125g kg^{-1} moisture and converted to Mg ha^{-1}, anthesis to silking interval (ASI) (days) calculated as SILK-AD, number of ears per plant (EPP) calculated as number of ears (NE) with at least one fully developed grain divided by NP.

Statistical Analyses

Individual analyses of variance were conducted for each trial with the PROC MIXED procedure from SAS (SAS Institute, 2005) with all factors (accessions, reps, blocks) being considered as random effects. Combined analyses of variance were conducted by means of PROC GLM in SAS (SAS Institute, 2005). Lattice-adjusted means were used to make comparisons between farmers' local landraces, ancestral OPVs from the USA, obsolete OPVs, and improved varieties. Heritabilities were calculated as the proportion of genetic variance over the total phenotypic variance (Fehr, 1987). Genotypic and phenotypic correlations were calculated between traits by considering the maize accessions as random effects. Heritability, genotypic and phenotypic correlations were calculated per location, per environment and across environments using SAS macros (Holland et al., 2003).

For each location, environment and across locations, biplots of the first two principal components were used to illustrate relationships between accessions and traits, grain yield and environments, and relationship between environments (Gabriel, 1971). Accessions, traits, or environments that are close together tend to be similar. The angle between two accessions, traits, or environments indicates the degree of association or correlation. Small angles indicate similarity, 90º angles indicate orthogonality and no association, and angles 90º indicate a negative correlation of genotype performance between these environments. The orthogonal projections of traits on accession or environment vectors indicate the relative performance of accession in a given environment; that is, the greater the projection of the genotype in the positive direction, the better the performance of that accession in that environment (Betran et al., 2003). The

biplots was generated using an excel add-in; Biplot v1.1 (Smith, 2004). http://www.stat.vt.edu/facstaff/epsmith.html.

Joint linear regression was used to estimate yield stability of the accessions (Eberhart and Russell, 1966). Stability analysis was performed using IRRISTAT (IRRI, 1998). A selection index based on grain yield, ASI and EPP under different environments was calculated for each accession. The selection index was used to account for grain yield, abiotic stress tolerance, and maturity. The upper 20% of the accessions that represented the agronomic diversity of the core set and had high selection indexes were identified. An excel add-in, Fieldbook, from CIMMYT was used to generate the selection indices (Vivek and Banziger, 2005) http://cimmyt.cgiar.org.

For cluster analysis, the agronomic data for each environment and across environments were standardized using the YBAR option of the Stand program from the NTSYS-pc 2.11 software (Exeter Software, Setauket, NY). The Simple Matching Coefficient (SM) was used to measure similarity among the accessions to perform cluster analysis on the whole set of varieties using the unweighted pair group method of arithmetic means (UPGMA) of NTSYS-pc version 2.1 software package (Exeter Software, Setauket, NY) Groups of accessions with similar characteristics were built using a hierarchical cluster analysis. Key agronomic traits were used to describe the characteristics of each of the groups formed from the cluster analysis. The degree of relationship between the distance estimates based on agronomic traits and SSRs (Chapter IV) was studied by comparing the clustering patterns produced by the agronomic traits versus that produced by SSR markers.

RESULTS AND DICSUSSION

Optimum Environments

During the growing season, the trial at Kadoma suffered from mid-season moisture stress (which also affects nutrient uptake) and thus for this analysis, this location was considered as random stressed. Only ART Farm comprised optimal fertilization and supplemental irrigation as needed to avoid moisture and nutrient stress and therefore only data from ART Farm location will be reported for the optimum environment. For the most important traits measured in this environment, highly significant differences

(P<0.01) were observed among the accessions in the trial. Average grain yield ranged from 3.26 to 8.204 Mg ha^{-1}(Figure 5.1). Days from planting to 50% anthesis ranged from 55.0 to 81.1 with a mean of 72.8 days. Anthesis to silking interval (ASI) ranged from -2.1 to 4.5 days with a mean of 1.1 days indicating that the trial was not stressed in terms moisture and fertilization. Number of ears per plant (EPP) was nonsignificant whereas plant height was significantly differently and ranged from 197.9 to 324.9 cm with a mean of 276.1 cm. Improved varieties in general outyielded landraces varieties under optimum conditions, but some landraces (e.g., Kanjilimane-1, Local-50 and Local-46) had grain yields comparable to some of the best improved varieties.

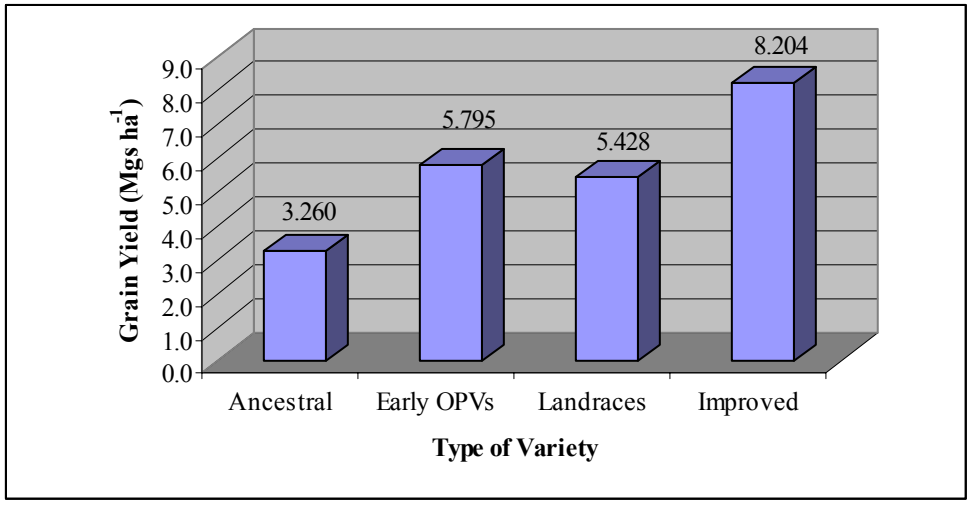

Fig. 5.1. Average grain yield of different types of maize accessions grown under optimum conditions at ART Farm, Zimbabwe during the 2004/2005 growing season.

Random Stress Environments

The trial planted at Lucydale failed due to severe drought stress and for this analysis, two locations, Makoholi and Kadoma were considered randomly stressed based on the growing conditions during the season. At each of these locations, the number of days from planting to 50% anthesis (AD) and the regression slope of grain yield versus AD was highly significant indicating a dependence of grain yield on AD. Analysis of covariance was therefore conducted in order to adjust yield for AD to enable valid

comparison of mean grain yields. For most of the important traits measured in this environment, highly significant differences ($P<0.01$) were observed among the

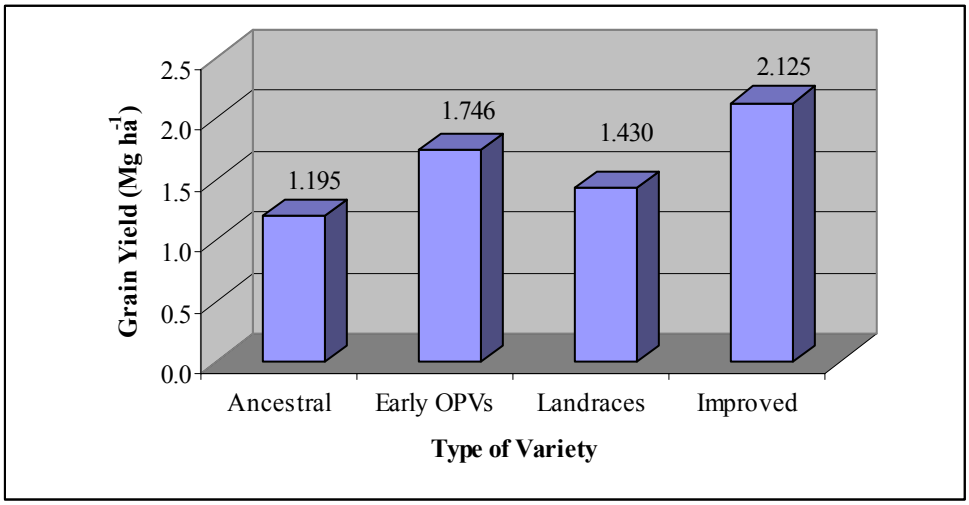

Fig. 5.2. Average grain yield of different types of maize accessions grown under random stress conditions at Kadoma and Makoholi, Zimbabwe during the 2004/2005 growing season.

accessions in the trial. Average grain yield ranged from 1.195 to 2.125 Mg ha^{-1} (Figure 5.2). In this environment, mean grain yields of the accessions tested was about 29% of grain yield under optimum conditions. Days from planting to 50% anthesis (AD) ranged from 51.3 to 81.3 with a mean of 70.2 days. Anthesis to silking interval (ASI) ranged from 4.7 to 10.9 days with a mean of 7.0 days while number of ears per plant (EPP) ranged from 0.64 to 0.93 with a mean of 0.78. The wide ranged in ASI coupled with the low yields and EPP (compared to optimum environment) may indicate that these two locations were subject to abiotic stress during the season. Plant height was also significantly differently and ranged from 159.2 to 190.2 cm with a mean of 175.8 cm. Compared with improved varieties, landraces were relatively more sensitive to the stresses that occurred at Kadoma and Makoholi than improved varieties. Improved varieties in general outyielded landraces varieties under random stress, but some landraces (e.g., Local-20, Local-46 and Kenya-3) had grain yields comparable to some of the best improved varieties.

Low N Stress Environments

At each of these low N locations (Harare and Golden Valley), the number of days from planting to 50% anthesis (AD) and the regression slope of grain yield versus AD were also highly significant indicating a dependence of grain yield on AD.

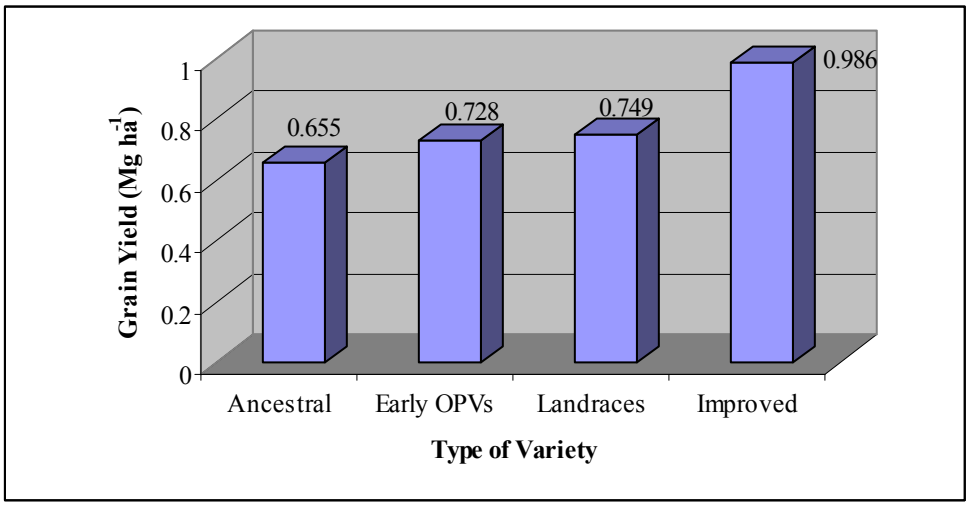

Fig. 5.3. Average grain yield of different types of maize accessions grown under low N stress conditions at Harare, Zimbabwe and Golden Valley, Zambia during the 2004/2005 growing season.

Analysis of covariance was therefore conducted in order to adjust yield for AD to enable valid comparison of mean grain yields. For most of the important traits measured in this environment, highly significant differences ($P<0.01$) were observed among the accessions in the trial. Average grain yield ranged from 0.665 to 0.986 Mg ha^{-1} (Figure 5.3). In this environment, mean grain yields of the accessions tested was about 17% of grain yield under optimum conditions. Such levels of intensity of stress observed for low soil nitrogen fall within the range of stress levels applied during selection of populations and inbred lines for tolerance to drought or low N (Bolanos and Edmeades, 1993). Days from planting to 50% anthesis (AD) ranged from 60.8 to 82.2 with a mean of 73.3 days. Anthesis to silking interval (ASI) ranged from 0.6 to 15.1 days with a mean of 4.8 days while number of ears per plant (EPP) ranged from 0.17 to 0.82 with a mean of 0.52. Again, the wide ranged in ASI coupled with the low yields and EPP (compared to Optimum) clearly indicates that these two locations were subject to low N stress during

the season. Plant height was significantly different only at Harare and ranged from 156.8 to 207.7 cm with a mean of 189.8 cm, while rate of leaf senescence was significant at Golden Valley and ranged from 3.1 to 9.3 with a mean of 4.6 on a 1-10 score. Compared with improved varieties, landraces were relatively more sensitive to low N stress compared to improved varieties in terms of grain yield, but six landraces (Kenya-3, Local-46, Local[Wine colored]-2, and Botoma 8-Line were in the top 20 fraction of the trial.

Drought Stressed Environments

Under drought stress highly significant differences ($P<0.01$) were observed among the accessions for the most important traits at Chiredzi while at Nanga only anthesis date and plant height were significant (Figure 4.4). Thus mainly data from Chiredzi will be used to represent the drought environment. At this location, the number of days from planting to 50% anthesis (AD) and the regression slope of grain yield versus AD were highly significant indicating a dependence of grain yield on AD. Analysis of covariance was therefore carried to adjust yield for AD to enable valid comparison of mean grain yields. For most of the important traits measured in this environment, highly significant differences ($P<0.01$) were observed among the accessions in the trial. Average grain yield ranged from 0.270 to 1.378 (Figure 5.4). In this environment, mean grain yields of the accessions tested was about 13% of grain yield under optimum conditions. Such levels of intensity of stress observed for drought fall within the range of stress levels applied during selection of populations and inbred lines for tolerance to drought or low N (Bolanos and Edmeades, 1993).

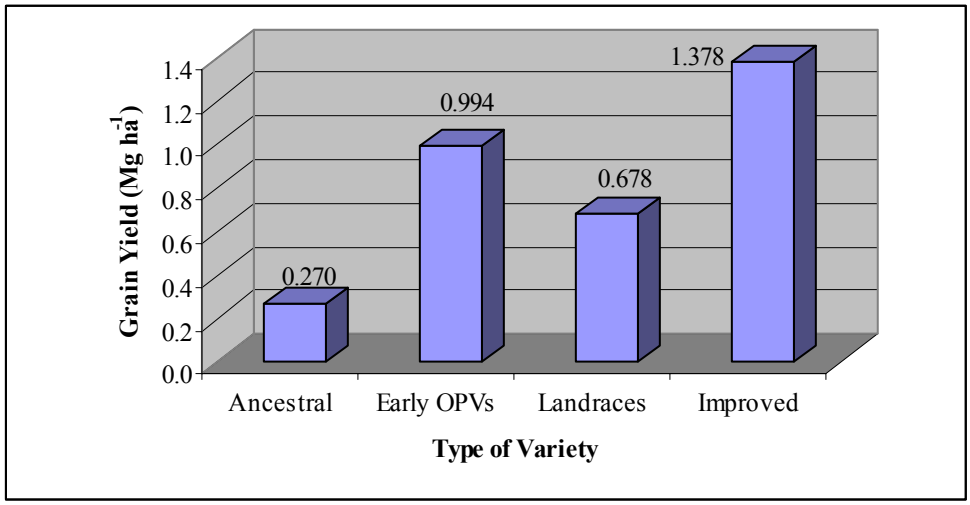

Fig. 5.4. Average grain yield of different types of maize accessions grown under drought stress conditions at Chiredzi, Zimbabwe and Nanga, Zambia during the 2004/2005 growing season.

Days from planting to 50% anthesis (AD) ranged from 77.1 to 104.1 with a mean of 93.2 days. The relatively higher AD in the drought environment compared to the other locations is probably due to the slow growth resulting from cooler during the winter. The other trials were all grown in the warm summer months. Anthesis to silking interval (ASI) ranged from -3.4 to 17.9 days with a mean of 10.0 days while number of ears per plant (EPP) ranged from 0.18 to 0.68 with a mean of 0.41. Again, the wide ranged in ASI coupled with the low yields and EPP (compared to optimum) clearly indicates that these two locations were subject to drought stress during the season. Plant height was significantly different and ranged from 184.4 to 209.5 cm with a mean of 196.6 cm, while rate of leaf senescence was significant at Chiredzi and ranged from 2.4 to 4.2 with a mean of 3.1 on a 1-10 score. In general, compared with improved varieties, landraces were relatively more sensitive to drought stress than improved varieties, but the highest yielding variety under this environment was a local landrace, Hickory King-10 collected from farmers in Gutu district of Zimbabwe. This fact confirms to some extent that smallholder farmers have succeeded in selecting some of the ancestral OPVs from USA for adaptation to their local growing conditions. In addition, data from the drought environment also shows that among the top 20 of the best yielders, nine were local

landraces (Hickory King-10, Red Local-1, Red Local-2, Mboni ya Sintu-5, Kenya-3, Kahilahila-2, Bhabadhla White Cob, Gankata 8-Lines, Chindawu-1) collected from smallholder farmers.

Low Soil pH Stress Environments

The trial planted at Marondera failed due to severe drought stress and for this analysis, only data from Kasama will be used to represent the low pH environment. For the most important traits measured, significant differences were observed only for grain yield and plant height. Average grain yield ranged from 1.082 to 3.209 Mg ha^{-1} (Figure 5.5). In this environment, mean grain yields of the accessions tested was about 38% of grain yield under optimum conditions. Such levels of intensity of stress observed for low soil pH fall within the range of stress levels applied during selection of populations and inbred lines for tolerance to drought or low N (Bolanos and Edmeades, 1993).

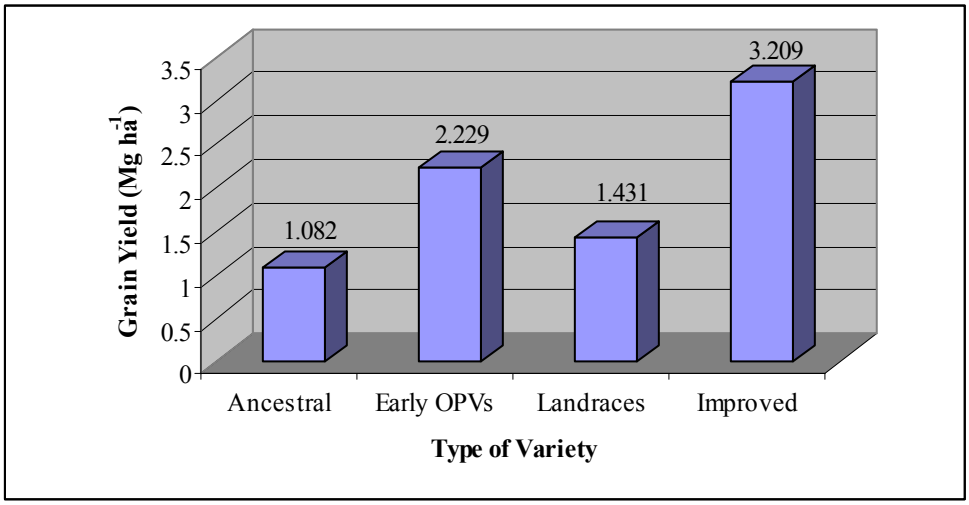

Fig. 5.5. Average grain yield of different types of maize accessions grown under drought stress conditions at Kasama, Zambia during the 2004/2005 growing season.

Plant height ranged from 124.2 to 219.9 cm with a mean of 179.6 cm. Compared with improved varieties, landraces were relatively more sensitive to low pH stress than improved varieties, but eight of the landraces (e.g., Hickory King-10, Mundele wa

Chintu-2, Local-20, Local[Wine Colored]-2, Kenya-3 and Red Local-2) had grain yields above the trial mean.

Across Environments

Genotype and genotype x environment (GxE) interactions were significant for grain yield of the accessions. Across environments, mean grain yields were lowest for ancestral OPVs, followed by their descendant OPVs, than landraces. Improved varieties had the highest grain yields (Figure 5.6). Maturity differences were detected across the range of accessions as well as within local landraces and improved varieties. Kenya-3, Local-46, Red Local-2, Local [Wine colored]-2, Local-20, and Botoma 8-Line all had grain yield comparable to some of the best entries across locations.

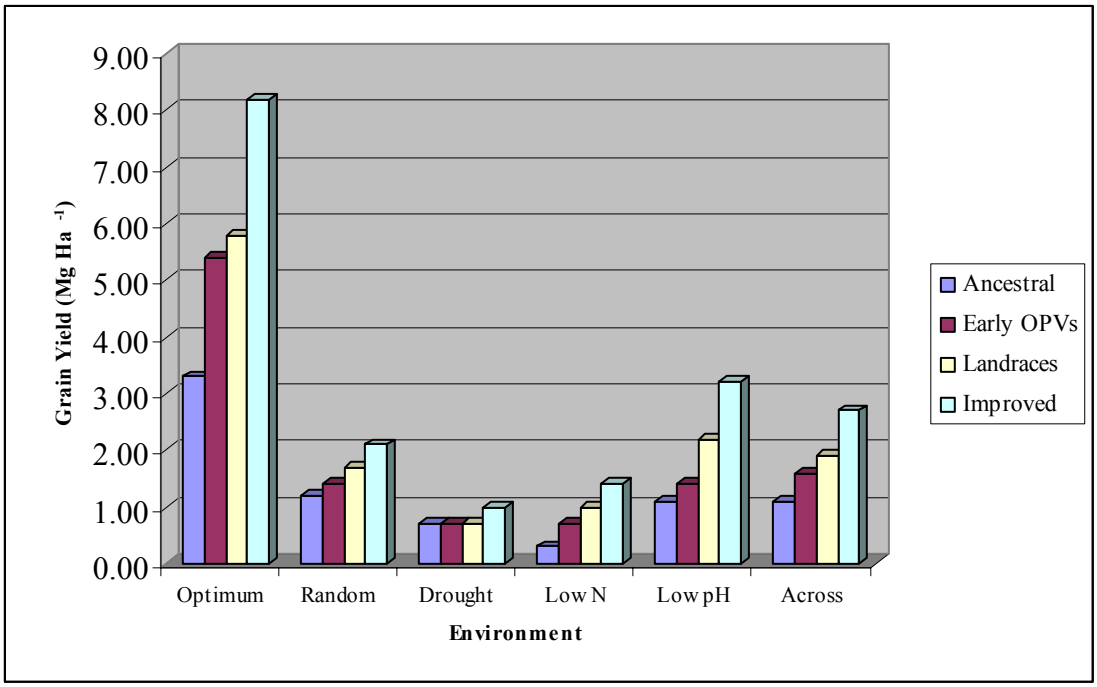

Fig. 5.6. Grain yield for 108 Zimbabwean maize varieties grown under different environments in Zimbabwe and Zambia in 2005.

Heritability Estimates

For grain yield, broad-sense heritability ranged from moderate to high across the different environments (Table 5.1). It was highest at ART Farm, the optimum location

(0.85±0.003) followed by Harare low N (0.81± 0.037) and Chiredzi drought environments (0.77 ±0.048). The two random stress sites had almost similar heritabilities (0.69±0071 and 0.62±0078). The high heritability estimates are probably due to high genetic variances and this indicates that breeding progress may be high at these locations. Similar results have been reported by other authors (Bolanos and Edmeades, 1996; Banziger et al., 2006). In this study genetic variance for grain yield is high probably due to the wide diversity in materials under study, ranging from ancestral OPVs from USA, obsolete OPVs, farmer local landraces and improved varieties from different breeding institutions.

In general heritabilities for grain yield were low and around 0.50 for the Zambian locations (Golden Valley low N, Nanga drought, and Kasama low pH). Bänziger et al. (2006) in a study on maize reported that under abiotic stress, broad-sense heritabilities for grain yield decreased compared to that under favorable growing environments. The low heritability estimates for grain yield suggest that progress in selecting for increased grain yield might be slow under these conditions. Days from planting to 50% anthesis (AD) dates showed high heritability at all environments and these data are in agreements with expected results reported by other authors (Hallauer and Miranda-Filho, 1988).

The highest heritability for anthesis to silking interval (ASI) was recorded under drought at Chiredzi (0.78±0.048). The random stressed sites (Kadoma and Makoholi) and Harare low N all had lower heritabilities around 0.60. In general, heritabilities for ASI were lowest at the Zambian locations (Golden Valley low N, Nanga drought, and Kasama low pH) and ranged from 0.16±0.526 to 0.36±0.128. Bolaños and Edmeades (1996) reported a broad-sense heritability of 0.60 and 0.69 for ASI measured in S_1 and S_2 progeny of tropical maize under well-watered conditions, while under severe stress broad-sense heritability was 0.51 and 0.71 for ASI of the same S_1 and S_2 progeny. For number of ears per plant (EPP), heritability was highest at Chiredzi (0.87±0.026) followed by Harare low N (0.82±0.035). Heritabilities for EPP were moderate for ART Farm (optimal), Golden Valley (low N), and Kadoma and Makoholi (random stressed) at 0.63±0.07, 0.52±0.10, 0.57±0.09, and 0.56±0.09, respectively.

Table 5.1. Broad-sense heritability estimates and standard errors for various traits measured on 108 maize accessions evaluated under different environments in Zimbabwe and Zambia in 2005.

	ART-OPT	KA-RAN	MK-RAN	HA-LN	GV-LN	CH-DR	NA-DR	KS-LP	ACROSS
GY	0.847±0.003	0.687±0.071	0.62±0.078	0.814±0.037	0.489±0.107	0.773±0.048	0.54±0.099	0.498±0.104	0.834±0.024
AD	0.947±0.012	0.819±0.038	0.873±0.026	0.923±0.016	0.789±0.042	0.904±0.02	0.991±0.002	0.893±0.055	0.963±0.005
ASI	0.613±0.074	0.663±0.068	0.599±0.078	0.584±0.081	0.36±0.128	0.776±0.046	0.199±0.16	0.162±0.526	0.745±0.039
PLHT	0.858±0.028	0.581±0.087	0.597±0.084	0.737±0.056	-	0.462±0.112	0.397±0.123	0.418±0.117	0.888±0.017
EPP	0.632±0.072	0.568±0.091	0.557±0.086	0.821±0.035	0.524±0.097	0.872±0.026	0.359±0.134	0.068±0.182	0.801±0.029
SEN	-	-	-	-	0.587±0.085	0.608±0.08	0.405±0.128	-	0.518±0.151

ART-OP= ART Farm optimum conditions, KA-RAN= Kadoma random stress conditions, MK-RAN= Makoholi random stress conditions, HA-LN = Harare low N stress, GV-LN= Golden Valley low N stress, CH-DR = Chiredzi drought stress, NA-DR=Nanga drought stress, KS-LP= Kasama low pH stress, Across=All environments combined; GY=grain yield, AD= Anthesis dates, ASI=Anthesis to silking Interval; PLHT= Plant height; EPP = number of ear per plant, SEN = rate of leaf senescence ….

The lower heritability of ASI and EPP at stressed environments is a result of reduced genotypic variance (Bänziger et al., 1997). A decrease in genetic variance under stress conditions has been reported in other crops e.g. barley (Cecarreli et al., 1991). The observed decrease in heritability in grain yield from optimum to stressed environments coupled with an increase in heritabilities for ASI and EPP indicates that under stressed environments more progress may be made by selecting for ASI and EPP as compared to selcting directly for grain yield, while under optimum growing conditions, rapid progress could be made by selecting directly for increased grain yield. Rate of leaf senescence (SEN) had heritabilities of 0.587±0.085, 0.608±0.08, 0.405±0.13 for Golden Valley low N, Chiredzi drought and Nanga drought locations respectively. Across all environments, high heritability estimates were recorded for all traits except SEN which had moderate heritability.

Genotypic and Phenotypic Correlations

Under optimum conditions, genotypic and phenotypic correlations between grain yield and days to 50% anthesis were positive but non-significant while both genetic and phenotypic correlations between grain yield and anthesis-silking interval (ASI) was negative but nonsignificant (Table 5.2). In contrast, the genetic and phenotypic correlations between grain yield and ears per plant were significant and positive (0.56 to 0.86). ASI was negatively correlated with ears per plant (-0.27 and -0.70 for phenotypic and genotypic correlations respectively).

Table 5.2. Genotypic (upper diagonal) and phenotypic (lower diagonal) correlations for various traits measured for 108 maize accessions grown at ART Farm, Zimbabwe during 2005.

	GY	AD	ASI	PLHT	EPP
GY		0.068	-0.436	0.006	0.860**
AD	0.026		0.208	0.851	-0.104
ASI	-0.197	0.990**		0.338	-0.695**
PLHT	0.079	0.678**	0.208		-0.284
EPP	0.563**	-0.138	-0.273**	-0.114	

GY=Grain yield, AD=days from planting to 50% pollen shed, ASI= anthesis-to-silking interval, PLHT=plant height, EPP=number of ears per plant, **=significantly different at 0.05 level

Under drought stress, both genetic and phenotypic correlations between grain yield and AD, and grain yield and ASI were significant and negative (Table 5.3) indicating that increasing AD and ASI cause a reduction in grain yield under this environment. ASI and AD were negatively correlated with grain yield across all environments studied by Bolaños and Edmeades (1996). ASI and AD were positive and significantly correlated while ASI and EPP were significantly and negatively correlated. The fact that ASI and EPP were negatively correlated indicates that reduced ASI can result in an increase in number of ears per plant (EPP). Delayed silking under a drought is related to less assimilate being partitioned to growing ears around anthesis, which results in lower ear growth rates, increased ear abortion and more barren plants (Edmeades et al., 1999). Grain yield was positively correlated with number of ears per plant (0.80 to 0.93 for phenotypic and genotypic correlations respectively). Similar resulted have been reported by other authors (Bolaños and Edmeades, 1996). The ability of a genotype to produce an ear under stress is one of the most important characteristic associated with drought tolerance.

Table 5.3. Genotypic (upper diagonal) and phenotypic (lower diagonal) correlations for various traits measured for 108 maize accessions grown under drought stress at Chiredzi, Zimbabwe and Nanga, Zambia during 2005.

	GY	AD	ASI	PLHT	EPP	SEN
GY		-0.681**	-0.829**	-0.358	0.936**	-0.057
AD	-0.534**		0.725**	0.298	-0.799	-0.109
ASI	-0.583**	0.605**		0.363	-0.934**	0.081
PLHT	-0.201	0.171	0.098		-0.366	-0.08
EPP	0.801**	-0.677	-0.741**	-0.219		-
SEN	-0.141	-0.148	0.062	0.158	-	

GY=Grain yield, AD=days from planting to 50% pollen shed, ASI=anthesis-to-silking interval, PLHT=plant height, EPP=number of ears per plant, SEN= rate of leaf senescence, **=significantly different at 0.05 level

Under low N stress (Table 5.4) and random stress (Table 5.5) correlations between the most important traits followed a similar pattern to that of drought stress, but were of lower magnitudes, especially under random stress. Both genetic and phenotypic correlations between grain yield and AD, and grain yield and ASI were significant and

negative (Tables 5.4 and 5.5) indicating that increasing AD and ASI cause a reduction in grain yield under this environment. ASI and AD were positive and significantly correlated while ASI and EPP were significantly and negatively correlated. Grain yield was positively correlated with number of ears per plant. Most of the correlations between measured traits under low pH were nonsignificant, probably because of large error variances recorded at this location (Table 5.6)

Table 5.4. Genotypic (upper diagonal) and phenotypic (lower diagonal) correlations for various traits measured for 108 maize accessions grown under low N stress at Harare, Zimbabwe and Golden Valley, Zambia during 2005.

	GY	AD	ASI	PLHT	EPP
GY		-0.346**	-0.751**	-0.367	0.918**
AD	-0.346**		0.567**	0.741**	-0.553**
ASI	-0.469**	0.297**		0.645**	-0.852**
PLHT	-0.070	0.422	0.296		-0.598**
EPP	0.819	-0.461	-0.486**	-0.287	

GY=Grain yield, AD=days from planting to 50% pollen shed, ASI=anthesis-to-silking interval, PLHT=plant height, EPP=number of ears per plant, **=significantly different at 0.05 level

Table 5.5. Genotypic (upper diagonal) and phenotypic (lower diagonal) correlations for various traits measured for 108 maize accessions grown under random stress at Kadoma and Makoholi, Zimbabwe during 2005.

	GY	AD	ASI	PLHT	EPP
GY		-0.467**	-0.918**	-0.684**	0.921**
AD	-0.349**		0.744**	0.869**	-0.546**
ASI	-0.520**	0.413**		-	-0.994**
PLHT	-0.205	0.258	0.395		-0.968**
EPP	0.623**	-0.361**	-0.541**	-0.248	

GY=Grain yield, AD=days from planting to 50% pollen shed, ASI=anthesis-to-silking interval, PLHT=plant height, EPP=number of ears per plant, **=significantly different at 0.05 level

Table 5.6. Genotypic (upper diagonal) and phenotypic correlation (lower diagonal) for various traits measured for 108 maize accessions grown under low soil pH stress at Kasama, Zambia during 2005.

	GY	AD	ASI	PLHT	EPP
GY		0.463**	-	-0.016	-0.501**
AD	-0.193	-	-	-	-
ASI	-0.010			-	-0.177
PLHT	0.205	0.337			0.176
EPP	-0.042	-	0.163	0.016	

GY=Grain yield, AD=days from planting to 50% pollen shed, ASI=anthesis-to-silking interval, PLHT=plant height, EPP=number of ears per plant, **=significantly different at 0.05 level

Genetic and phenotypic correlations across all environments presented in Table 5.7 shows that grain yield had negative and low correlation with AD (about -0.2) but positive and high correlation with EPP (0.5 to 0.8). Correlation between grain yield and ASI interval were negative and ranged from 0.243 to 0.734 for phenotypic and genotypiccorrelations, respectively. Correlation between ASI and AD were significant and positive while correlations between ASI and EPP were moderate to high and negative (-0.4). Data presented by other authors has shown negative correlation between grain yield and ASI under stress conditions (Bolaños and Edmeades, 1996) while other studies have shown also the importance of the relationship between ASI and EPP (Betrán et al., 2003).

Table 5.7. Genotypic (upper diagonal) and phenotypic (lower diagonal) correlations for various traits measured for 108 maize accessions grown under different environments in Zimbabwe and Zambia during 2005.

	GY	AD	ASI	PLHT	EPP	SEN
GY		-0.241	-0.734**	-0.383	0.887**	-0.727**
AD	-0.218		0.803**	0.756**	-0.615**	-0.772**
ASI	-0.243	0.356**		0.809**	-	-0.144
PLHT	0.018	0.282	0.173		-0.702	-0.278
EPP	0.494**	-0.348**	-0.434**	-0.137		0.076
SEN	-0.303	-0.202	0.071	-0.039	-0.173	

GY=Grain yield, AD=days from planting to 50% pollen shed, ASI=anthesis-to-silking interval, PLHT=plant height, EPP=number of ears per plant, SEN= rate of leaf senescence, **=significantly different at 0.05 level

Biplot Analyses

Biplots were used to visualize depict the relationships between accessions and measured traits under different environments and the relationship between the testing environments in terms of discriminating accessions based on grain yield. In these biplots, accessions are represented as points (labeled with the entry number for each accession) and traits are represented by vectors. In general, the biplots confirm the results of the correlation analysis, but give more detail to enhance the interpretation of the data from the trials.

The first biplot (Figure 5.7) shows that under optimum growing conditions, grain yield was positively correlated with number of ears per plant while it is negatively correlated with anthesis-silking interval. Days to 50% anthesis and plant height were not positively correlated to grain yield as depicted by the larger than 90º angle between the vectors of these two traits. Accessions 108 (SC719) and 82 (DK8031) were among the highest yielders while accessions 1 (Booney County White), 40 (Chindawu) and 5 (Eureka) were amongst the lowest yielders.

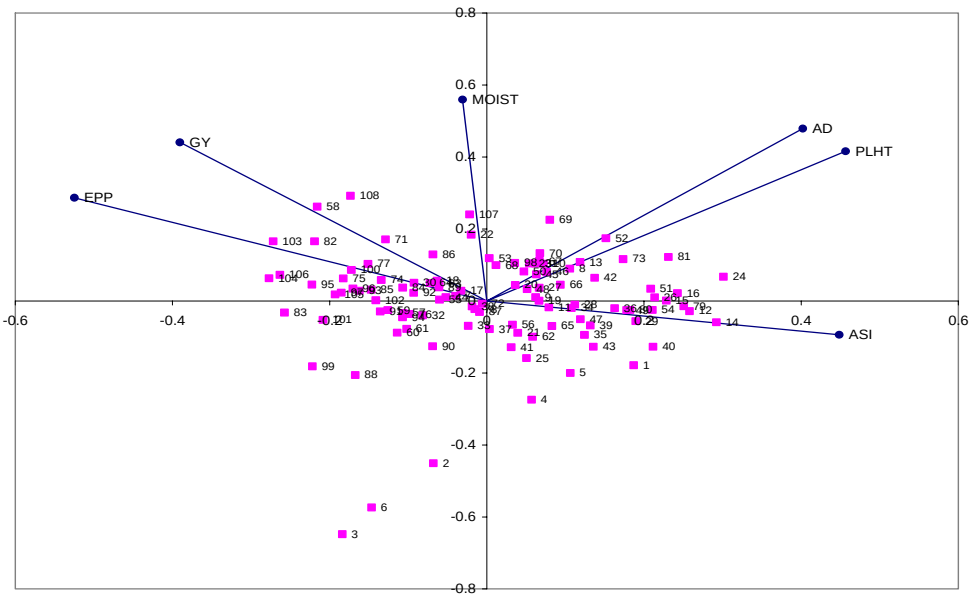

Fig. 5.7. Singular value decomposition biplot showing the relationships among traits and accessions for 108 maize accessions grown under optimum conditions at ART Farm, Zimbabwe during the 2005 season.

GY= grain yield, EPP=number of ears per plant, AD=days from planting to 50% pollen shed, PLHT=plant height, ASI=anthesis-to-silking interval, MOIST=grain moisture at shelling.

Under random stress, low N and drought, the biplots (Figures 5.8 to 5.10) showed that grain yield was positively correlated with yield, negatively correlated with ASI and moderately related to number of days to 50% anthesis. However, under low soil pH (Figure 5.11), there were weak relationships between grain yield and ASI, and grain yield with AD. Surprisingly grain yield was negatively correlated with number of ears per plant (EPP). In the random stressed environment, the accessions 108 (SC719), and 103 (ZM623#) were among the highest yielders while 5 and 1 were among the lowest yielders. Under low N stress, 108 (SC719), and 107 (SC637) were the highest yielders while 5, and 4 were amongst the lowest yielders. In the drought stressed environment, 41 (Hickory King), and 106 (SC525) were among the highest yielders, while 34 (Local-mixed black and white), and 70 (Local) were among the lowest yielders. It is worthy noting that the best yielding accession under drought (41) is a Hickory King-type landrace accession collected from local farmers in Zimbabwe.

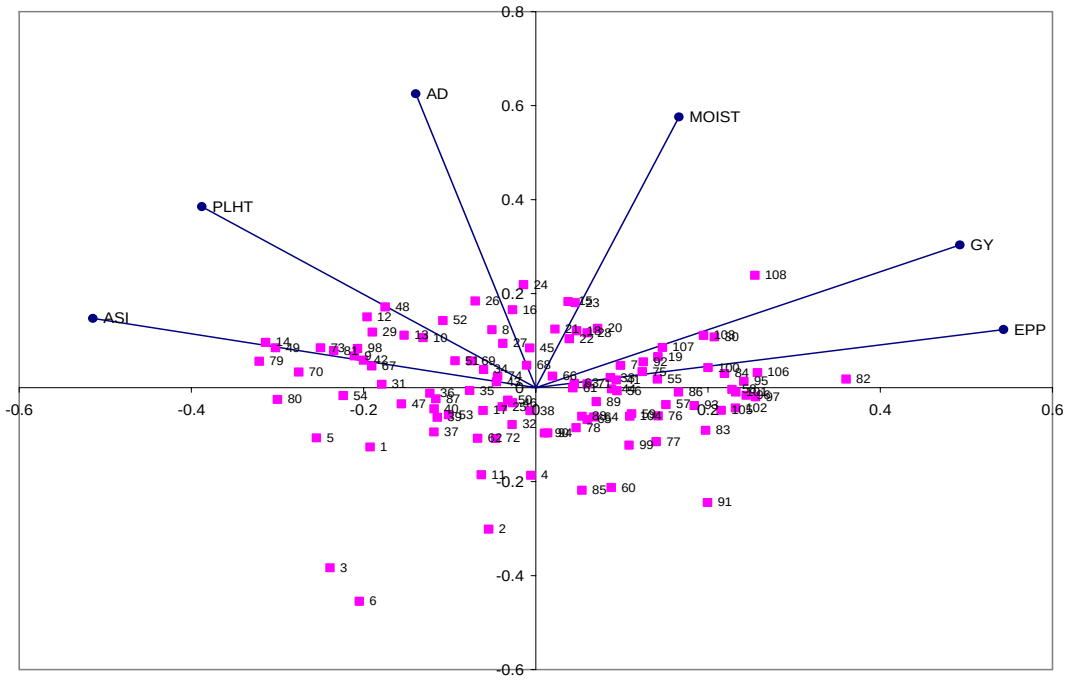

Fig. 5.8. Singular value decomposition biplot showing the relationships among traits and accessions for 108 maize accessions grown under random stress conditions at Kadoma and Makoholi, Zimbabwe during the 2005 season.

GY= grain yield, EPP=number of ears per plant, AD=days from planting to 50% pollen shed, PLHT=plant height, ASI=anthesis-to-silking interval, MOIST=grain moisture at shelling.

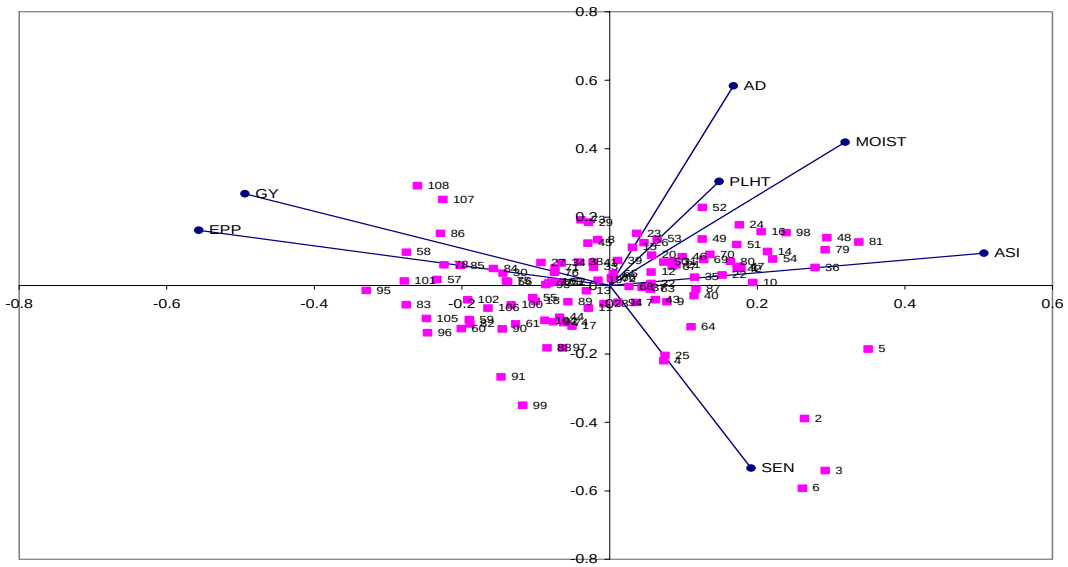

Fig. 5.9. Singular value decomposition biplot showing the relationships among traits and accessions for 108 maize accessions grown under low N stress conditions at Harare, Zimbabwe and Golden Valley, Zambia during the 2005 season.

GY= grain yield, EPP=number of ears per plant, AD=days from planting to 50% pollen shed, PLHT=plant height, ASI=anthesis-to-silking interval, MOIST=grain moisture at shelling, SEN=rate of leaf senescence.

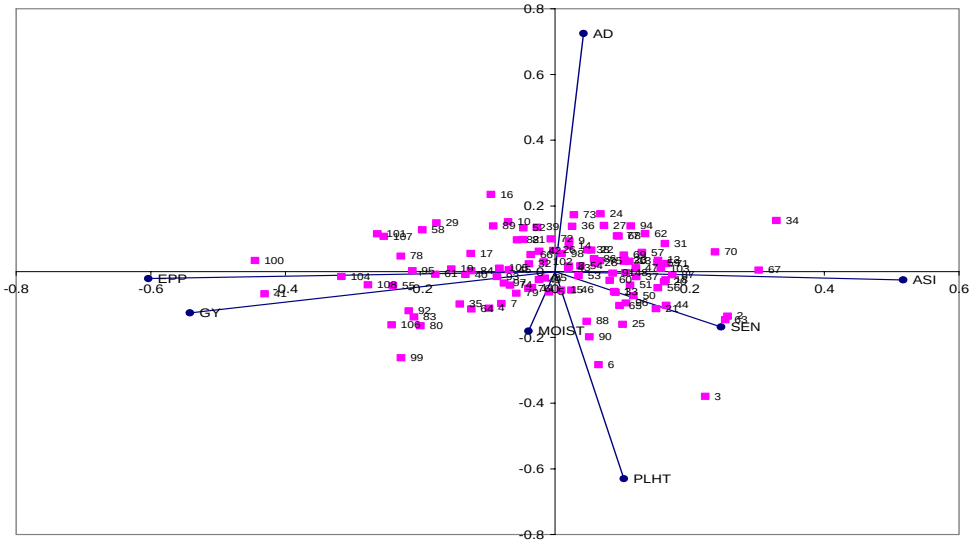

Fig. 5.10. Singular value decomposition biplot showing the relationships among traits and accessions for 108 maize accessions grown under drought stress conditions at Chiredzi, Zimbabwe and Nanga, Zambia during the 2005 season.

GY= grain yield, EPP=number of ears per plant, AD=days from planting to 50% pollen shed, PLHT=plant height, ASI=anthesis-to-silking interval, MOIST=grain moisture at shelling, SEN=rate of leaf senescence.

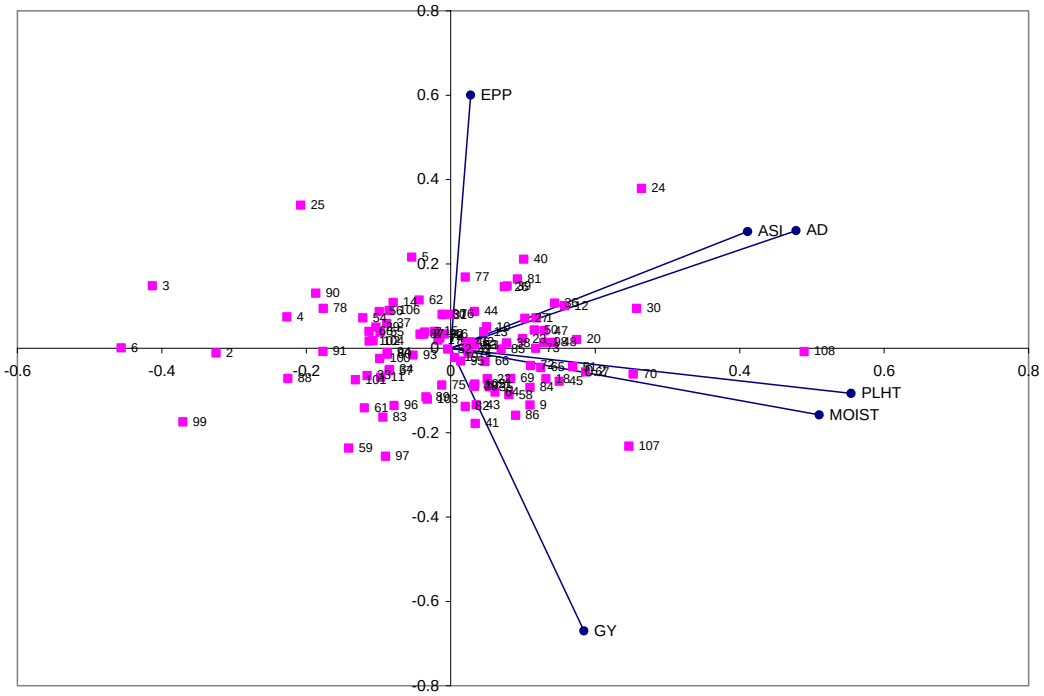

Fig. 5.11. Singular value decomposition biplot showing the relationships among traits and accessions for 108 maize accessions grown under low soil pH stress conditions at Kasama, Zambia during the 2005 season.

GY= grain yield, EPP=number of ears per plant, AD=days from planting to 50% pollen shed, PLHT=plant height, ASI=anthesis-to-silking interval, MOIST=grain moisture at shelling.

The biplot for grain yield under different growing conditions is shown in Figure 5.12. Environment vectors at 90^{o} or greater indicate that discrimination among genotypes from these environment differ. The angles between the optimal, low N, low pH and random stress vectors were narrow indicating that these environments discriminated the accessions in a similar fashion. However, the angle between these environments versus the drought environment was greater that 90°, a clear demonstration that the drought environment discriminated the accessions in a different manner compared to the rest of the environments. Possible reasons for these results may be due to the fact that trials conducted for the optimal, low N, random stress, and low pH are conducted during the summer season whereas the drought trials are conducted during the winter season. In winter environments, it takes longer to reach maturity because of a slower rate of accumulation of growing degree units. Futhermore, drought trials are conducted in mega-environment E (dry lowlands) while the rest of the trials are conducted in mega-

environments A and C (both mid-altitude). Results similar to these were reported by Betran et al. (2003) for tropical and subtropical maize in Mexico.

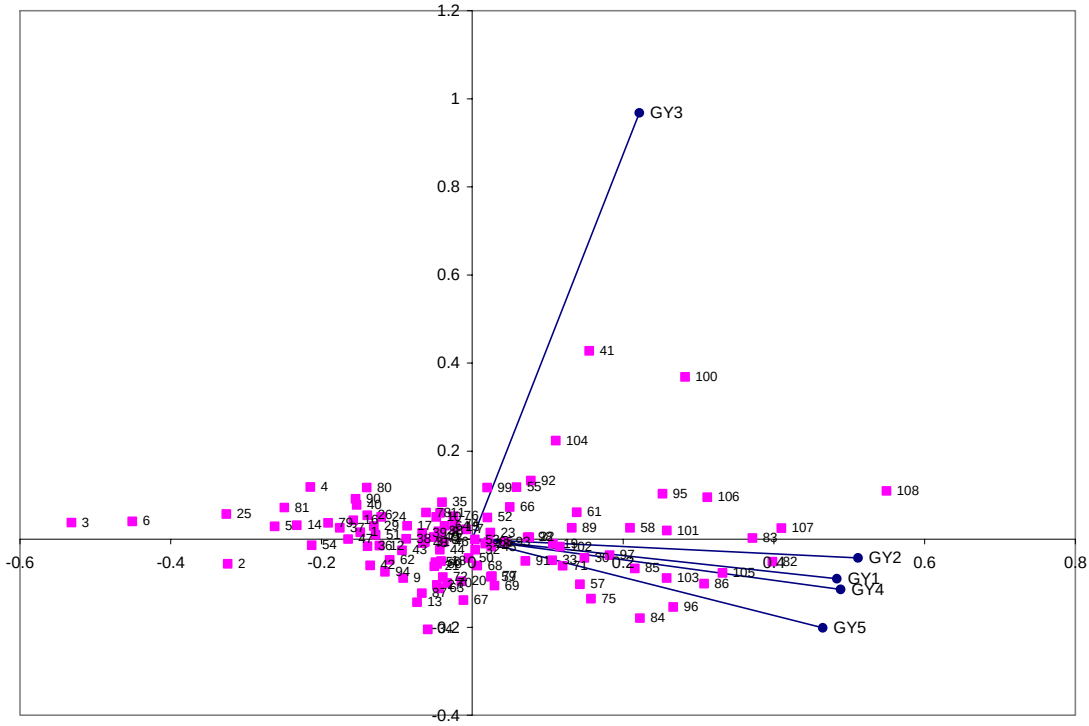

Fig. 5.12. Singular value decomposition biplot showing the relationships among test environments for grain yield of accessions for 108 maize accessions grown under different conditions in Zimbabwe and Zambia during the 2005 season.

GY1= grain yield under optimum growing conditions, GY2=grain yield under random stress, GY3=grain yield under drought stress, GY4=grain yield under low N stress, GY5=grain yield under low soil pH stress.

Stability Analysis

The combined analysis of variance across locations identified the existence of genotype x environment interaction (GEI) for most of the measured traits from this trial. Since the GEI variance for grain yield was found to be significant, stability analysis was conducted to assess the performance of the accessions across different environments. According to the Eberhart-Russell (1966) regression method of measuring stability, a stable variety is defined as one with a slope = 1 and a deviation from regression = 0. The results from this study showed a range of 0.285 to 1.894 for slope and 0.000 to 1.150 for

deviation from regression indicating a wide range in stability among the accessions. Based on the above criteria, accessions 32 (Local (Maroon w/ white tips), 88 (MMV400), 59 (Kaile-1), 63 (Gankata-1), 61 (Red Local-2), 50 (Mofati-2) and 46 (Local-54) were classified as stable while accessions 3 (Leaming), 4 (Boone Country white), 25 (Local-33), 98 (0101-M01SP), 105 (SC411), 107 (SC637) and 108 (SC719) among others were classified as the most unstable. Interestingly the most stable accessions were mainly local landraces while the most unstable were mainly improved and some ancestral varieties. However, the mean yield of the varieties should also be considered when choosing stable varieties. These data confirm the fact that smallholder farmers in southern Africa have been selecting for yield stability rather than high yield per se.

Selection Index

A selection index that sought to increase grain yield and number of ears per plant under all environments, while reducing anthesis-silking interval under abiotic stress conditions was calculated for each accession. Furthermore, the index sought to maintain anthesis date to avoid bias towards early or late maturing accessions. The upper 25% of the accessions (27 in total) represents those accessions that combine superior yield potential with abiotic stress tolerance. Results of the selection index show that among the top 25%, 8 were landraces while the remaining 19 were improved varieties. This study showed that there are eight landraces which deserve special attention from the breeders' point of view and are potential candidates for further investigation in a pre-breeding program. Landraces Local-46, Hickory King-10, Red Local-1, Local (Wine Colored)-2, Kenya-3, Red Local-2, Kanjiliame-1 and Local-20 combine high grain yield per environment with increased number per ears per plant and low values for anthesis-silking interval (ASI) and rate of leaf senescence under abiotic stress conditions. These traits are important in a breeding program to develop varieties for smallholder farmers in southern Africa.

The germplasm under selection or evaluation plays a critical role in defining relationships among environments and ultimately in the relative efficiency of direct vs. indirect selection. In growing areas in the tropics, maize frequently suffers from more

than one abiotic stress factor during a single growing season. For example where drought stress is common, farmers reduce the application of N fertilizer, or in acidic soils under high rainfall intensity, N fertilizer is frequently lost due to high leaching. The identification of accessions with superior performance under different abiotic stresses could enhance maize production in the tropics, even though the drought stress and the other stress environments were clustered in different groups. In addition, no yield penalty should result if the selected accessions are grown under a favorable environment, provided the selection index also incorporates higher yield under optimum growing conditions. Additional data on diseases, pests and some agronomic traits such as lodging can then be used to establish the best accessions for further breeding activities. As shown by the results of the selection index, some accessions performed well across stress levels, indicating that it is possible to combine stress tolerance and yield potential in tropical maize hybrids. Similar results have been reported with tropical maize (Betran et al., 2003) and temperate maize hybrids where improvements for tolerance to abiotic and biotic stresses have been associated with the ability to maximize grain yield under nonstress growing conditions (Duvick, 1997).

Cluster Analysis

Since the clustering of test environments revealed separation of the drought site from the other stress sites, for abiotic stress environments, two cluster analyses were done, one for drought stress and another for all locations. It was expected that the combined cluster would be similar to the one for all stresses combined except for the drought environment. Cluster analysis based on across location performance of the genotypes was also conducted. Lattice-adjusted means for GY, AD, ASI, PLHT, EPP and SEN were used in classifying the varieties. Different members within a cluster were assumed to be more closely related in terms of the traits under consideration with each other than those members in different clusters.

Similarly, members in clusters with non-significant distance were assumed to have more close relationships with each other than they are with those in significantly distant clusters. In general, when the agronomic performance of the maize varieties under different environments was used to create dendrograms (Figures 5.13 to 5.15), the

materials clustered into groups based on respective performance under specific environments. According to the dendrogram of the accessions under drought (Figure 5.13), two main groups of accessions and smaller one were obtained by cluster analysis of the 108 accessions tested. Cluster 1 (C1) was comprised of a mixture of local landraces, ancestral populations and the first OPV developed in Zimbabwe, while C2 was comprised mostly of improved varieties and a few local landraces. Comparisons of the agronomic relationships of the maize varieties under this environment with SSR clustering (Figure 4.5) showed that that the improved varieties in Figure 5.13 were located in the same cluster in the SSR dendrogram in Figure 4.5. When compared with the agronomic data under this environment, it was apparent that the best entries clustered together based on both SSR and agronomic data. Thus, the improved varieties demonstrated a significant association between agronomic performance and genetic diversity determined using SSR markers.

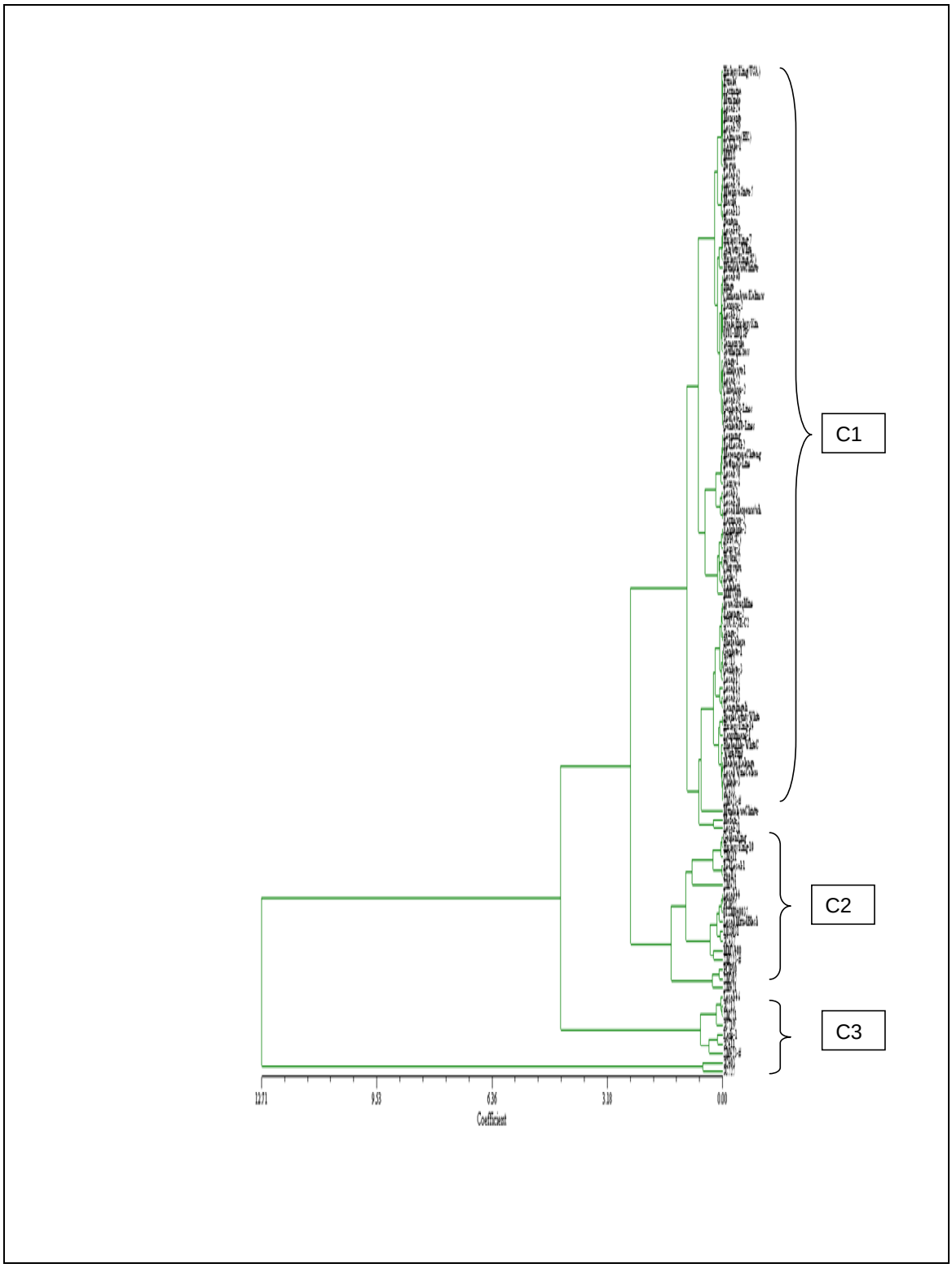

Fig. 5.13. Cluster Analysis based on agronomic traits for 108 maize varieties grown under drought stress in Zimbabwe, and Zambia during the 2004/2005 growing season.

The dendrogram of the varieties under a combination of random stress, low N and low soil pH also revealed two main clusters (Figure 5.9). Cluster 1 (C1) was also comprised of a mixture of local landraces, ancestral populations and the first OPV developed in Zimbabwe, while C2 was comprised mostly of improved varieties and a few local landraces. Comparisons of the agronomic relationships of the maize varieties under this environment with SSR clustering (Figure 4.5) showed that that the improved varieties and the few landraces that clustered together (Figure 5.9) were also located in the same cluster in the SSR dendrogram in Figure 4.5, although the overall clustering patterns between the two methods were somewhat different. When compared with the agronomic data under this environment, it was clear that the best entries clustered together based on both SSR and agronomic data. Thus, the improved varieties demonstrated a significant association between agronomic performance and genetic diversity determined using SSR markers.

The dendrogram for the accessions across different environment (Figure 4.10) revealed a closely related pattern to the one for random stress, low N and low soil pH (Figure 4.9). However, three main groups could be identified. Cluster 1 (C1) was comprised mostly of local landraces, ancestral populations from the USA and the first open pollinated varieties developed in Zimbabwe. C2 was comprised mostly of improved varieties, both hybrids and OPVs, and a few landraces, while C3 was also entirely of improved OPVs. Entries in C2 were mostly intermediate to late maturing while those in C3 were mostly early maturing materials. Comparisons of the agronomic relationships of the maize varieties under this environment with SSR clustering (Figure 4.5) showed that the improved varieties and the best performing local landraces (Figure 5.10) were located in the same cluster in the SSR dendrogram in Figure 3.5. Results from this cluster analysis showed that the best entries clustered together based on both SSR and agronomic data, even through the clustering patterns produced by the two methods were not exactly similar. Thus, the improved varieties demonstrated a significant association between good agronomic performance and genetic diversity determined using SSR markers.

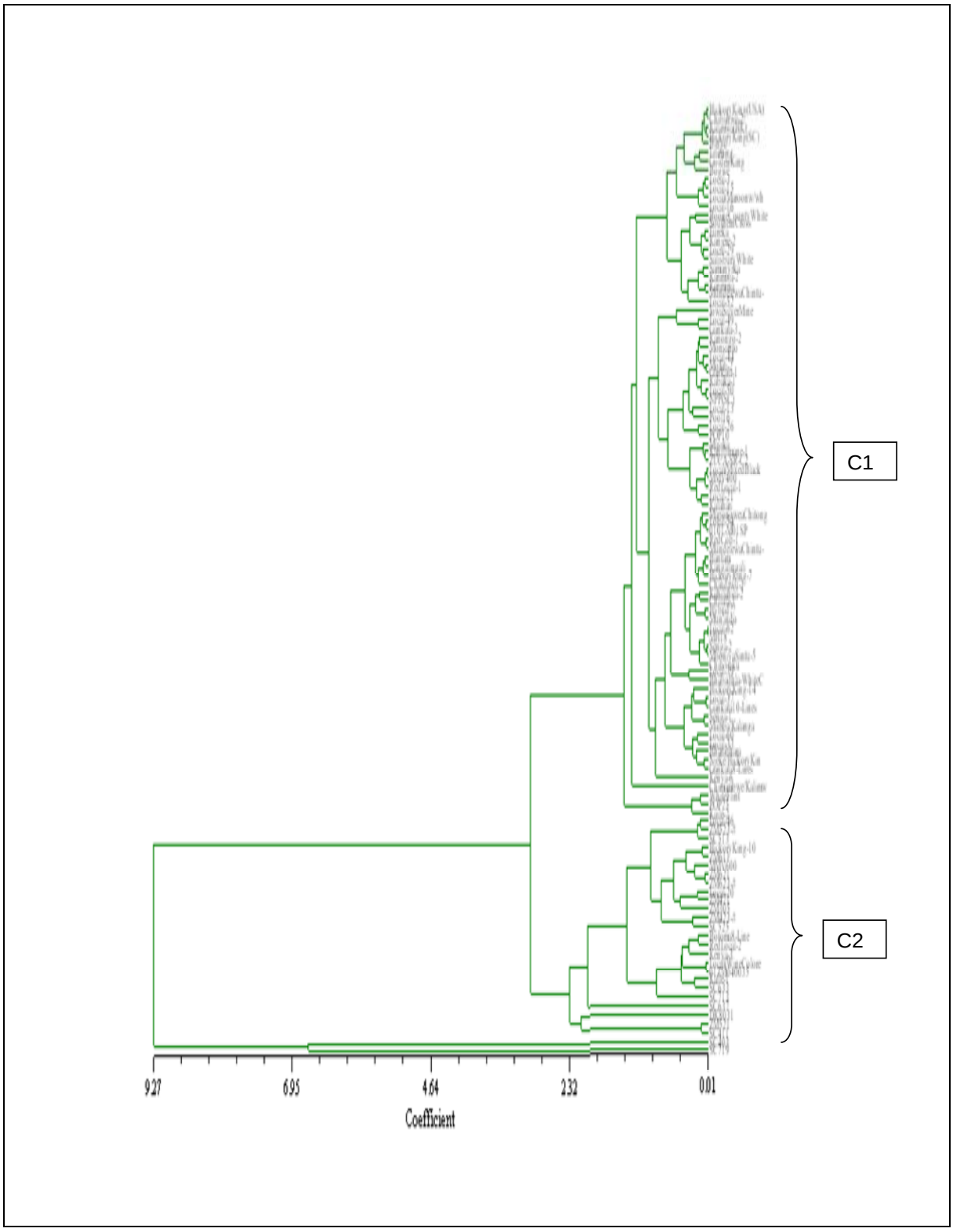

Fig. 5.14. Cluster Analysis based on agronomic traits for 108 maize varieties grown under low N, low pH and random stress in Zimbabwe, and Zambia during the 2004/2005 growing season.

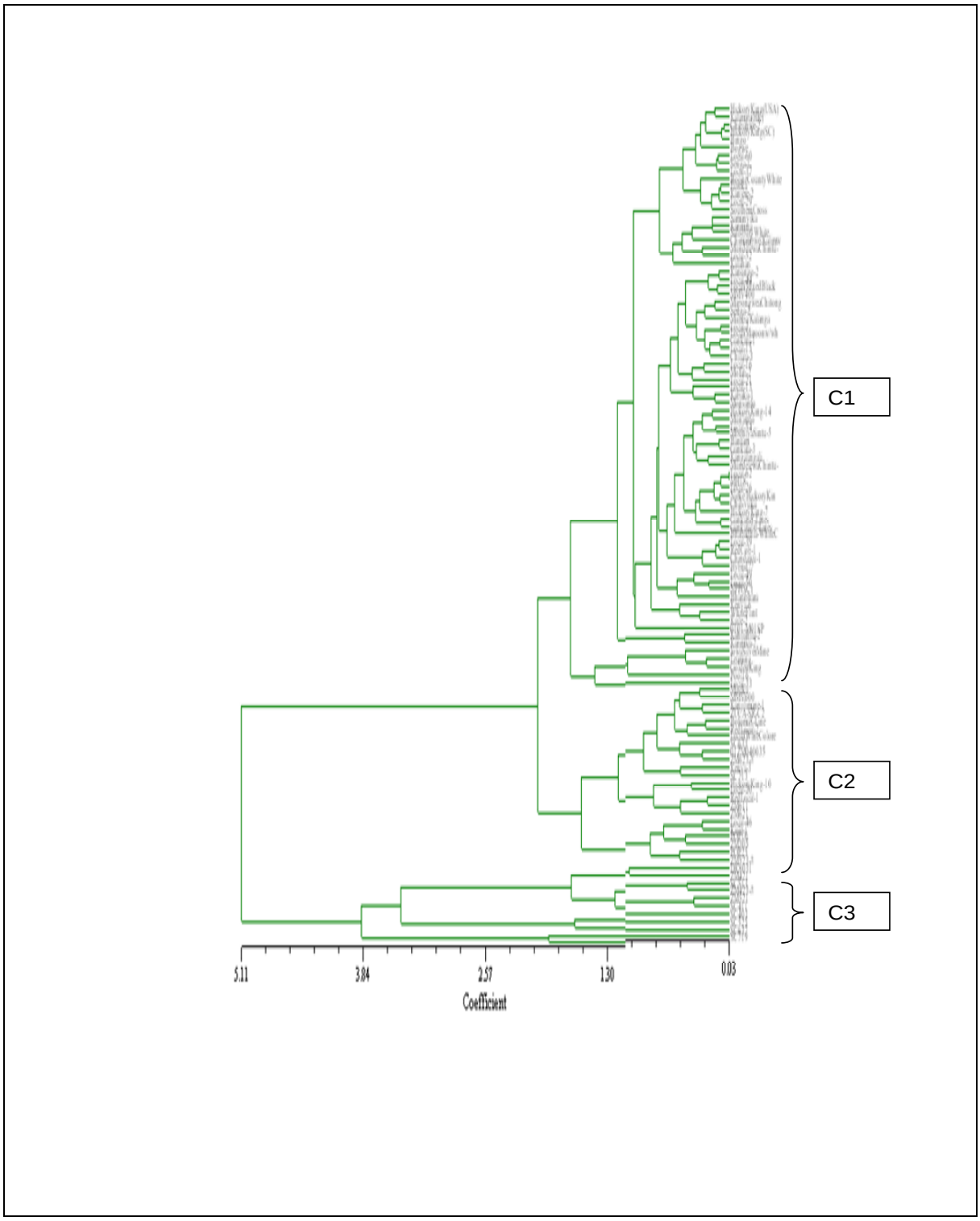

Fig. 5.15. Cluster Analysis based on agronomic traits for 108 maize varieties grown under different environments in Zimbabwe, and Zambia during the 2004/2005 growing season.

The current study included a wide range of genotypes representing more than one ecotype; therefore, it is not surprising that the associations between SSR grouping and clusters based agronomic performance were higher. Comparison of molecular to morphological and physiological data in cactus species in Texas also produced generally similar conclusions of relatedness among accessions, confirming the utility of either characterization analysis (Wang and Larkins, 2001). The close association between the SSR diversity analysis and agronomic performance under different environments should accelerate the usefulness of these data to maize breeders. Genetically different genotypes (a few landraces and some improved varieties) were identified with similar agronomic performance under abiotic stress. It is reasonable to assume that the genetic basis of stress tolerance in these materials is different, particularly when comparing landraces and improved varieties. Maize breeders can, therefore, combine these different sources of genetic variability for stress tolerance in their breeding programs. The efficiency of crossing can be increased, as closely related genotypes, determined on the basis of the SSR analysis, need not be crossed.

CONCLUSIONS

The objective of this study was to assess the variability of a core set of maize varieties under low soil nitrogen, low soil pH, drought stress, random stress, and under optimum growing conditions. The results of the present study showed that there exists considerable genetic variation in agronomic traits under different abiotic stresses commonly encountered in southern Africa. Differences among the accessions, type of accessions (landraces, ancestors, early OPVs and improved) were significant for most of the studied. Significant genotypes x environment interactions were also present. Estimates of genetic broad sense heritabilities varied depending on traits and testing environments. However, estimates were generally larger for each trait in the environment where the variance of that trait was highest. Genetic and phenotypic correlation coefficients also varied depending on traits and testing environments. Biplots constructed revealed which traits were closely related for each environment. Biplot analysis also separated the environments according to the season when trials were conducted.

Generally improved varieties outperformed landraces under all environments, but there were notable exceptions with many landraces yielding as much as improved varieties. Landraces were more stable than improved varieties across test environments, but improved varieties were more responsive to favorable growing conditions. From selection index conducted, the most promising landraces for pre-breeding and further investigation were identified. In general, when the agronomic performance of the maize varieties under different environments was used to create dendrograms, the materials clustered into groups based on respective performance under specific environments. The clustering pattern was different from SSR markers, but in general the genotypes groupings were consistent across the two methods of measuring diversity.

CHAPTER VI

GENETIC YIELD IMPROVEMENT

INTRODUCTION

Sub-Saharan Africa is staggering under the weight of its failure in food and agriculture, the sector that employs two out of every three people on the continent. After more than 25 years of independence for most of the countries, the region faces a growing food production gap, a loss of world market shares of many of its agricultural exports, and pervasive rural poverty. Africa is currently importing one-third of its maize consumption (FAOSTAT, 2005).

The story of maize production and improvement in Zimbabwe started with the introduction of open pollinated populations towards the end of the 19th century, mainly from the USA. A list of some of introduced varieties is provided by Weinamann (1972). The second major event was the development of the first local open pollinated varieties from direct selection of the introduced OPVs, or by inter-crossing some of the introductions to produce varietal hybrids that were more adapted to the growing conditions in the region. The next major event was the initiation of a hybrids maize program in the country in 1932 (Mashingaidze, 1994). Numerous hybrids, especially double crosses such as SR1 and SR11 were produced and used by commercial farmers, but hybrids did not become popular until the release of SR52, a highly productive single cross that was released in 1960. SR52 was a late maturing variety which required a long season with adequate moisture and fertilizer to realize its potential and was therefore not suited to farmers operating in short-season sandy soils which were less productive and prone to drought and low soil fertility. This constraint led to breeders of that time to expand their activities to include short-season varieties and beginning in the early 1970s, three-way hybrids were released. These were more heterogeneous compared to SR52 and hence could tolerate to some extent the drought occurring in marginal areas. They were short season and fitted well with the season length in these areas. Since then, various hybrids, mostly single crosses and three-way hybrids have been developed and released in the country. Today more than 90% of Zimbabwe's maize area is under hybrids and short-season hybrids are outcompeting other food grains in many low rainfall areas.

Numerous studies in maize have described long term trends in yield and physiological traits associated with the gains in yield improvement for the cultivars developed over time in the USA, Canada and Argentina. Most of these investigations have compared original open-pollinated pollinated varieties with different types hybrids released over time in each country. The main focus has been temperate maize. A survey of literature has shown that few have focused on tropical maize, e.g. in Brazil (Sangoi et al., 2002), and none have dealt with southern Africa.

Average maize grain yield per area increased dramatically during the second half of last century (Duvick, 1997). This yield gain has been attributed to genetic improvement, climate change, switches in management practices and greater tolerance of modern-hybrids to stresses imposed by low soil moisture in the field (Dwyer et al., 1992), weed interference (Tollenaar et al., 1997), and high plant population densities (Duvick, 1997; Tollenaar and Wu, 1999). Knowledge of the changes in physiological traits associated with genetic gains in yield potential is essential to improve the understanding of yield-limiting factors and to inform future breeding strategies, especially for abiotic stress prone areas of southern Africa.

The objectives of this section of the study was to (i) determine the genetic gain in Zimbabwean maize varieties released and widely grown since 1900 up to 2004, and (ii) to identify physiological traits associated with genetic gains in grain yield of these varieties in Zimbabwe.

LITERATURE REVIEW

Maize Breeding in Zimbabwe

The development of improved varieties of maize in Zimbabwe started in the early 1900s, when a Department of Agriculture was established to re-organize agricultural production through insights from agrarian sciences. In 1919, commercial farmers founded the Maize Breeders Association to promote selection and production of better seed, and scientific maize breeding started in 1932 (Mashingaidze, 1994). The first hybrid maize varieties bred outside the United States were produced to fit the country's climate. Commercial farmers established the Seed Maize Association of Southern Rhodesia in 1940 to ensure the timely production and supply of high-quality seed. Experiments in the

post-1945 period showed that these new hybrids provided significantly higher yields in both normal and drought years (Rattrary, 1956). A major in achievement in the agrarian sciences in Zimbabwe was the release in 1960 of SR52, the world's first single-cross hybrid that could be produced economically on a commercial scale. By 1970, 98% of Zimbabwe's commercial maize area was planted to SR52. In the late 1960s, attention shifted to breeding three-way-cross hybrids, such as R200, R201 and R215, which showed good adaptation to areas of unreliable rainfall and sandy soils (Mashingaidze, 1994). In 1973, the Plant Breeders' Right Act was passed to protect ownership of maize varieties in the country. Subsequently, the Seed Maize Association established the country's first private research station in 1974, which tested experimental varieties that came out of public research programs.

After independence in 1980, the state-funded maize-breeding program in Zimbabwe was decimated by loss of experienced staff and severe funding reductions. Public sector breeding efforts were boosted in 1985 with the arrival of CIMMYT in Harare, which introduced both expertise and germplasm. In 1983, the Zimbabwe Seed Maize Association and the Crop Seeds Association merged to form the Seed Co-operative Company of Zimbabwe (Seed Co), which initially worked in co-operation with government. Changes in policy in 1995 cleared the way for increased foreign investment in Zimbabwe's maize seed industry. Although Seedco now faces competition from large international seed companies (e.g. Pioneer, Pannar, Monsanto), which invest more resources in maize breeding than the government and CIMMYT combined, it remains the most important player in Zimbabwe's maize seed industry.

The most dramatic change in the early post-independence period in Zimbabwe was in the pattern of usage of hybrids. Between 1950 and 1975, adoption was largely limited to large scale commercial farmers. Subsequently, agricultural extension workers started to encourage the adoption of hybrids among communal farmers as a way to ensure national food self-sufficiency. These efforts were complemented by government investments in rural infrastructure. In the post-independence period, adoption of R201 and R215 (first generation hybrids) skyrocketed (Rohrbach, 1988), with dramatic increases in yield (Eicher, 1995).

In the last fifteen years, most maize breeding programs in the country have paid more attention to resistance to diseases of concern to commercial farmers and improved drought tolerance, rather than emphasizing increased yields. Consequently, many seed companies now produce a wide variety of hybrid maize seeds (second generation hybrids) with these improved traits, although these improvements may not be visible to all farmers. Examples include the SC40x, SC50x and SC60x series of seeds from Seedco (Bourdillon et al., 2002). These new varieties are marketed to farmers in a number of ways, including sponsoring field days and trial or demonstration units, advertisements in the print and electronic media and the production and dissemination of seed manuals written in both local languages and English. Institutions that provide inputs or input loans (such as the Grain Marketing Board) also play a part in the diffusion of new hybrid varieties as does the government's Department of Agriculture Research and Extension Services (AREX). With the introduction of newer hybrids, old hybrids such as R201 and R2015 have been discontinued.

Genetic Gain

Many studies in maize have described long-term trends in grain yield, tolerance to biotic and abiotic stresses, and agronomic characters for the cultivars developed over time in selected regions of the world, mainly the USA, Canada and South America. Most of these investigations have compared early OPVs and hybrids from temperate regions. Few have focused on tropical maize and less still fewer still have dealt with African maize cultivars.

Previous studies of US maize hybrids over time have consistently shown linear increases in grain yield from the oldest to the newest hybrids (Duvick, 1997). Genetic yield gain has been continual and constant (in amount) over the years. The studies also consistently show that the oldest hybrids make their maximum yields at low plant densities typical of maize farming in the earl decades of the last century, whereas newer hybrids yield the most at higher densities typical of current cultural practices. In another study on maize, Tollenaar (1991) examined genetic advances in maize varieties grown in Ontario, Canada from the 1950s to the 1980s and found genetic gains of 1.7% yr^{-1}. The current cultivars consistently had higher grain yields than the old cultivars. In Argentina,

genetic gains in grain yield for seven maize hybrids developed for the central region of Argentina between 1965 and 1997 was 13.2 g m^{-2} yr^{-1}(Luque et al., 2006). In this study, varieties were cropped in the field at five stand densities (from almost isolated plants to supra-optimal levels) during two contrasting growing seasons under optimal growing conditions.

In a study to compare the agronomic performance of maize varieties that represented 10-year eras from OPVs of pre-hybrid time to modern hybrids of the 1980s, Russell (1984) showed inconsistent trends over the years for grain yield. The first single cross hybrids (1930) yielded significantly more than the OPVs, but subsequent hybrids (1940-1950) yielded significantly less than the 1930 single crosses. After the 1950s there was significant and consistent yield increases. Castleberry et al. (1984) studied the trend in maize yield for a set of maize varieties grown from the 1930s to the 1980s and found that even under varying soil fertility and climatic conditions, yields of newer hybrids were consistently higher than those of old released. These authors concluded that US maize production will be best served by the continued development and deployment of improved single cross maize hybrids even if less favorable soil fertility or climatic conditions should occur.

Physiological Traits Associated With Genetic Gain in Maize

Genetic gains in grain yield and related phenotypic attributes have been extensively documented in many crops including maize, cotton, wheat and soybean, but the effect of breeding on the physiological determinants of grain yield is still poorly understood in most of these crops. For the USA, Duvick (1992) attributed the consistent increase in maize grain yield to a continued and linear improvement in plant defensive traits such as reduced rate of leaf senescence, better root and stalk lodging, a narrow interval between pollen shedding and silking (ASI) and reduced barrenness. In Canada, Tollenaar and Lee (2002) concluded that recent genetic improvements of grain yield in maize were due to increased stress tolerance of new varieties, which has been obtained through selection for yield stability across target environments and was not negatively related to high grain yields. These authors were in agreement with Duvick (1997), who

had previously considered that grain yield potential has not increased over time when considering plants under no resource competition.

Many studies have shown that grain yield increases have been mostly correlated with an increase in number of kernels per unit land area. This fact is related to the performance of certain key plant traits during critical developmental periods that affect final kernel number, such as silk growth rate during the critical period (Banziger et al., 2002) or ASI (Edmeades et al., 1999). Breeding effects on kernel weight determination deserved less attention, probably based on evidence that trade-off effects between grain yield components did not impair increases in the number of kernels to translate into improved grain yields. Russell (1984) showed that breeding for improved grain yield promoted an increase in harvest index, but this trend was not registered by Tollenaar (1989) among hybrids bred for the short-season, high-latitude environment of Canada. The response of grain yield to different secondary traits depended upon each particular genotype per environment interaction.

In Zimbabwe, most part of maize production relies on the mid-altitude region for which relative maturities between 120 and 150 are currently recommended. This region has always concentrated the main breeding efforts of most seed companies in the country, and it was not until recent years that they started special programs aimed to the drought prone and less fertile environments located at lower altitudes. In addition, since the beginning of maize varietal development in the country, little is known about the genetic gain and adaptive traits responsible of the genetic gains between 1900 and 2004. Therefore, the objective of this study was to estimate the genetic gain in Zimbabwean maize varieties released and widely grown since 1900 up to 2004.

MATERIALS AND METHODS

Germplasm

A set of 48 maize accessions were chosen (Table 6.1). Seeds were obtained from the Crop Breeding Institute (CBI) of the Zimbabwe Agriculture Research and Extension Service (AREX), Seedco's Rattray Arnold Research Station, and CIMMYT, Harare. The accessions were selected on the basis of meeting, as much as possible, a combination of

time or release, wide cultivation by farmers, and availability of seeds. Each of these varieties was among the topmost cultivated hybrids in Zimbabwe for at least 5 yr after their release. The first six varieties originating from the USA are considered the ancestral populations for maize breeding in Zimbabwe. Twelve of the varieties corresponded to the same seed company, Seedco, while fourteen were from AREX. The Seedco breeding program was initiated partly from the AREX materials and may be considered an extension of the breeding efforts of AREX. This unique setup allowed for a better understanding of breeding effects than usually possible when hybrids from completely different breeding programs are tested. CIMMYT varieties were included to demonstrate the impact of a new dimension in maize breeding where the emphasis is placed on selection under managed abiotic stresses.

Environments and Stress Management

The set of 48 varieties were evaluated in Zimbabwe at ART Farm near Harare (optimum conditions), CIMMYT Harare (low N) and Chiredzi Research Station (drought stress). At Chiredzi, drought stress was achieved by withholding water from 3 weeks before silking to the end of the flowering period. This location is largely rain free during the winter season, allowing the control of drought stress intensity by withdrawing or delaying irrigation for varying lengths of time during flowering and grain filling stages (Edmeades et al., 1999). Fertilizer rate at each location (except Harare low N) were adjusted to reflect the agronomic recommendations for each location. At Harare, low nitrogen stress conditions were achieved by growing the trial in a field that had previously had continuous cropping of maize without N fertilizer application for several years. Standard cultural and agronomic practices were followed in trial management at each location.

Table 6.1. Maize varieties tested, year or release, type of variety and their origins.

Variety	YOR	Breeding Period	Type of Variety	Origin
Hickory King (USA)	1900	1900	OPV	USA
Iowa Silver Mine	1900	1900	OPV	USA
Leaming	1900	1900	OPV	USA
Boone County White	1900	1900	OPV	USA
Eureka	1900	1900	OPV	USA
Golden King	1900	1900	OPV	USA
Salisbury White	1909	1910	OPV	AREX-Zimbabwe
Hickory King (ZIM)	1909	1910	OPV	AREX-Zimbabwe
Southern Cross	1915	1910	OPV	AREX-Zimbabwe
Natal Potschestoom Pearl	1918	1910	OPV	AREX-Zimbabwe
SR52	1960	1970	Hybrid	AREX-Zimbabwe
R200	1970	1970	Hybrid	AREX-Zimbabwe
R201	1973	1970	Hybrid	AREX-Zimbabwe
R215	1975	1970	Hybrid	AREX-Zimbabwe
ZS107	1984	1980	Hybrid	AREX-Zimbabwe
ZS206	1984	1980	Hybrid	AREX-Zimbabwe
ZS225	1984	1980	Hybrid	AREX-Zimbabwe
ZS233	1984	1980	Hybrid	AREX-Zimbabwe
ZS232	1984	1980	Hybrid	AREX-Zimbabwe
SC401	1984	1980	Hybrid	Seedco Zimbabwe
ZS240	1992	1990	Hybrid	AREX-Zimbabwe
ZS251	1992	1990	Hybrid	AREX-Zimbabwe
SC621	1994	1990	Hybrid	Seedco Zimbabwe
SC701	1994	1990	Hybrid	Seedco Zimbabwe
SC403	1997	1990	Hybrid	Seedco Zimbabwe
SC709	1997	1990	Hybrid	Seedco Zimbabwe
SC407	1999	1990	Hybrid	Seedco Zimbabwe
SC513	1999	1990	Hybrid	Seedco Zimbabwe
SC517	1999	1990	Hybrid	Seedco Zimbabwe
SC506	1999	1990	Hybrid	Seedco Zimbabwe
SC604	1999	1990	Hybrid	Seedco Zimbabwe
SC713	1999	1990	Hybrid	Seedco Zimbabwe
SC715	2000	2000	Hybrid	Seedco Zimbabwe
ZM621-#	2000	2000	OPV	CIMMYT-Zimbabwe
ZM305	2001	2000	OPV	CIMMYT-Zimbabwe
ZM611-F3	2001	2000	OPV	CIMMYT-Zimbabwe
Syn01E2	2002	2000	OPV	CIMMYT-Zimbabwe
ZM421	2002	2000	OPV	CIMMYT-Zimbabwe
ZM423-#	2002	2000	OPV	CIMMYT-Zimbabwe
ZM521	2002	2000	OPV	CIMMYT-Zimbabwe
Kalahari Early Pearl	2003	2000	OPV	Seedco Zimbabwe
SC635	2003	2000	Hybrid	Seedco Zimbabwe
OBATANPA-ZMSR	2003	2000	OPV	CIMMYT-Zimbabwe
SC411	2004	2000	Hybrid	Seedco Zimbabwe
SC525	2004	2000	Hybrid	Seedco Zimbabwe
SC637	2004	2000	Hybrid	Seedco Zimbabwe
SC719	2004	2000	Hybrid	Seedco Zimbabwe
ZM623-#	2005	2000	OPV	CIMMYT-Zimbabwe

YOR=Year of Release, OPV=Open pollinated variety, AREX=Department of Agriculture Research and Extension Services, Seedco = Seed Company of Zimbabwe

Experimental Design and Data Collection

The trials for optimum conditions and low N stress were grown during the 2004/2005 rainy season, while the drought stress trials were grown during the rain-free winter months of 2005. Experimental design was a split-plot alpha-lattice design with plant densities as main plots and varieties as subplot. Incomplete block sizes were six entries for each subplot. Experiments were planted with two replicates for higher plant density and three replicates for the lower plant density. Plots were overplanted and thinned to one plant every 25cm (for the high density main plot) one plant every 40cm (for the lower density main plots). Plot sizes were two rows of 4 m length set at 75 cm apart. The low plant density (37,000 plants ha^{-1}) was chosen to represent the standard density used in the early days of maize cultivation in the country while the higher plant density (53,333 plant ha^{-1}) represents the current norm.

During the growing season, data was collected as follows: number of days from planting to 50% of the plants shedding pollen (AD); number of days from plating to 50% of the plants having silks at least 1 cm long (SILK); plant height (PLHT)(cm) to the flag leaf insertion, and ear height (EHT) (cm) at the upper ear insertion node. Traits measured after harvest on a plot basis were: number of harvested plants (NP), number of ears (EN), ear weight (EWT) (kg), and shelled grain weight (GWT) (kg). For the drought and low N trials, rate of leaf was also recorded (SEN) on a 1 to 10 scale (1=10% dead leaf area 10=100% dead leaf area). Additional variables calculated from direct measurements were: grain yield (YLD) calculated as shelled grain weight per plot adjusted to 125g kg^{-1} moisture and converted to Mg ha^{-1}, anthesis to silking interval (ASI) (days) calculated as SILK-AD, and number of ears per plant (EPP) calculated as number of ears (NE) with at least one fully developed grain divided by NP and ear position calculated as the ration of EHT to PLHT.

Statistical Analysis

Variety and density effects were evaluated by ANOVA, and a t-test was used to determine significant differences between means. Individual analyses of variance were conducted for each trial with the PROC MIXED procedure from SAS (SAS Institute, 2005) with all factors (accessions, reps, blocks, densities) being considered as random

effects. Phenotypic correlations per breeding period were calculated between traits by considering the maize accessions as random effects. Combined analyses of variance were conducted by means of PROC GLM while correlations were done using PROC CORR in SAS (SAS Institute, 2005). Mean decade group yields were regressed as dependent variables on either the midpoints of the appropriate decade (breeding period) as independent variables. Difference in b values were evaluated by t test. Regression analysis using simple linear models was conducted using SPSS software (SPSS, 2004).

Breeding effects were estimated as the genetic gain for the attributes under study. For this analysis, six temporal breeding periods were defined to reflect the major breeding efforts of maize in Zimbabwe. Period I entries were released and grown before 1920 and is comprised mostly of the original open pollinated maize populations introduced into Zimbabwe by white settlers. Period II entries are those OPVs developed directly from selections or crosses between the OPV from Period I and these comprise the first varieties developed in the country. Period III entries were the first modern hybrids developed in the country mainly from the lines extracted from the period II OPVs. Period IV entries were the first post-independence hybrids grown in Zimbabwe, while periods V and VI reflect entries developed and widely grown during the last 15 years. Genetic gain for grain yield was computed for each breeding period as the response of the grain yield to the year of release (YOR) of hybrids included in the analysis.

RESULTS AND DISCUSSION

The ANOVA showed that plant density by variety interaction was non-significant under all environments. Therefore, for each environment data was combined across plant densities and analyzed as simple alpha lattice. Where necessary, analysis of covariance was conducted to adjust grain yield for different maturities to enable valid comparison of the mean grain yields. Mean decade performances for the most important traits are presented in table 6.2.

Under optimum soil fertility and moisture growing conditions, variety effects and breeding period effects were significant for grain yield (GY), days to 50% anthesis (AD), number of ears per plant (EPP), but not for anthesis-silking-interval (ASI). The fact that

ASI was non-significant under optimum fertilizer and moisture conditions serves to confirm that this trial was conducted under non-stress conditions for fertilizer and water availability. Although the range in AD was wide across all the varieties tested, the range per breeding period was small enough to allow meaningful comparison of yields without undue concern about maturity. Mean grain yield consistently increased for each decade group (breeding period) beginning at 2.458 Mg ha^{-1} for the OPVs grown in the 1900s to 10.993 Mg ha^{-1} for hybrids grown during the 2000s (Figure 6.1). However, the trend for other plant traits, except EPP was not consistent. Just like grain yield, there was a consistent increase in EPP from a low of 0.88 in the ancestral OPVs of the 1900 to 1.1 for varieties grown in the 2000s. Percent root and stalk lodged plants was higher in the earlier breeding periods especially OPVs (ranging from 8 to 43%) compared to later breeding periods (below 3%), but the trend was less apparent beginning in the hybrid breeding period.

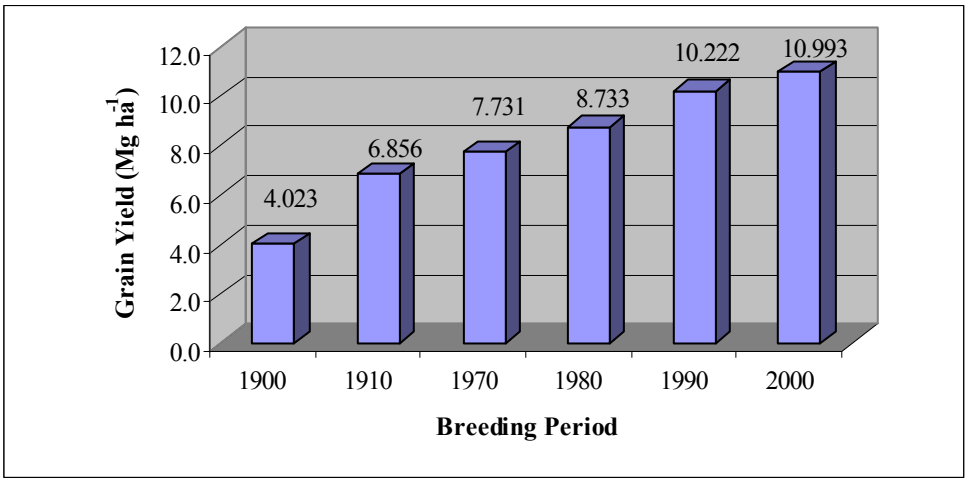

Fig. 6.1. Grain yield under optimum conditions for 48 Zimbabwean maize varieties developed and released from 1900 to 2004.

Results of the regression analysis of mean breeding period group yields on the decade of use for the individual environments are shown in table 6.3 and figure 6.2. The correlation between grain yield and breeding period (YOR) was statistically significant (0.924) at the 0.01 level, and coefficient of determination (R^2) and the b values (yearly rates of genetic improvement of yield) were all positive (Figure 6.2). The regression

shows that mean grain yields have been increasing at an average rate of 55kg ha^{-1} yr^{-1} from 1900 up to 2004.

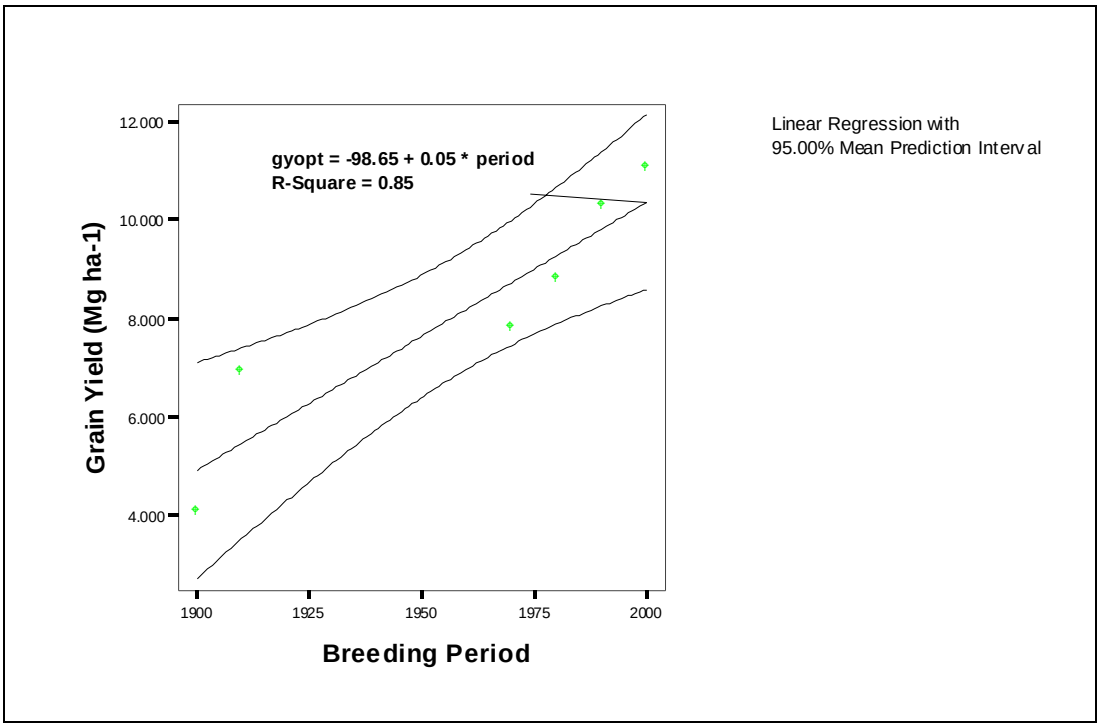

Fig. 6.2. Regression of grain yields of maize varieties grown under optimum conditions on decade (breeding periods) at ART Farm, Zimbabwe during the 2004/2005 growing season.

The effect of low soil N on the relationship between mean decade (breeding period) group yields, averaged over years is presented in figure 6.3. The pattern of consistently higher yields for later decades was maintained under low soil N conditions but with a smaller advantage as compared to optimum conditions. The average rate of yield increase due to genetic improvement (b values) was 14 kg ha^{-1} yr^{-1}, also positive under low soil N, but less than under optimal fertility and moisture conditions (Figure 6.4). In general, secondary traits associated with grain yield showed consistent changes with breeding period. The number or ears per plant (EPP) increased steadily from 0.38 for OPVs to 0.77 for later decade hybrids while ASI declined from a high of about 4.7 days for earlier decade varieties to 2.4 days for later decade varieties.

Table 6.2. Mean decade performances for different for 48 maize varieties evlauated in Zimbabwe duringthe 2004/05 growing season.

Period	AD (days)			ASI (days)			PLHT (cm)			EPOS (%)			RL (%)			EPP (No.)			SEN (Score)	
	OPT	LN	DR	OPT	LN	DR	OPT	LN	DR	OPT	LN	DR	OPT	LN	DR	OPT	LN	DR	LN	DR
1900	66	72	89	2	4	8	227	164	203	0.53	0.42	0.54	43.69	8.69	7.60	0.88	0.38	0.54	8.33	4.32
1910	73	78	94	1	5	10	252	174	196	0.56	0.43	0.52	5.35	8.64	4.41	0.92	0.44	0.35	5.93	2.91
1970	71	76	93	1	3	4	247	181	201	0.56	0.41	0.54	5.96	6.57	5.54	0.97	0.65	0.57	5.89	2.66
1980	71	76	92	1	4	5	249	179	206	0.55	0.41	0.54	10.93	5.27	4.27	0.98	0.66	0.54	5.57	2.88
1990	71	77	93	1	3	4	250	181	215	0.54	0.41	0.54	7.05	4.23	1.47	1.06	0.78	0.62	4.98	2.90
2000	72	78	95	0	2	5	254	180	214	0.56	0.45	0.55	2.69	1.11	1.68	1.12	0.77	0.67	5.21	3.03

AD = Anthesis date; ASI= Anthesis to silking interval, PLHT = Plant height; EPOS= Ear position; RL = Root lodging; EPP = Number of ears per plant; SEN=Rate of leaf senescence; OPT=optimum growing conditions, LN= low soil N stress, DR= drought stress conditions

Under low soil N, the rate of leaf senescence (SEN) also declined from 9.9 for early decade varieties to 5.2 for later decade varieties on a 1-10 scale.

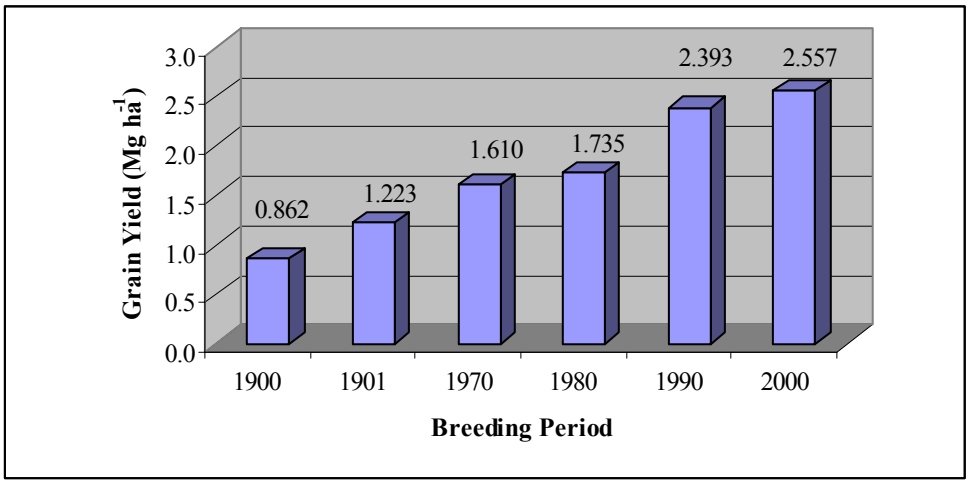

Fig. 6.3. Grain yield under low soil N conditions for 48 Zimbabwean maize varieties developed and released from 1900 to 2004.

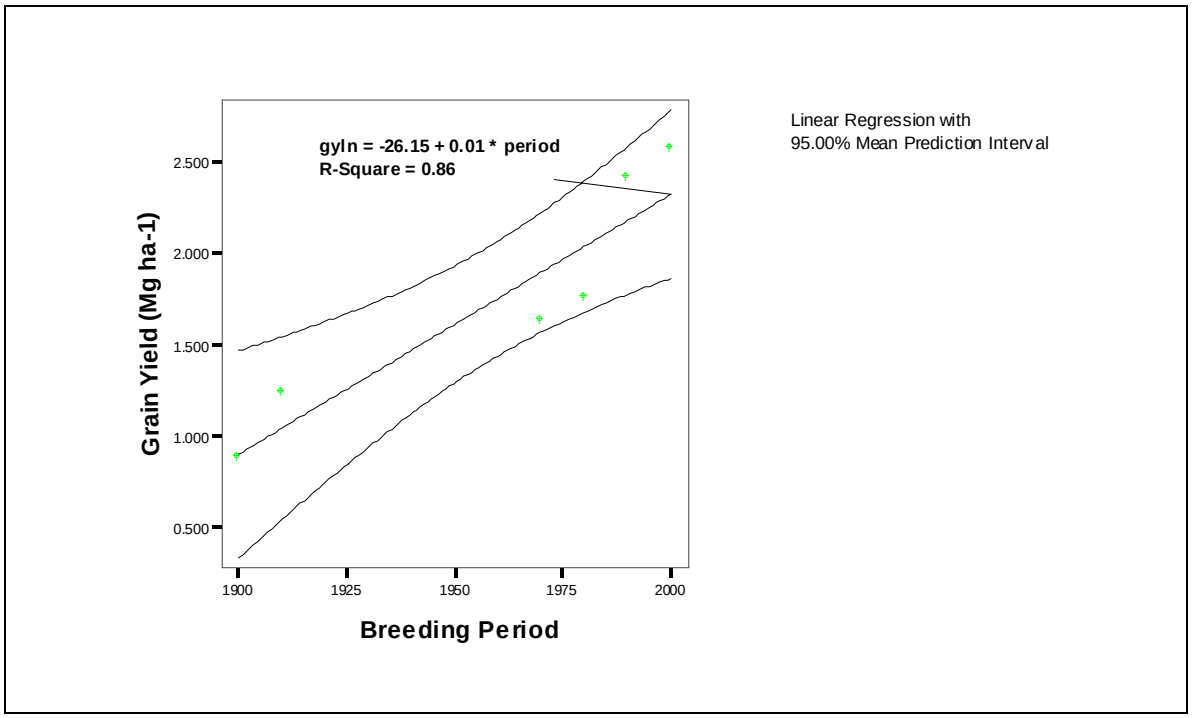

Fig. 6.4. Regression of grain yields of maize varieties grown under low soil conditions on mean decade (breeding periods) at Harare, Zimbabwe during the 2004/2005 growing season.

The effect of drought stress at Chiredzi on the relationship between mean decade group yields is presented in Figure 6.5.

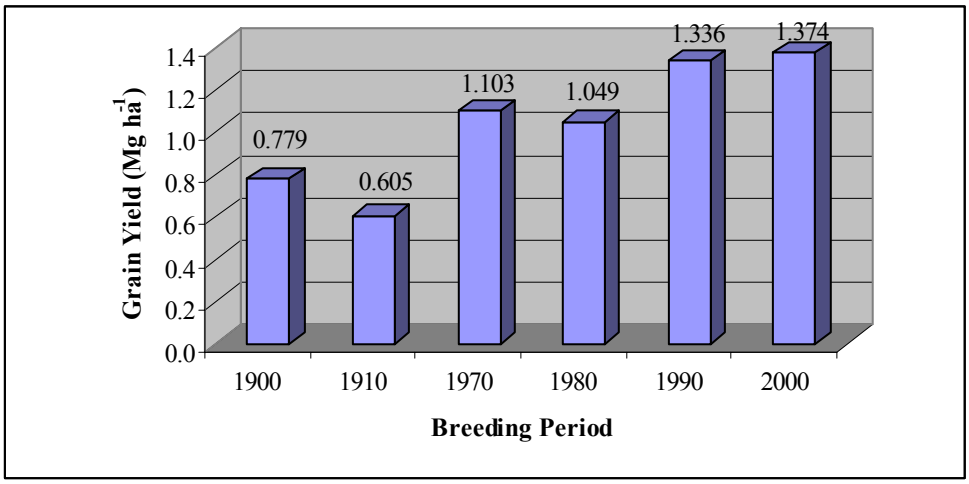

Fig. 6.5. Grain yield under drought stress conditions for 48 Zimbabwean maize varieties developed and released from 1900 to 2004.

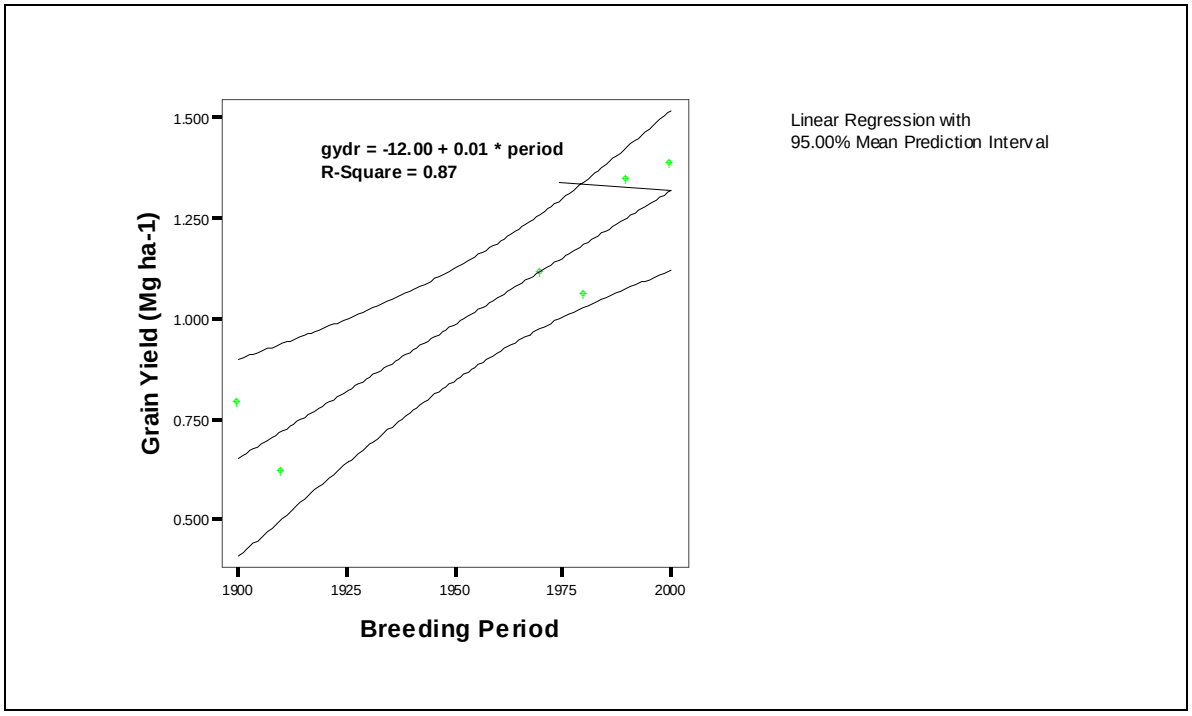

Fig. 6.6. Regression of grain yields of maize varieties grown under drought stress conditions on mean decade (breeding periods) at Chiredzi, Zimbabwe during the 2004/2005 growing season.

Drought stress effects on the relationship were similar to that of low soil N stress. The absolute rate of yield increase due to genetic improvement (b values) was 7 kg ha^{-1} yr^{-1} and positive under drought stress (Figure 6.6) but less than under optimal conditions and low N stress conditions (Figures 6.2 and 6.4). Trends in secondary traits related to grain yield were similar to those for low N environment.

Regression analysis of mean decade group yields on an across environments basis gave the relationship shown in Fig 6.7. The average rate of yield increase due to genetic improvement (b values) was 25 kg ha^{-1} yr^{-1}.

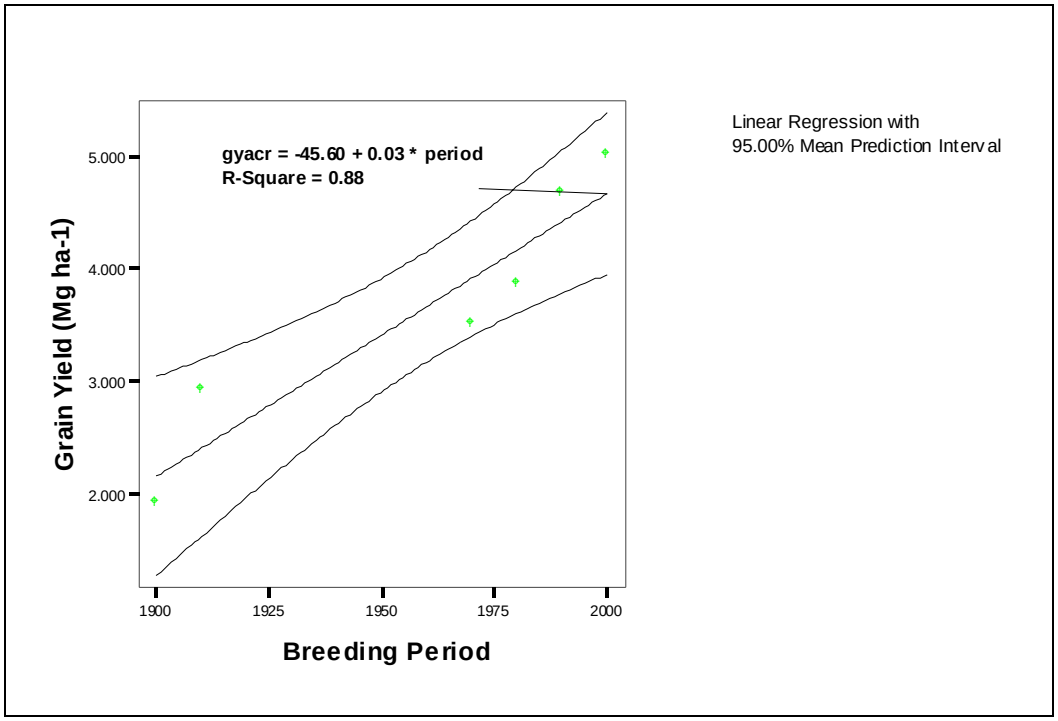

Fig. 6.7. Regression of grain yields of maize varieties grown across optimum conditions, low N and drought stress conditions on mean decade (breeding periods), in Zimbabwe during the 2004/2005 growing season.

Correlations under optimal growing conditions of various agronomic traits with grain yield indicate that high yield was associated with later maturity and reduced barrenness (EPP), while under low N conditions, high grain yield was associated with later maturity, reduced barrenness and rate of leaf senescence (Table 6.3). High grain yield was also associated with shorter plants under both optimum and low N conditions.

Under drought stress, high grain yield was associated with a reduced anthesis-to-silking interval and reduced lodging.

Table 6.3. Correlations of several traits with grain yield under optimum, low N and drought conditions for 48 maize varieties.

Character	Correlation coefficient(r)		
	Optimum	Low N	Drought
Days to 50% anthesis	0.66***	0.69***	-0.11^{ns}
Anthesis to silking interval (days)	0.15^{ns}	0.25^{ns}	-0.57***
Number of ears per plant	0.57***	-0.45**	0.29*
Plant height (cm)	-0.43**	-0.42**	-0.04^{ns}
Root lodged plants (%)	0.12^{ns}	0.32*	-0.32*
Rate of leaf senescence (1-10 score)	-	0.60***	-.008^{ns}

*, **, and *** significant at the 0.1, 0.05 and 0.001 levels respectively, ns=non-significant

In this study the more recent maize varieties showed a consistent improvement over older varieties. The apparent yearly rate of yield increase due to genetic improvement (b values) was positive in all environments tested and characterized by high coefficients of determination. Substantially higher grain yields were obtained with modern hybrids even under yield limiting conditions such as low soil N and drought stress conditions. In addition more recent hybrids were responsive to favourable growing conditions. The most recent maize hybrids evaluated in this study provided the best yield response to favourable environments, while maintaining better yields under poor conditions than older hybrids or varieties. These results are in general agreement with those previously reported for temperate maize by Castleberry et al. (1984), Russell (1984) and Duvick (1997). Similar results were also found for the high yield varieties of rice and wheat associated with the green revolution (Plucknett and Smith, 1982), and for cotton in the US (Bayles et al., 2005).

Although the results from studies of this type are somewhat dependent on the varieties and environments sampled, the general consistency of results reached in this study should allow some general conclusions concerning maize production in Zimbabwe. The transition of Zimbabwean farmers from OPVs to hybrids, and the associated changes in emphasis in maize breeding programs to higher yielding management responsive

hybrids has not led to increased vulnerability in unfavourable conditions. In fact, as demonstrated by CIMMYT (2002), modern competitive hybrids that can tolerate low soil fertility and drought stress without any yield penalty under favourable conditions have been developed.

The apparent yearly rates of genetic improvement in yield (b values) observed in this study were strongly dependent on the environment in which they were determined. Thus some caution must be exercised in interpreting estimates of yearly improvement rates due to genetics since the environments sampled as well as the genotypes included can substantially affect the estimates. The average estimates of yield increase due to genetic improvement (b value) under optimum growing conditions was 55 kg ha^{-1} yr^{-1} in this study. This represents 72 % of the 76 kg ha^{-1} yr^{-1} in average US maize hybrids since 1930 as reported by Duvick (1997). However, it should be noted that in the study by Duvick, only hybrids, beginning with double crosses from the 1930s, were used as the baseline to determine the genetic gain, while in this study OPVs from as early as the 1900s were used here as the baseline.

The gain in yield due to genetic factors under both low N and drought stress conditions in this study suggests that constant progress can been made in developing maize varieties (both OPVs and hybrids) that more efficiently utilize available soil nitrogen and water. In addition there is no yield penalty when the varieties are grown under optimum fertilizer and moisture conditions. In breeding work statistically significant yield differences are usually easy to identify in high yield environments which typically give higher heritability estimates than stress environments. In the study reported here, the absolute yield separation between the cultivars or groups of cultivars was consistently greater in higher yielding environments. These factors suggest that maize breeders may be able to select more efficiently for overall yield performance by conducting evaluations for yield potential in high yield environments while conducting evaluations for stress tolerance in separate trials or nurseries. The important secondary traits that should aid breeder in selecting the best genotypes under different environments have also been demonstrated from this study.

In this study, all cultivars were grown under modern cultural practices, and the use of modern cultural practices on all genotypes caused the slope estimate of yield

improvement to contain both the genetic improvement component and the genetic improvement by improved cultural practices interaction. Reaction to diseases was not measured in this study. Earlier decade varieties encountered different disease during their time period compared to modern time cultivars. Climatic changes have also occurred in the last 100 years that can have a bearing on the results obtained in this study. At the moment it is not possible to replicate the climatic conditions of the past. In addition it should also be pointed out that only one environment was used but more trials are still underway. This will then allow an estimation of genetic gain across year and more environments.

CONCLUSIONS

In Zimbabwe, the more recent maize varieties showed a consistent improvement over older cultivars in grain yield this study. The apparent yearly rate of yield increase due to genetic improvement (b values) was positive in all environments tested and characterized by high coefficients of determination. The absolute rate of yield increase due to genetic improvement was higher in high yielding environments. Physiological traits such as ASI, EPP and SEN were some of the key traits that were associated with genetic gain in Zimbabwean maize varieties over 100 years under different environments but especially under stress conditions.

CHAPTER VII

TRENDS IN GENETIC DIVERSITY

INTRODUCTION

Analyzing changes over time in genetic diversity of major crops is important for understanding the impact of plant breeding on crop genetic diversity and setting up of baseline indicators for the genetic diversity and conservation of genetic resources. However, in southern Africa such information is lacking for most crops, particularly the main food crops such as maize and sorghum.

There have been many reports recently on the impact of plant breeding on crop genetic diversity especially in developing countries. Some researchers, e.g. Donini et al. (2000), reported a reduction in wheat genetic diversity accompanying plant improvement between 1934 and 1965. There study was based on SSR and AFLP-based analysis of 55 UK wheat varieties and they concluded that plant breeding had resulted in qualitative rather than quantitative changes in the diversity of this crop. Base on an SSR analysis of 96 Canadian oat cultivars released from 1886 to 2001, Fu et al. (2003) observed a significant decrease in allele diversity at specific loci after the 1970s and associated this change with plant breeding practices. In another SSR-based study of 559 wheat varieties released and grown in France from 1800 to 2000, Roussel et al. (2004) detected a significant decrease in allelic diversity at the end of the 1960s. However, these changes in diversity could be partly be explained by the sample size of the accessions examined, the use of different molecular markers, and geographical origin of the accessions. Contrasting results were reported by other authors. For example, Reif et al. (2005b) concluded that genetic diversity of bread wheat has actually increased from 1990 to 1997 based on a comprehensive SSR characterization of wheat accession originating from many different parts of the world.

During the last 100 years, various players in the maize breeding sector in Zimbabwe have had a tremendous impact on maize improvement in the country and in neighboring countries such as Zambia and Malawi. Maize was introduced as OPVs on a large scale in Zimbabwe by European settlers about 100 year ago. The main source of

these large-scale introductions was white dent materials with large kernels sourced from the USA. From historical records (McCann, 2005), varieties such as Hickory King, Horsetooth, Iowa Silver Mine, etc formed the initial sources of maize germplasm for varietal improvement in the country. From these original introductions, farmers and breeders formed the first locally improved OPVs either by selecting the best adapted plants from these introductions or by crossing two or more OPVs to form varietal crosses. Examples of the resultant improvement were a new version of Hickory King adapted to southern Africa, and varieties such as Salisbury White (SW), Southern Cross (SC) and Natal Potchestroom Pearl (NPP) (Weinamann, 1972).

Hybrid maize breeding started after 1930 in Zimbabwe following news of the success of maize hybrids in the USA. Naturally the first source populations for developing inbred lines were the currently grown OPVs (Mashingaidze, 1994). The first hybrids released were double-crosses but hybrids were did not become popular with farmers until the release in 1960 of the world's first commercially successful single cross hybrid, SR52 (McCann, 2005). Since then, many types of hybrids including varietal hybrids, three-way hybrids, modified single cross and modified three way hybrids have been released by the government breeding program and several seed companies, and grown by both smallholder and commercial farmers in different parts of the country. Today, over 90% of the maize area is sown to hybrids or recycled hybrid maize seed in the country. With the arrival of CIMMYT in Zimbabwe in 1985, newer and more abiotic stress tolerant OPVs have been released to cater for smallholder farmers located in areas marginal to maize production the country. Zimbabwe's maize germplasm is therefore exceptionally suitable for investigation whether breeding has reduced genetic diversity in maize in a detrimental manner. Examining genetic diversity of maize over time would enhance the understanding of maize introduction to Africa and the change from traditional landraces to modern hybrid and OPVs. Over 70 years of scientific maize breeding requires molecular analyses that incorporate representative samples of ancestral varieties, obsolete OPVs, traditional landraces and their progenitors, modern hybrids and improved OPVs.

The main goal of this section of the study was to monitor the temporal trends in genetic diversity over the past 100 years among maize cultivars with the largest number

of hectares in Zimbabwe. Specific objectives were: (i) to characterize the allelic diversity of a set of maize varieties representing different eras of breeding in Zimbabwe from 1900 to 2004; (ii) to assess changes in allelic diversity in Zimbabwean maize varieties over time; (iii) to investigate how much of the original genetic diversity present in ancestral OPVs introduced from the USA about 100 years has been captured in the elite maize hybrids and OPVs released and grown in Zimbabwe for the last 100 years.

LITERATURE REVIEW

Genetic Erosion and Its Consequences

More than 30 yr have passed since in a landmark study, the National Research Council (1972) alerted the scientific community and the public about the dangers of restricting crop improvement to a narrow collection of germplasm. In the USA, fears were raised by an epidemic of corn leaf blight that struck the U.S. corn crop in 1970. The epidemic resulted from genetic uniformity in T male-sterile cytoplasm, in which a mutant form of *Bipolaris maydis* (Nisikado & Miyake) Shoethat maker (*Helminthosporium maydis* Nisikado & Miseed yake) found a welcome home. In that same year, Jack Harlan applied the term "genetic erosion" (Zeven, 1998) to describe what he viewed as a diminishing global stock of "landraces," or traditional forms of cultivated crop plants still grown in parts of the developing world. By referring to the stock of crop germplasm as resource economists refer to a nonrenewable natural resource, he drew attention to the economic value associated with rare alleles or unique gene complexes that may be found in such landraces.

A popular hypothesis is that an extended period of plant breeding and intensive selection have further reduced genetic diversity among cultivars, narrowing the germplasm base available for future breeding advances (Tanksley and McCouch, 1997). Cultivation of germplasm with a narrow genetic base entails a risk due to genetic vulnerability. This risk is that mutations in pest populations or changes in environmental conditions may bring about stresses that the cultivar could not cope with and, therefore, could lead to severe crop losses. This risk was brought sharply into focus in 1970 with the outbreak of the southern corn leaf blight (Anonymous 1972). The first signs that

germplasm with a narrow genetic base might also lead to disasters in wheat came from several severe epidemics of shoot fly (*Atherigona* spp.) and karnal bunt (*Tilletia indica*) in India in the 1970s (Dalrymple 1986). Nevertheless, plant breeding does not inevitably lead to a loss of genetic diversity. Reduction in diversity caused by intensive selection can be counterbalanced by introgression of novel germplasm.

Genetic erosion became synonymous with the displacement of landraces by modern cultivars. In 1970, Frankel called for urgent collection expeditions to fore- stall "the loss of ancient patterns of diversity in the Vavilovian centers," since modern cultivars contain "a minimum of genetic variation" and "in many instances have a narrow genetic base" (Frankel, 1970). Harlan asserted that the "destruction of genetic resources is caused primarily by the very success of modern plant breeding programs" (Frankel, 1970).

Genetic Diversity Changes Over Time

With the advent of the first maize hybrids around the world, maize cultivation has undergone a complete change. Many landraces and OPVs with adaptation to certain geographical areas have been replaced by a limited number of hybrids bred from a large genetic basis. Currently, the predominant maize hybrids marketed in the world involve a restricted number of key inbred lines. Therefore, genetic diversity of those cultivars is almost certainly limited, in comparison to the large genetic diversity available in genebanks (Gay, 1984). In the past, the threat of genetic erosion led to a significant interest in the assessment of genetic diversity in germplasm collections and a huge number of studies on various crops. American breeders were already concerned by the genetic diversity among their maize hybrids after the Southern corn leaf blight of 1970 (Williams and Hallauer, 2000). Maize breeders want to be assured that the genetic base of their cultivars has not become too narrow to face unexpected environmental stresses. Until now, numerous studies of maize genetic diversity have been carried out to analyze mainly populations from the Americas (Warburton et al., 2002) and Europe (Dubreuil and Charcosset, 1998). On the contrary, fewer investigations have been done on African maize germplasm (e.g. Beyene, 2005 on Ethiopian Highland maize landraces), and at the moment none have been done for southern African maize populations.

Few studies have documented an increase in crop genetic diversity over time. An example is the study of Reif et al. (2005) who studied 253 CIMMYT or CIMMYT-related modern wheat cultivars, landraces, and *Triticum tauschii* accessions, using 90 simple sequence repeat (SSR) markers dispersed across the wheat genome. Wheat's genetic diversity was narrowed from 1950 to 1989, but was enhanced from 1990 to 1997 indicating that breeders averted the narrowing of the wheat germplasm base and subsequently increased the genetic diversity through the introgression of novel materials. Moreover, since national programs in developing countries cross CIMMYT lines with their own materials before releasing them, the genetic diversity in their cultivars is at least as great as that present among CIMMYT lines. More surprisingly, Maccaferri et al. (2003) demonstrated that the level of genetic diversity present in modern varieties of durum wheat was increasing over time. In addition, as reported by Donini et al. (2000) working on UK wheat, no significant narrowing of genetic diversity was detected among winter wheat varieties cultivated between 1934 and 1994. In an analysis of 75 Nordic spring wheat cultivars released from 1901 to 1993, Christiansen et al. (2002) found an increase in genetic diversity from 1901 to 1940, followed by a decrease from 1940 to 1960, and a second increase again from 1960 onwards.

In contrast, Fu et al. (2003) detected a significant decrease in allele diversity at specific loci in 96 Canadian wheat cultivars released and grown from 1886 to 2001 and linked these changes to breeding practices. From a more comprehensive study of 559 wheat cultivars spanning released and grown in France from 1800 to 2000, Roussel et al. (2004) clearly demonstrated a significant decrease in allelic diversity at the end of the 1960s. SSR markers were used in these two studies. Results from a study of 133 maize varieties grown and released in France for the last five decades showed that the genetic diversity has been reduced by about 10% in the maize cultivars bred before 1976 compared to those bred after 1985 (Le Clerc et al., 2005). According to the authors, the very low differentiation observed among the maize cultivars of the last two decades should alert French maize breeders to enlarge genetic basis in their variety breeding programs. Similar results were presented by Manifesto et al. (2001) working on 105 Argentinean wheat cultivars released between 1932 and 1995.

In summary, the findings from the above studies appear to provide inconsistent information for understanding the impact of plant breeding on the genetic diversity of wheat cultivars. Thus, further effort is warranted to assess the diversity changes in existing gene pools of cultivated plants.

Maize Breeding in Zimbabwe

Scientific maize breeding in Zimbabwe began in 1920 with the goal to shift from OPVs to modern hybrids (Weinamann, 1972). By 1932, this breeding effort had produced some double cross hybrids, SR1, SR11 (Mashingaidze, 1994). Initially, high yielding parental lines were developed by selfing mostly the southern African derivatives of the USA-introduced OPVs such as HK, (Weinamann, 1972). Inbred lines from the different OPVs such as Salisbury White and Southern Cross were then crossed to form the first heterotic groups. Therefore, it can be conjectured that (i) a bottleneck occurred in the original Zimbabwean gene pool during the transition from OPVs to hybrids, and (ii) OPVs, which did not serve as germplasm source for the original maize inbred contain untapped allelic variation useful for future breeding progress. Detailed information about a reduction in genetic diversity could help to emphasize the importance of identifying germplasm sources for broadening the elite breeding pools.

In the second phase of hybrid breeding, new lines were primarily developed by recycling of breeding lines, i.e., from crosses among elite inbreds within heterotic groups. Continuous breeding efforts generated the high-yielding and productive single cross, SR52 released in 1960. SR52 dominated the southern African maize production for than five decades (McCann, 2005). Because SR52 was late maturing required high inputs many farmers located in semi-marginal areas with sandy soils could not successfully use this hybrid. As a result, in the early 1970s, early maturing three-way hybrids such as R200, R201 and R215 were developed and released in the country. With the devastating maize streak and mottle virus appearing in the farming system from the 1980s, breeding was directed more toward selection for MSV resistance, which resulted in several high-yielding cultivars with resistance, such as SC709 and SC715 by 1998 (Bourdillon et al., 2002).

After Zimbabwe's independence in 1980, there was shift towards breeding for stability under smallholder farmer conditions (Mashingaidze, 1994). Since then the national breeding program has produced several highly successful cultivars, such ZS206, ZS233, ZS225 (Mashingaidze, 1994). Following the liberalization of the seed sector in Zimbabwe, new players such as Pioneer, Pannar, Monsanto and others, and many new varieties were released by these companies. In the mid 1980s, an additional maize breeding effort was made with the arrival of CIMMYT, which resulted in the generation of several abiotic stress tolerant OPVs such as ZM421, ZM521, ZM621 in 2000. Over the last two decades, selection has been aimed more at the improvement of productivity, resistance to biotic and abiotic stresses, and end-use quality such as increased grain protein.

In many breeding programs, outstanding elite lines were shared as parents of different commercial hybrids and coupled with intensive selection, this is expected to result in a reduced genetic diversity in the breeding pools and even more seriously in the varieties cultivated by farmers. Thus, the risk of genetic erosion does not only depend on plant breeding practices but also on the system that delivers the final products of plant breeding to the market. In Zimbabwe, this includes the regulations to register new varieties and the marketing of registered varieties. Statutory testing of new varieties is required to register them on the national lists (UPOV, 2002). Afterwards, their acceptance by farmers depends on the amount and quality of the marketing effort of breeding companies but also on further series of voluntarily recommended lists based on regional trials. Consequently, only a few of the registered varieties are grown on a large scale. Monitoring the genetic diversity available to farmers is important, because plant breeding practices, the registration procedures, and the marketing of new varieties could have caused a potential genetic erosion and, consequently, a potential increased genetic vulnerability of cultivated varieties. Snap-shots of the diversity present in maize breeding programs were reported (e.g., Messmer et al., 1992). In addition, the temporal trend of genetic diversity was investigated for a single US breeding program (Duvick et al., 2004) as well as for important public US lines (Lu and Bernardo, 2001). However, no information is available on the temporal trends in genetic diversity of important Central European maize varieties cultivated by farmers.

Early maize breeding efforts in Zimbabwe were largely carried out at research stations of Department of Agriculture Zimbabwe and in agricultural colleges (e.g. Gwebi). Currently, there are ten companies and institutions across Zimbabwe with about 30 breeders devoted to maize improvement in ten different market classes, each with unique end-use suitability parameters. To date, breeding programs have developed and released over 100 maize cultivars, most of which have had significant impacts on the economy of the country (Vivek and Banziger, 2005). In spite of impressive achievements in grain yield (Chapter VI), biotic and abiotic stress tolerance and resistance, there is concern about the narrowing of the maize gene pool because selection has been based on a limited number of OPVs over the century of breeding, with a few introgressions here and there. Up to now, no comprehensive study of the genetic diversity in Zimbabwean maize cultivars has ever been conducted. Microsatellite (or simple sequence repeat; SSR) markers have proven to be important tools in maize genetics and germplasm research. In recent years, these markers have also been applied to analyze diversity changes in maize germplasm released over time (Donini et al., 2000; Le Clerc et al., 2005).

MATERIALS AND METHODS

Plant Materials

In this study, five temporal breeding periods were defined to reflect the major breeding efforts of maize in Zimbabwe. Period I comprised entries that were released and grown before 1920 and is comprised mostly of the original open pollinated maize populations introduced into Zimbabwe by white settlers. Period II comprised OPVs developed directly from selections or crosses between the OPV from Period I and these represent the first varieties developed in the country. Period III comprised the first modern hybrids developed in the country mainly from the lines extracted from the period II OPVs. Period IV comprised the first post-independence hybrids grown in Zimbabwe, while periods V and VI comprised entries developed and widely grown during the last 15 years. A set of 48 maize accessions were chosen (Table 6.1). The number of accession in ranged from 4-8 for each temporal group depending on the availability of seeds from the chosen suppliers. Seeds were obtained from the Crop Breeding Institute (CBI) of the

Zimbabwe Agriculture Research and Extension Service (AREX), Seedco's Rattray Arnold Research Station, and CIMMYT, Harare. The accessions were selected on the basis of meeting, as much as possible, a combination of time or release, wide cultivation by farmers, and availability of seeds. Unfortunately, because the specific organization of the institutes involved in breeding tasks in the country was limited and the fact that a lot of passport data were unavailable (breeders' origin, pedigrees), it was not always possible to fully satisfy the three criteria. However, the fact that some important varieties could no longer be found is an historical reality and cannot be considered to be a factor that carries a risk of biased sampling.

DNA Isolation and Fragment Analysis

For DNA isolation and SSR fragment analyses, the procedures and PCR conditions described in detail in Chapter III were followed. The same twenty-three SSR markers used in Chapter III were used for the 38 maize accessions. Details of the SSRs are given in Chapter IV.

Statistical Analysis

Data were transformed to a binary code based on the presence (1) or absence (0) of each allele with columns representing the variety and rows the different SSR markers. The resulting matrix was analyzed with NTSYS-pc version 2.1 software package (Exeter Software, Setauket, NY) to estimate the genetic similarities among all pairs of varieties using Dice's coefficient of similarity as follows:

$$\mathbf{GS_{ij} = 2\ N_{ij}/(N_i + N_j)}$$

where N_{ij} is the number of alleles (scored bands) shared by lines i and j, and N_i and N_j are the total number of scored bands in lines i and j, respectively. Standard statistics for characterizing genetic variability were computed for each locus and for the whole set of accessions: the total number of alleles, the number of unique alleles, and PIC were calculated. Accessions were then grouped according to their period of registration in order to calculate the allelic richness, number of alleles and GS within and between

different breeding periods. A dendrogram on the basis of similarity matrix was generated following unweighted pair group method with arithmetic average (UPGMA) (Sneath and Sokal, 1973).

RESULTS AND DISCUSSION

Overall Diversity

Standard statistics are summarized in Table 6.2. A total of 155 alleles were detected from the 23 amplified loci for the 38 varieties. The number of alleles per marker ranged from two to twelve, with an average value of 6.74. Primer pair umc1332 detected 12 alleles (the largest number), and umc2250 only 2 alleles, the smallest number. The microsatellite markers used showed different levels of gene diversity: the genetic similarity index of Dice ranged from 0.346 to 0.863, with an average of 0.613 for all populations. The highest genetic similarity (0.91) was observed between the AREX hybrids, ZS240 and Z2332 leading to speculation that these hybrids may actually have parents that are very closely related. Genetic similarities between original ancestral maize population and their descendant OPVs were also high, clear evidence supporting the historical data presented in literature, e.g. the Dice similarity coefficient between the Hickory King introduced from the USA and locally selected OPVs such as Salisbury White, Southern Cross and Natal Potschestroom Pearl were all around 0.8.

The mean number of alleles detected on the 38 cultivars (6.7) was higher than the one obtained by Lu and Bernardo (2004) on 40 US maize inbreds (4.9) and by Senior et al. (1998) on 94 US inbreds (5.2) but was consistent with the one reported by Matsuoka et al. (2002) on 101 inbreds (6.9). Variations in mean number of alleles can be due to the predominant type of SSRs used in a study. Dinucleotide repeats in general display a higher number of alleles than tri- and tetranucleotide repeats. However, in this study, only one dinucleotide repeat primer, phi112 was used, and it can thus be stated that allelic diversity was high for the varieties genotyped.

Temporal Variation -Allelic Richness

Figure 7.1 shows the data for allelic variation among the temporal groups. Since the number of varieties genotyped per ancestral group was slightly different, the average allelic richness per group is presented and best describes the allelic variation for this sample of varieties.

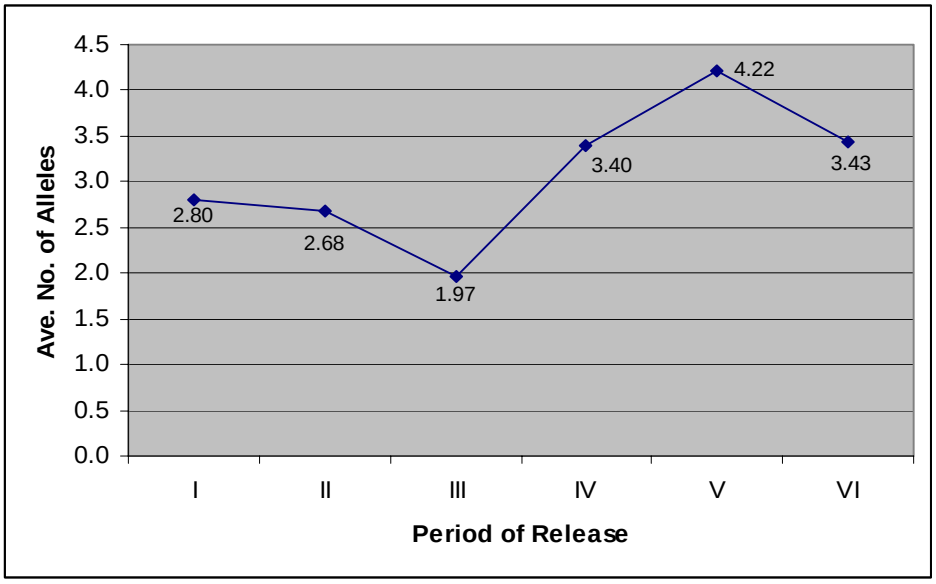

Fig. 7.1. Allelic richness per breeding period for 38 Zimbabwean maize varieties genotyped with 23 SSR markers.

The allelic richness of periods I and II were close and higher compared with that of period III. This reflects changes that occurred in maize breeding in Zimbabwe. The most important reduction in allelic richness was observed between historical open pollinated cultivars of period II and modern hybrid cultivars of period III. Period III marks the beginning of hybrids maize release and cultivation in the country. With the advent of such hybrids as SR52, R200, and R201, OPVs such as Hickory King, Salisbury White and Southern Cross were progressively replaced. Consequently, the maize varieties became more and more homogeneous. However, as suggested by Allard (1996), the reduction in allelic diversity may not only due to plant breeding, but also largely to the elimination of deleterious alleles by selection rather than erosion.

From period IV allelic richness started to increase peaking at period V at 4.22 alleles per locus. Periods V and VI only differ in the type of varieties involved; period V

entries were exclusively hybrids produced by one seed company Seedco, while period VI entries were OPVs from CIMMYT. Thus, for this discussion, these periods will be considered as one since the varieties were released and grown in the same time period. A probable reason for the increase in allelic diversity from period IV onwards may be the introduction of new genetic material into the breeding programs after the country obtained independence in 1980. Seed companies and the national program now had access to a wider range of sources of germplasm than before. In addition, CIMMYT set up a breeding program in the country in 1985, and with it came new sources of alleles that were readily available to all maize breeders in the country.

The change in percentage of unique alleles (those that occur once) in the 38 varieties over time is shown in Figure 7.2. The largest increase occurred from period II to period III, a 50% increase in unique alleles. This was then followed by a drastic decline from period III to period IV, close to 50% decrease. Thereafter, the percentage of unique alleles has somewhat remained constant over the last three periods.

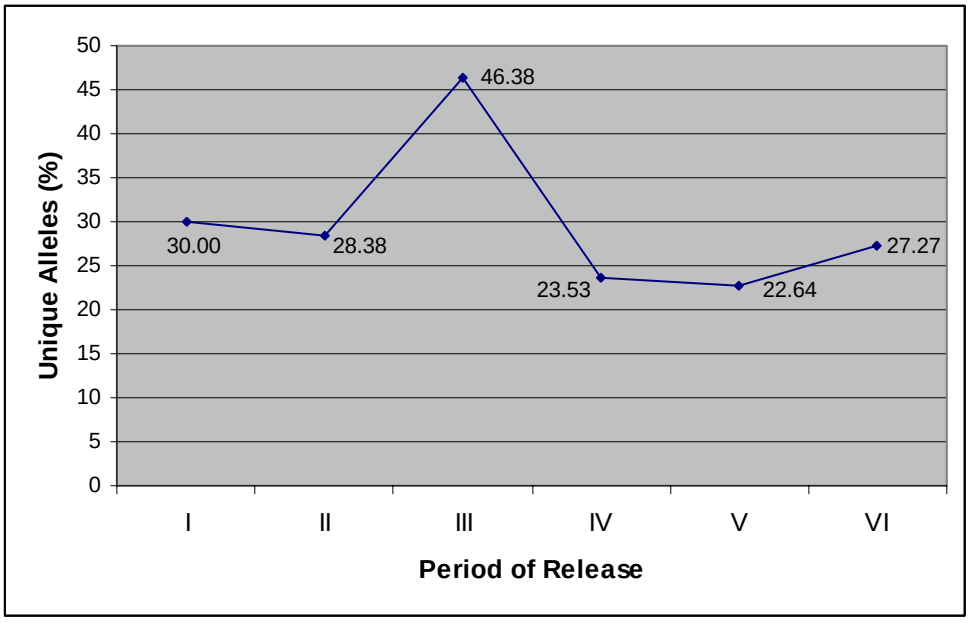

Fig. 7.2. Changes in unique alleles over time for 38 Zimbabwean maize varieties genotyped with 23 SSR markers.

No major difference was detected in terms of unique alleles between periods I and II. The original varieties introduced in period I from the USA were the direct parents of

varieties released in period II, i.e. the first OPVs developed and released in Zimbabwe (period II) were either direct selections of OPVs introduced from USA, or were crosses among those USA OPVs followed by selection for local adaptation (MaCann, 2005), thus very little differences could be expected in terms of unique alleles between these two groups. The high peak in period III signifies the beginning of hybrid maize in Zimbabwe. Breeder became aware of the phenomena of heterosis between widely differing inbred parents in hybrid combinations. As a result new germplasm was introduced to from other areas of the world to complement the USA introductions, thus many new unique alleles came with these new introductions. In fact, as detailed in the maize breeding history of Zimbabwe (McCann, 2005), new introductions of materials were made for Mexico and South Africa. Therefore, we state that no drastic reduction in genetic diversity has occurred during the last few decades. Moreover, the advent of new alleles in modern cultivars gives evidence of the introduction of new genetic material in breeding programs.

In order to identify qualitative variations in allelic diversity over time, the number of alleles lost or introduced was also analyzed in each temporal group. When examining the number of alleles specific to period I versus periods V and VI, 38 alleles (24.5%) of the total number of alleles observed in period I was not recovered in periods V and IV, whereas 37 new alleles (23.87%) were detected in cultivars of periods V and VI. About 51.61% of the alleles detected in period I were recovered in periods V and VI. The advent of new alleles in modern cultivars gives evidence of the introduction of new genetic material in breeding programs. As explained previously, the main forms of cultivars for the last two periods have been hybrids (more than 80%), whereas before 1960, populations were predominant. A comparison of the alleles specific to Seedco hybrids versus CIMMYT OPVs (period V vs. period VI) shows that relatively few alleles (<20%) are common across the two groups, probably reflecting differences in origin of source germplasm.

Gene Diversity Changes Over Time

Figure 7.3 shows an erratic evolution of Dice's genetic similarity over time: there was a low average gene diversity in period I, then a strong increase in this parameter

from period I to period II, a decline in period III and finally a tendency for average gene diversity to stabilize for the other periods. One striking fact was that late cultivars of the periods III to VI, were more closely related than the early (period II) and very early (period I) varieties. This may suggest that the genetic basis employed for the selection of recent varieties may be narrower than that used for early ones.

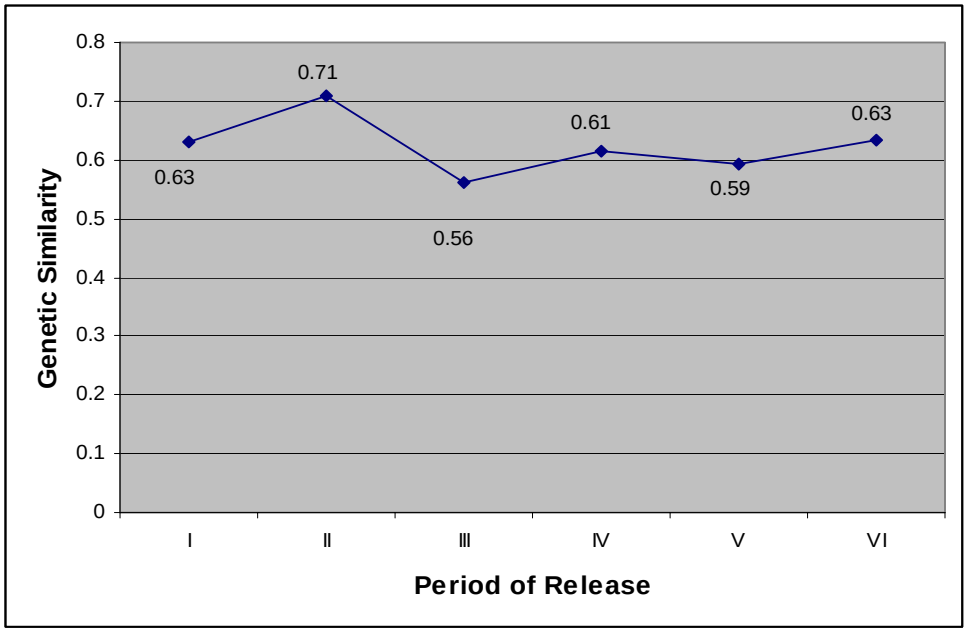

Fig. 7.3. Average genetic similarities per release period for 38 Zimbabwean maize varieties genotyped with 23 SSR markers.

This conclusion is slightly different from those of Donini et al. (2000) and Koebner et al. (2003) on UK wheat and barley, respectively: both authors concluded that plant breeding has resulted, over time, in a qualitative, rather than a quantitative, shift in the diversity of the respective species studied. These investigators probably arrived at this conclusion because their respective studies involved fewer polymorphic markers and only a small number of accessions originating from the same homogenous geographical area and, consequently, from related breeding programs.

Cluster Analysis

The dendrogram ensuing from the cluster analysis, based on the genetic distance matrix, discriminated all of the cultivars tested, and at least four major groups were distinguished. Group C1 consisted mostly of a mixture of ancestral varieties introduced from USA and their immediate descendants.

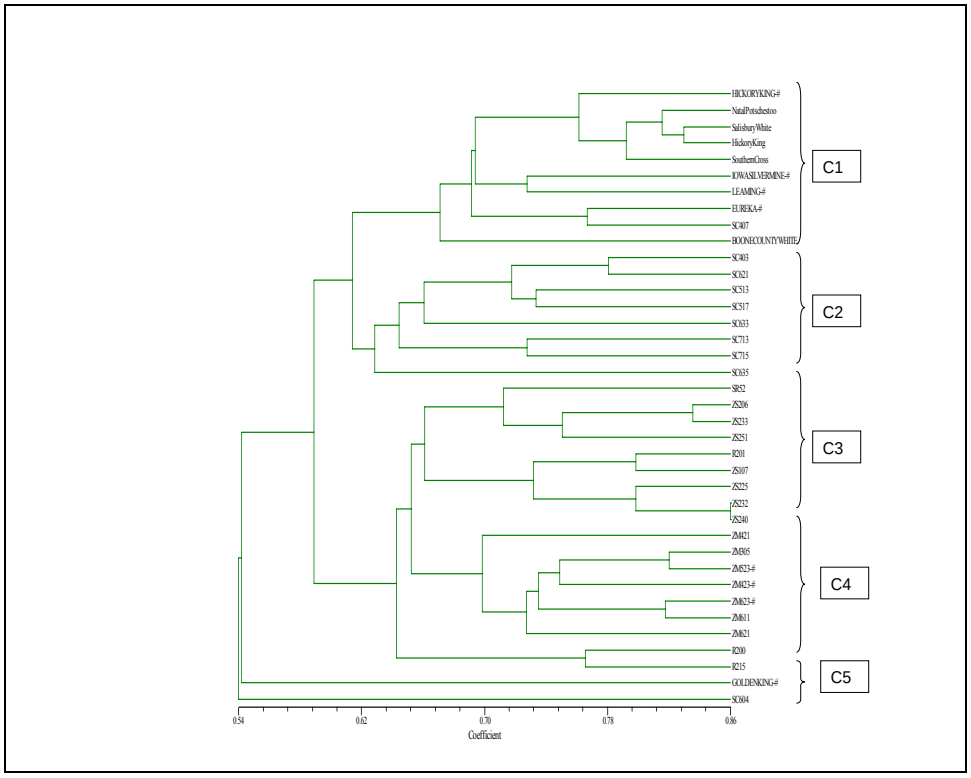

Fig. 7.4. UPGMA dendrogram of Dice's genetic similarities between 38 maize accessions from Zimbabwe.

The fact that there were close relationships between the USA types and the first OPVs developed in Zimbabwe confirms, on a molecular basis, the fact that the original OPVs introduced from USA into Zimbabwe were the progenitors of these first Zimbabwean OPVs and perhaps the other hybrids later developed early in the 20th century. Group C2 consisted mostly of varieties developed by Seedco, while group C3 was composed mostly of the varieties developed by AREX both in the pre and post-

independence period. Group C4 was dominated mainly by CIMMYT developed varieties. The clustering pattern also shows that Seedco developed varieties are perhaps the most closely related with ancestral varieties and pre-hybrids Zimbabwean OPVs, followed by AREX developed hybrids. CIMMYT developed varieties appear were perhaps the most distantly related group from the ancestral varieties introduced from USA. This is probably because of the fact that CIMMYT mainly used germplasm imported form Mexico and South America. A smaller group, (C5), does not have a clear pattern like those four groups reported previously. It consists of just two varieties, R201 and Golden King.

The hierarchical tree also separates the Zimbabwean varieties into groups that also reflect the release period of the genotyped varieties. This last result is exactly the same as that observed by Roussel et al. (2004) on French wheat cultivars released and grown over a long time period. The observation that period IV varieties are separated from the period III varieties may indicate a recent change in breeding goals. As indicated earlier, modern maize breeders in Zimbabwe now focus, not only on yield potential under optimum growing conditions, but also on abiotic stress tolerance especially drought and low N conditions. This fact may be responsible for such specific clustering.

CONCLUSIONS

A total of 38 maize varieties of historical and economic importance during the last 100 years in Zimbabwe were examined with 23 SSR markers. The results obtained from allelic richness, genetic diversity, differentiation parameters, and cluster analysis are consistent. In general genetic diversity in Zimbabwean maize has neither significantly decreased nor increased over time. The advent of new alleles in modern cultivars gives evidence of the introduction of new genetic material in breeding programs. However, a great proportion of the genetic diversity is conserved in each period. The genetic diversity maintained in the historical cultivars is not exactly the same as the one conserved in the modern cultivars. Nevertheless, temporal changes are more qualitative than quantitative. It is important to mention that the present analyzed genetic diversity was only expected to be representative of the major varieties produced by two main breeding programs, that of

AREX and Seedco and not representative of the entire maize diversity available in from other breeding program such as new private seed companies that have been established during the two decades. At the national level, the effects of breeding practices and agriculture policies led to a slight but significant evolution in maize diversity, which is not only qualitative but also quantitative. The distinct contrast in diversity observed between and early and recent varieties might be the result of the combined effects of both adaptation by the initial germplasm to different environmental conditions and specific breeding practices. The main consequence of these results concerns present-day Zimbabwean maize breeder, who should increase their exchange of genetic resources in order to expand genetic material and improve new cultivars. Otherwise, the present evolution could be prejudicial to the long-term maintenance of maize genetic diversity in Zimbabwe. CIMMYT germplam contains numerous unique alleles that were absent in the elite maize breeding pools of both AREX and Seedco. Consequently, CIMMYT developed OPVs could present useful sources for broadening the genetic base of elite maize breeding germplasm in Zimbabwe.

CHAPTER VIII

SUMMARY AND CONCLUSIONS

STUDY I: COLLECTION AND DOCUMENTATION OF LANDRACES

A total of 267 distinct traditional landraces of maize were collected from smallholder farmers in Zimbabwe, Zambia and Malawi for conservation and further studies. The objective of conserving this collection is to preserve the diversity in the maize landraces before much loss as farmers are shifting to planting modern hybrids. This study presents the first report of the range of variability of maize landraces and traditional varieties in the three countries and provides important baseline data for future diversity assessments. The main factors that favor continued cultivation of the landraces within farming systems include the heterogeneity in the physical, economic, and cultural contexts of local smallholder agriculture. The results from this survey also showed that landrace diversity could be associated with diversity for abiotic stress tolerance since the geographic areas where the landraces were collected are frequently subjected to different types and intensities of drought, and low soil fertility – the major abiotic stresses occurring in the three countries.

STUDY II: MORPHO-PHENOLOGICAL DIVERSITY

This study confirmed that traditional maize populations and improved varieties from southern Africa display a large range of phenotypic variation for morphological, agronomic and phonological traits, and that within the landraces exist, a vast amount of variation, much of which is not present in improved varieties. Three groups with different agro-morphological traits could be identified: (i) local landraces characterized by low yields, late flowering and intermediate seed size, (ii) Hickory King types consisting of tall and late flowering plants, with few kernel rows per ear and large seed size, and (iii) improved varieties and some landraces including “creolized” types consisting of short and early flowering plants, with more kernel rows per ear and higher yields. From the three clusters identified from this study, the upper 25% of the landraces representing those with the highest average diversity of the clusters were used to form a subset for

molecular analysis and further field evaluation under drought, low soil fertility and acid soils alongside improved and ancestral varieties.

STUDY III: SSR DIVERSITY

SSR analysis of the core sample (formed from the previous study) revealed high allelic richness, high frequencies of rare and unique alleles, and high gene diversity values both within populations and mega-environments, confirming the broad genetic diversity and the relationships of the maize landraces and improved varieties sampled as expected from historical information, phenotypic diversity and pedigree data where known. The data from this study also showed that the genetic diversity introduced from the original gene pool from USA about 100 years ago is still found in both the descendant landraces and commercially-bred varieties and that the plant material grown for a long time in southern Africa and maintained by local farmers through yearly selection has resulted in many different landraces identifiable by different names and with different traits.

STUDY IV: AGRONOMIC PERFORMANCE

The results of agronomic evaluations of the core sample set (formed in previous sections) under low soil nitrogen, low soil pH, drought stress, random stress, and under optimum growing conditions showed that there exists considerable genetic variation in agronomic traits under different abiotic stresses commonly encountered in southern Africa. Differences among the accessions, type of accessions (landraces, ancestors, early OPVs and improved) were significant for most of the studied. Significant genotypes x environment interactions were also present. Generally improved varieties outperformed landraces under all environments, but there were notable exceptions with many landraces yielding as much as improved varieties. Landraces were more stable than improved varieties across test environments, but improved varieties were more responsive to favorable growing conditions. From a selection index conducted, the most promising landraces for pre-breeding and further investigation were identified. Finally, when the agronomic performance of the maize varieties under different environments was used to

create dendrograms, the materials clustered into groups based on respective performance under specific environments. The clustering pattern was different from SSR markers, but in general the genotypes groupings were consistent across the two methods of measuring diversity.

STUDY V: GENETIC YIELD IMPROVEMENT

The objectives of this study was to determine the genetic gain in Zimbabwean maize varieties released and widely grown since 1900 up to 2004, and (ii) to identify physiological traits associated with genetic gains in grain yield of these varieties in Zimbabwe. The results showed that the more recent maize varieties showed a consistent improvement over older cultivars in grain yield this study. The apparent yearly rate of yield increase due to genetic improvement (b values) was positive in all environments tested and characterized by high coefficients of determination. The absolute rate of yield increase due to genetic improvement was higher in high yielding environments. Physiological traits such as anthesis to silking interval (ASI), number of ears per plant (EPP) and rate of leaf senescence (SEN) were some of the key traits that were associated with genetic gain in Zimbabwean maize varieties over 100 years under different environments but especially under stress conditions.

STUDY VI: TRENDS IN GENETIC DIVERSITY

The goal of this study was to monitor the temporal trends in SSR diversity over the past 100 years among maize cultivars with the largest number of hectares in Zimbabwe. The results obtained from allelic richness, genetic diversity, differentiation parameters, and cluster analysis are consistent. In general genetic diversity in Zimbabwean maize has neither significantly decreased nor increased over time. The advent of new alleles in modern cultivars gives evidence of the introduction of new genetic material in breeding programs. However, a great proportion of the genetic diversity is conserved in each period. The genetic diversity maintained in the historical cultivars is not exactly the same as the one conserved in the modern cultivars. Nevertheless, temporal changes are more qualitative than quantitative. It is important to

mention that the present analyzed genetic diversity was only expected to be representative of the major varieties produced by two main breeding programs, that of AREX and Seedco and not representative of the entire maize diversity available in from other breeding program such as new private seed companies that have been established during the two decades. At the national level, the effects of breeding practices and agriculture policies led to a slight but significant evolution in maize diversity, which is not only qualitative but also quantitative. The distinct contrast in diversity observed between and early and recent varieties might be the result of the combined effects of both adaptation by the initial germplasm to different environmental conditions and specific breeding practices. The main consequence of these results concerns present-day Zimbabwean maize breeder, who should increase their exchange of genetic resources in order to expand genetic material and improve new cultivars. Otherwise, the present evolution could be prejudicial to the long-term maintenance of maize genetic diversity in Zimbabwe. CIMMYT germplam contains numerous unique alleles that were absent in the elite maize breeding pools of both AREX and Seedco. Consequently, CIMMYT developed OPVs could present useful sources for broadening the genetic base of elite maize breeding germplasm in Zimbabwe.

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