GENETIC CHARACTERIZATION, ASSOCIATION MAPPING AND EVALUATION OF HETEROTIC PATTERNS OF TROPICAL MAIZE (*Zea mays* L.) INBRED LINES UNDER DROUGHT AND NON-DROUGHT CONDITIONS

By

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DECLARATION

I hereby declare that except for references to works of other researchers, which have been duly cited, this work is my original research and that neither part nor whole has been presented elsewhere for the award of a degree.

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ABSTRACT

Genetic diversity provides the capacity for plants to meet changing environments and farmers' preferences, and to establish heterotic patterns, essential for an efficient and sustainable hybrid breeding program. Group interviews and formal surveys were conducted in North and South-Sudan agro-ecological zones of Burkina Faso to assess farmers' preferences and evaluate their perception of constraints to maize production. Fifty-nine INERA and forty one elite CIMMYT, IITA, U.S and European maize inbred lines were characterized using 1151 SNP markers to determine the level of genetic diversity in the INERA inbred lines and examine the genetic difference between local and exotic germplasms. A subset of 54 diverse maize inbred lines were selected to generate 100 hybrids using a line by tester mating design. Inbred parents and hybrids were evaluated under both well-watered and water-stressed conditions in two years (2011 and 2012) and under well-watered and high plant density condition in one year (2013), to examine genetic variation in grain yield and drought tolerance traits, and the heterotic patterns of the inbred lines.

The participatory rural appraisal (PRA) indicated that the farmers' maize "ideotype" includes traits such as yield potential, earliness, tolerance drought and *Striga*. Model-based population structure and principal component analyses of the 100 lines genotyped revealed the presence of 5 groups consistent, to some extent, with the origin of the germplasm. There was genetic diversity among INERA inbred lines which were less closely related and showed a low level of heterozygosity. These lines were divided into 3 majors groups and a mixed group consistent with the source population of the lines. Pairwise comparisons between local and exotic germplasms showed that the temperate lines and some IITA lines were differentiated from INERA lines. There appeared to be substantial levels of genetic variation between local and exotic germplasms as revealed by missing and unique alleles. Association mapping with phenotypic traits showed that 22 SNPs were consistently associated with at least one drought-related trait in individual and across environments. The contribution of GCA to total genetic variance was higher than SCA for grain yield and secondary traits under drought and non-drought conditions. However, the contribution of SCA for number of ears per plant (EPP) and leaf rolling (LR) was predominant under drought. The average relative contribution of SCA, indicative of non-additive gene effects, to total genetic variance for grain yield under drought accounted for 28% and 35% under non-drought conditions. The average high parent heterosis of CIMMYT (testers) x INERA (lines) and IITA (testers) x INERA (lines) were 299 % and 200 %, respectively. Based on SCA effects and testcross mean grain yield across environments, the two CIMMYT testers were able to separate 54 % of CIMMYT and INERA white lines into two opposite heterotic groups while the two IITA testers assigned all the IITA and INERA yellow lines. Based on this classification, 2 complementary heterotic groups were formed with VL0511298 and T02058 as testers for white lines and TZEI17 and ELN45111 as testers for yellow lines.

Four white hybrids, VL0511298xT020-58, VL0511298xVL057967, VL0511298xVL05616, VL0511298 x VL054794, and three yellow hybrids, TZEI17 x ELN45111, TZEI17 x TZEI146 and TZEI10 x ELN39427, with an average grain yield of 3 t/ha, high parent heterosis between 200 to 500 % and with favorable drought tolerance and stability indices, were identified as high yielding, and drought tolerant stable hybrids with a consistent heterotic response .

The results from this study will be useful to breeders in designing inbred-hybrid breeding program, association mapping studies and marker assisted breeding.

DEDICATION

In memory of my father, Seydou

and my sisters, Korotimi & Zouwairatou

To my mother, K. Alizeta

To my sisters and brothers

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AATF :	African Agricultural Technology Foundation
AMMI :	Additive Main effect and Multiplicative Interactions
ANOVA :	Analysis of variance
CAADP :	Comprehensive Africa Agriculture Development Programme
CIMMYT :	International Maize and Wheat Improvement Centre
DGSPA :	Direction General des Statistiques et des Prévisions Agricoles
DTMA :	Drought Tolerant Maize for Africa
ECOWAS :	Economic Community of West African States
FAOSTAT :	Food and Agriculture Organization of United Nations, Statistics Department
FBML :	Farako-Bâ Maize Line
GCA :	General Combining Ability
GDP :	Gross Domestic Product
GIS :	Geographic Information System
GLM :	Generalized Linear Model
GMO :	Genetically Modified Organism
IITA :	International Institute of Tropical Agriculture
INERA :	Institut de l'Environnement et de Recherches Agricoles
INRA :	Institut National de la Recherche Agronomique
IPCA :	Interaction Principal Component Analysis
IRAD :	Institut de Recherche Agricole pour le Développement
IRAT :	Institut de Recherche en Agriculture Tropicale
KASPar :	KBiosciences Competitive Allele-Specific PCR SNP genotyping system

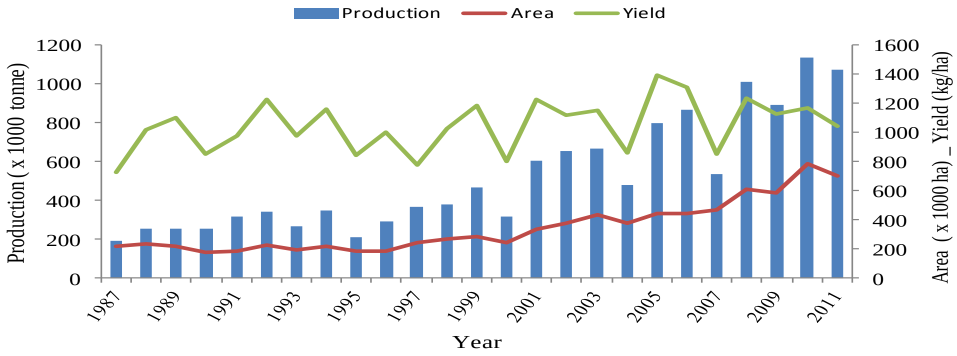
LD :	Linkage Disequilibrium
MAF :	Minor Allele Frequency
MAHRH :	Ministère de l'Agriculture, de l'hydraulique et des Ressources Halieutiques
METs :	Multi-Environment Trials
MSEs :	Managed drought Stress Environments
NARS :	National Agricultural Research System
NEPAD :	New Partnership for Africa's Development
NGO :	Non-Government Organization`
OPVs :	Open Pollinated Varieties
PRA:	Participatory Rural Appraisal
QTL :	Quantitative Trait Loci
RFLP :	Restriction fragment length polymorphism
SAFGRAD :	Semi-Arid Food Grain Research and Development
SAS :	Statistical Analysis Software
SCA :	Specific Combining Ability
SNP :	Single Nucleotide Polymorphism
SSA :	Sub-Saharan Africa
SSR :	Simple Sequence Repeats
TASSEL :	Trait Analysis by aSSociation, Evolution and Linkage
WEMA :	Water Efficient Maize for Africa
WS :	Water-stressed
WW :	Well-watered
WWHPD :	Well-watered high plant density

CHAPTER ONE

1.0 Introduction

Maize or corn (*Zea mays* L.) is a cereal crop that is grown widely throughout the world in a range of agro-ecological environments. It is third behind wheat and rice in terms of total area of world production. About 20 % of the area harvested is in sub-Saharan Africa (SSA) (FAOSTAT, 2012), where maize is widely grown for direct human consumption.

In Burkina Faso, maize is an important staple food, currently it is third in total national cereal production. The importance of maize has increased lately replacing traditional cereal crops, particularly sorghum and millet, and it has also become a major source of cash for farmers. Five to17.2% of the average annual growth rate of cereal crops from 1995 to 2003 was estimated to be due to maize (MAHRH, 2008). Trends in maize production indicate a steady growth rate. However, maize grain yield has not increased uniformly over 25 years (1987 -2011) (Figure 1.1). It fluctuated between 725 to 1396 kg/ha with a variation of 17% indicating that the increased production is largely due to the expansion of the area. The fluctuation of maize yield and the low rate of increase can be explained by factors related to production and yield potential of the maize varieties grown.



Source : (DGPSA, 2012)

Figure 1. 1. Evolution of maize production, yield and area harvested in Burkina Faso over a period of 25 years (1987-2011).

Maize production in Burkina Faso, is constrained by many factors including stresses, both biotic and abiotic, and socio-economic factors. Drought is a major cause of food insecurity for many households and has been estimated to cause annual maize yield loss of 24 million tons in the developing world (Edmeades *et al*., 1995). Most of the maize varieties grown in Burkina Faso are open pollinated varieties (OPVs) and this accounts for the slow yield increase. In United States (U.S), for instance, before the introduction of hybrids in the 1930s, there was almost no increase in maize yield per acre over 70 years but the transition from open-pollinated varieties to hybrid maize resulted in significant yield increase. The adoption of hybrids was surprisingly rapid. In Iowa, for instance, the proportion of the area under hybrid maize grew from less than 10% in 1935 to well over 90% 4 years later (Crow, 1998). Although external factors including increasing use of fertilizer, herbicide and better equipment have contributed to this increase, 60% or more of the increased performance was due to genetics (Russell, 1974; Duvick, 1977). Therefore, development and commercialization of drought tolerant, high-yielding and stable hybrid maize is essential for Burkina Faso to boost yield and meet the increasing demand for maize products. A program called "Operation Bondofa", initiated in 2011 by the government to adress the food crisis due to erratic rainfall of that year , has contributed to the increased demand for hybrid maize. In this program, farmers were encouraged and supported to grow Bondofa (varietal hybrid maize) during the off-season on irrigated land. Cognizant of this fact, INERA maize breeding program has been devoted to developing and assembling germplasm from various sources. A large numbers of inbred lines were generated from different adapted and adopted by farmers OPVs. Inbred lines are classified mainly based on their performance *per se* and source population. There is no clear established heterotic grouping, this has important implications in a comprehensive breeding program where outputs of different products are the ultimate objective. It assists exploitation of heterosis in an efficient and consistent manner through isolation of complementary lines; assertion of diversity and creation of new heterotic groups for hybrid program enhancement and the development of different products (Russell, 1991; Cheres *et al*., 2000 cited by Legesse *et al*., 2011). There is a gap in knowledge on heterotic groupings of lines developed in Burkina maize breeding program and the heterotic relationships between these lines and exotic germplasm especially from CIMMYT and IITA which have been regularly introduced into the country. Studies showed that the heterotic response of inbred lines in combination could change from environment to environment (Menkir *et al*., 2003; Agbaje *et al*., 2008), which implies the need to evaluate the combining ability of inbred lines in drought and non-drought environments in order to identify lines with consistent heterotic responses to unpredictable rainfall conditions. In addition, genetic study, which is one of the integral components and driving forces of success in plant breeding, as it estimates the mode of gene action, the genetic association and inheritance of different traits is needed in order to identify useful inbred lines and design a suitable breeding strategy.

Although the conventional method of breeding remains the best method for plant improvement, molecular markers are more accurate and faster in identifying differences among breeding materials and detecting genes or QTL associated with tolerance to many stresses including drought. Despite the importance of molecular markers for maize improvement worldwide, very little information is available in a Burkina maize breeding context. In addition, the potential of tropical maize inbred lines for association mapping, which does not require a biparental mapping population and an extensive and complicated process of fine mapping (Setter *et al*., 2011), has been demonstrated (Warburton *et al*., 2008; Wen *et al*., 2011). However, few studies have been carried out to identify genetic variation associated with drought-related traits using association analysis tools in sub-Saharan African. The agro-ecological zone where farmers are located has an impacts on their preferences for varieties since the constraints differ from one zone to the other. As the entire of maize grain production in Burkina Faso is used for human consumption, the ability in processing grain influences the preferences of farmers as well as consumers. There is no recent published report on of farmers' preference and constrains to maize production in Burkina Faso.

The overall goal of this research was to develop new hybrid maize varieties which are highly productive under both drought and non-drought conditions, in order to reduce food insecurity in Burkina Faso.

The specific objectives were to :

1. identify maize varieties grown by farmers, farmers' preferences and constraints to maize production.
2. assess the genetic diversity among the maize inbred lines developed at INERA and compare them to temperate elite maize lines and CIMMYT and IITA inbred lines.
3. identify the genetic variations underlying drought tolerance using association analysis
4. determine heritability and the interrelationship between yield and its associated traits
5. determine the mode of gene action for grain yield under drought and non-drought conditions.
6. examine the heterotic relationships among the inbred lines and classify them into heterotic groups
7. identify drought tolerant hybrids with high grain yield and stability.

The present study was undertaken with the following assumptions:

1. Famers have their own ways to identify and select maize varieties, and to recognize the constraints from which a breeding strategy can be formulated.
2. There is sufficient genetic diversity among local maize inbred lines and genetic differences between local and exotic lines which can be exploited to develop new varieties.
3. The genetic variation present in the tropical inbred lines tested can be used to identify genes associated with drought tolerance.
4. Yield and its associated traits of testcross hybrids are related and heritable under drought and non-drought conditions
5. The additive gene action of maize grain yield is more predominant than non-additive gene action under drought and non-drought conditions.
6. Maize inbred lines including local and tropical exotic lines shows combining ability in testcrosses and the testers can separate the lines into heterotic groups
7. High yielding drought tolerant hybrids can be selected from testcross hybrids.

CHAPTER TWO

2.0 Literature Review

This chapter provides a context for the research by a) reviewing importance and production of maize in sub-Saharan Africa and showing the major factors hampering production, b) reviewing progress in breeding on drought in terms of methodologies developed and achievements, c) reviewing literature on molecular diversity of maize and the potential for the use of this diversity in association mapping, and d) reviewing theory relevant to maize heterotic patterns, heterosis, combining ability, heritability and genetic associations.

2.1 Maize in sub-Saharan Africa

2.1.1 Importance and production

Maize is the region's principal cereal crop. Farmers harvest some 25 million ha, producing about 35 million tons of maize grain each year,. This accounts for 40% of the region's cereal production. Nine-tenths of the grain goes directly for human consumption (Bänziger and Diallo, 2001). The grains are rich in vitamins A, C and E, carbohydrates, and essential minerals, and contain 9% protein. They are also rich in dietary fiber and calories which are good sources of energy. Of the 23 countries in the world with the highest per capita consumption of maize as food, 16 are in sub-Saharan Africa. Maize provides 50% of the calories in diets in southern Africa, 30% in Eastern Africa, and about 15% in West and Central Africa (Bänziger and Diallo, 2001). The Economic Community of West African States (ECOWAS) which covers 17% of the area of the continent is one of the economic communities most populous in Africa with a regional population totaling about 300 million in 2008, of which 57% live in rural areas. Maize production in this region has progressively increased in the last 5 years with an important contribution of Nigeria about 50%. When Nigeria is excluded the average production over the last five years indicate that Burkina Faso is third top country in maize production after Ghana and Mali (FAOSTAT, 2008-2012). Maize production in Burkina Faso is increasingly important (Figure 1.1) and the local consumption guarantees a domestic market.

2.1.2 Constraints to maize production

Maize yield in Africa was estimated at 2.1 t/ha in 2012 which was more than two times less than the global (4.9 t/ha), European (5.1 t/ha) and Asian (5.0 t/ha) yields and three times less than yields in America (6.3 t/ha) (FAOSTAT, 2012). Several factors explain the low yield of maize in Sub-Saharan Africa including abiotic and biotic constraints, and socio-economic factors.

In a given season, crops can be damaged or ruined by one or several important constraints. **Drought:** which can be due to insufficient or poorly distributed rainfall reduces maize output across the region. Severe droughts periodically cause deficits in cereal production leading to food crisis particularly in the Sahelian countries. In 2004-2005 season the deficit in cereal production in Burkina Faso was estimated at 436 013 tonnes (PANA, 2007). Drought affects all the agro-ecological zones even in the high-yield potential regions where crops may be affected by mid- and late-season droughts. Drought affects maize yields by restricting season length and through unpredictable stress that can occur at any time during the cropping cycle (Edmeades *et al*., 1994a). Drought occurring at flowering leads to greater yield losses than when it occurs at other developmental stages (Grant *et al*., 1989). In addition, by flowering time farmers can no longer adjust management practices, such as fertilizer application, weed control and replanting (Myers, 1985). Water deficit lasting only one or two days during tasselling or pollination may cause as much as 22% reduction in yield (Hall *et al.*, 1981). Less than 7% of agricultural area in sub-

Saharan Africa is irrigated. Only 24 000 hectars out of 233 500 potential area in Burkina Faso are irrigated.

Soil nutrient deficiency: Nutrient depletion and soil fertility decline are widespread in smallholder farming systems, as many farmers cannot afford or do not have access to organic and inorganic fertilizers. Kumwenda *et al*. (1995 and 1996) reviewed the causes and extent of soil fertility decline in the maize-based cropping systems of southern Africa and concluded that the challenge is so large that farmers will need to combine gains from improved germplasm with improvements in their management of soil fertility. Soil fertility decline is generally linked with increased population pressure, especially in areas with fragile ecosystems, such as the Sahel. Lack of phosphorus, low levels of nitrogen and poor organic matter content have been identified as the main problems affecting soils in Burkina Faso (Elshout *et al*., 2001).

The parasitic weed: *Striga hermonthica* is one of the most important biotic factors constraining the grain yield of maize in the savanna zones of sub-Saharan Africa . Yield losses attributable to this obligate hemiparasite may range from 10 to 100% depending on the genotype grown, climatic conditions, soil fertility status and levels of infestation (Lagoke 1998; Kroschel 1999 cited by Akaogu *et al*., 2012). Increasing soil fertility is the best measure against this pest, but often out-of-reach to resource-poor farmers (Bänziger and Diallo, 2001).

Socioeconomic factors: Among the socioeconomic and political limitations on food production are public policies and investments that are biased against poor farmers and consumers, women and less-favored areas; inadequate infrastructure; inequitable access to land and other critical resources; poorly functioning and poorly integrated markets; and lack of access to credit and technical assistance; the few facilities and services provided are most of the time primarily made available for men than women farmers although they produce about 75% of the domestically grown food in Sub-Saharan Africa (Marc, 2005). Less accessibility to education and to labor, fertilizer and other inputs than men do, have resulted in lower yields of women's plots of about 20– 30% (Marc, 2005). In Burkina Faso, a study showed that total household agricultural output could increase by 10– 20% if currently used inputs were reallocated from men's to women's plots (Quisumbing, 2003). Seed recycling is another cmmon pratice of African small-scale farmers in which a portion of the harvested crop is set aside for the following year's planting (Moola, 2010), resulting in the reduction of the yield potential of the crop. Understanding this societal dynamics is important for plant breeders in designing a breeding program. In Burkina Faso there are few reports on farmers' preferences and constraints to maize production, therefore an update of such information would be favorable for the national maize breeding program.

2.2. Breeding research on drought

2.2.1 Breeding methods/strategies.

Plant breeders have amassed considerable knowledge on the improvement of drought tolerance in maize (Boyer, 1996). Most of the maize breeding strategies that are now available for improving drought tolerance were developed and refined over a considerable period (Heisey and Edmeades, 1999). A number of reviews have addressed the options for developing drought tolerant maize including conventional and molecular breeding approaches (Beck *et al.*, 1997; Vasal *et al.*, 1997; Bruce *et al.*, 2001; Tuberosa *et al.*, 2007; Araus *et al.*, 2008).

2.2.1.1 Conventional breeding approach

Drought escape : One of the first principles of crop improvement is to fit the variety to the growing season (Ludlow and Muchow, 1990). An early maturing cultivar will escape terminal drought stress more often than its later flowering counterpart. But, early maturing cultivars lack yield potential for good years when, contrary to expectations, rains are plentiful.

Indirect strategies: elite progenies are tested in multi-environment trials (METs) that represent a random selection of the potential variation in drought stress that a cultivar may encounter in its target environment (Rosielle and Hamblin 1981). It is believed that genes for drought tolerance are present in elite high-yielding genotype, even after the number of genotypes has been narrowed to the few evaluated under random stress, and that selection under optimum growing conditions can also increase performance in sub-optimum conditions (Russell, 1974). However drought in most maize target population of environments (TPE) is a randomly occurring event that gives rise to genotype x year interactions that are hard to interpret (Edmeades, 2013).

Managed drought stress environments (MSEs): In MSEs, the selection is done under different levels of managed drought stress to ensure that performance under unfavorable conditions does not compromise performance when water is available (Edmeades *et al.* 1997a; Boyer 1996). The approach has been well described and successfully employed by CIMMYT to improved germplam for drought tolerance (Bolaños and Edmeades, 1996; Edmeades *et al.;* 2000; 2006; 2008; Bänziger *et al.,* 2000; 2006); and has been adopted by National research programs and leading commercial companies (Edmeades, 2013).

Alternative strategy : High plant density and inbreeding, in which male and female flowering must coincide on the same plant, is a strategy for maize improvement aimed at "general" stress tolerance (Vasal *et al.,* 1997). This practical method of exposing maize to an abiotic stress has been particularly exploited in temperate maize (Troyer, 1983; Duvick, 1997), as the mechanisms of tolerance to drought and to high plant density appear to be related (Dow *et al.,* 1984).

2.2.1.2 Value of Secondary Traits and Selection criteria

Genetic improvement can be achieved through direct selection for a primary trait (such as grain yield) in a target environment (Ceccarelli and Grando, 1996) and indirectly, for a secondary trait that must be putatively related to higher yield potential and/or to improved behavior of the crop when grown in a stressful environment (Araus *et al.*, 2008). For a secondary trait to be useful in a program, it must meet several requirements (Bänziger *et al*., 2000; Araus *et al*., 2002). It must be genetically variable and genetically associated with grain yield in the target environment, that is to say, the relationship with yield must be causal not casual. However if the correlation between the trait and yield is near 1.0 then it is usually simpler and cheaper to measure yield alone (Edmeades *et al*.,1997b). It must be highly heritable. If the product of square root of heritability of the secondary trait and the genetic correlation between the secondary trait and grain yield exceeds the square root of the heritability of grain yield, then faster progress for grain yield can be made by selecting for the secondary trait alone (Falconer, 1981). It must be cheaper or faster to measure than grain yield. According to Edmeades *et al*. (1997b) secondary traits should be preferably observed at or before flowering, since this allows the breeder to eliminate drought-susceptible lines from his crossing nursery and reduces the time required to complete a selection cycle and provides an estimate of yield potential before final harvest. For instance in the situation where grain yield under drought is strongly related to barrenness, ASI may be a better measure of yield potential under drought than yield itself, if yield is affected by disease, lodging, cold, etc., during grain filling since anthesis-silking interval (ASI) is a good indicator of barrenness (Bolaños and Edmeades, 1993).

Over the years, CIMMYT's research program has determined the value of several secondary traits (Edmeades *et al*., 1994b 1997b) including anthesis-silking interval (ASI), leaf and stem elongation rate under drought versus well-watered conditions (RLE), canopy temperature (CT), leaf rolling and erectness scores (LRS; LES), leaf chlorophyll concentration (CHL), staygreen score (LDS), tassel size (TS), leaf osmotic concentration (OSM) and lodging (LOD).

Relationships between yield and yield components (weight kernel^{-1}, kernels ear^{-1} (WPK, KPE, EPP)) have also been determined. Correlations between some of these traits and grain yield under drought are: large (0.5-0.9) for EPP, KPE, PH, TS, and ASI; low (0.1-0.4) for WPK, CT, LDS, CHL and OSM; and <0.1 for LOD, LRS, RLE, LES and TBN. Reduced ASI under stress is a simple external indicator of changes in ear growth rate at flowering. The process of favoring ear growth can be accelerated by actively selecting for small tassel size, reduced ASI, and to some degree, for shorter plants, as adjuncts to selection for increased grain yield. Improved radiation interception by leaves which do not roll should also favor increased productivity under drought, while erect leaves are believed to be more water-use-efficient than horizontal leaves. Where resources for collecting these types of data are limited, the measurement of shelled grain yield, anthesis date and ears per plant are considered the minimum dataset required for efficient selection. Many proposed selection criteria for crop drought resistance including drought susceptibility index (DSI) (Fischer and Maurer, 1978) the mean productivity (MP) and stress tolerance (TOL) (Rosielle and Hamblin 1981); drought resistance index (DI) and stress tolerance index (STI) Fernandez (1992), emphasize on sole grain yield and do not include the secondary traits. CIMMYT has established a selection index method based on yield and secondary traits (Bänzinger *et al*., 2000) which was improved by Hao *et al*. (2011a).

2.2.1.3 Molecular breeding approaches

Molecular breeding approach include Marker assisted selection (MAS) and transgenic selection methods. The approach of MAS has been used to develop accurate estimates of QTL effects within a relatively narrow reference population and the estimates has been used in the application of MAS, after assessing that the effects of the desirable QTL alleles will not vary after a number of selection cycles. MAS based on a strategy combining both anthesis-silking interval (ASI) and grain yield QTL identified under drought is thought to be a powerful tool to improve drought tolerance in tropical maize inbred lines (Bänziger *et al*., 2000). QTLs mapping studies in tropical maize to identify QTL associated with drought tolerance traits especially ASI and grain yield have been reported (Messmer *et al*., 2009; Almeida *et al*., 2013; Semagn *et al*., 2013). In addition to MAS, important research on development of transgenic drought tolerance maize is ongoing in sub-Saharan Africa in collaboration with international private compagnies such as Monsato (Edmeades, 2013). Basically, the strategy has employed the use of key genes which have been observed to induce abiotic stress in other plants or microorganisms, to generate transgenic maize.

2.2.2 Achievements in improving drought tolerance in maize

Breeding research carried out and numerous results achieved in sub-saharan Africa have been possible thanks to donors (Rockefeller, Bill and Melinda Gate foundation...), politics (NEPAD-CAADP), international centres (CIMMYT, IITA), established projects (DTMA, WEMA, AATF) private sector (Monsanto, national seed companies) and national research programs and their partnership. Substantial progress has been made in drought tolerance breeding in subtropical and tropical maize. Based on conventional breeding, CIMMYT has selected for drought tolerance in tropical maize using rainfree tropical locations and irrigation to create its managed drought stress environments (MSEs) during the past 38 years and this is summarized (Edmeades, 2013) as follows: recurrent selection using an index of traits was conducted for 2-9 cycles in six improved tropical populations, normally evaluated under well- watered and two distinct drought stress regimes in Mexico. Evaluations in multilocation trials under optimal, water stress (70% yield reduction) and low N environments (31% yield reduction) showed consistent gains averaging 164 and 99 kg/ha/cycle under drought and optimal conditions. Barrenness and ASI were also reduced under drought. Gains in drought tolerance from selection under MSEs were significantly greater than those from selection under multilocation testing alone. Gains transferred well to other environments such as moderately low N (Bänziger *et al.*, 1999), and showed only moderate levels of G x E. This suggests that selection resulted in a constitutive change in floral behavior and reproductive efficiency through changes in biomass partitioning to and within the ear (Edmeades, 2008).

The Drought Tolerant Maize for Africa (DTMA) Project has produced conventionally selected hybrids as well as several OPVs that have been released in 13 African countries: Angola, Benin, Ethiopia, Kenya, Malawi, Mali, Mozambique, Nigeria, Tanzania, Uganda, Zambia, Zimbabwe and Ghana. The seed production of these varieties in 2011/12 season was close to 30,000 metric tons (MT) and more than 62% of the seed produced was hybrids and nearly 38% Open Pollinated Varieties (OPVs), depending on the country (DTMA, 2012). WEMA has recommended 16 drought tolerant hybrids in Kenya, 8 in Uganda and 5 in Tanzania for the National Performance Trials; There are several drought tolerant hybrids already released by collaborating private seed companies in the regions that contain all or some lines derived from CIMMYT's long involvement in the region (Edmeades, 2013).

Drought escape strategies were also employed with success in development of early maturing varieties such as the R200 series of hybrids in Zimbabwe (Mashingaidze, 1994) and the Katumani Composite-derived varieties in Kenya (Mugo and Njoroge, 1997). Several early maturing drought tolerant OPVs employed in West Africa developed in frame the international Institute of Tropical Agriculture - Semi-Arid Food Grain Research and Development (IITA-SAFGRAD) project (Badu-Apraku *et al.*, 1997).

A number of QTLs in maize have been identified for drought response but no superior varieties have been commercialized using this powerful approach (Xoconostle-Cazarcs *et al.*, 2011).

However reviews on molecular breeding approach have outlined the distinct advantages of utilizing MAS to improve quantitative traits (Turberosa *et al.,* 2007; Edmeades, 2013). Besides, it is noted that DTMA and WEMA have created a unique database comprising 5000 lines from 27 inter-related populations that provides an excellent basis for genetic studies on drought tolerance in tropical germplasm, and will facilitate a flow of improved drought tolerant hybrids for several years to come (Edmeades, 2013). The generation of transgenic maize, based on the expression of selective markers will positively impact the market of maize production (Xoconostle-Cazarcs *et al*., 2011). Monsanto remains the leader in transgenic research for drought tolerance in maize, and is scheduled to commence commercial sales of transgenic Droughtgard™ hybrids in 2013. South Africa will be the first African country to benefit from transgenic drought tolerance. The time line for release could be as early as 2015 and 2017 in Uganda and perhaps Kenya (Edmeades, 2013). Transgenic drought tolerance is likely to encounter adoption challenges, the lack of an established regulatory framework is one of the current constraint in developing countries (Edmeades, 2008). Generally, this review showed that there are important ongoing research on drought using both conventional and molecular breeding approaches in sub-Saharan Africa. But there is very little published information for research conducted in Burkina Faso.

Burkina Faso's national research centre has established a breeding program for selecting for drought tolerance selection based on conventional breeding approach using managed drought conditions and secondary traits. In addition, Sauvaire and Sanou (1986) identifed three major zones of adaptation based on rainfall patterns for early maturing (zone with rainfall below to 900 mm), intermediate maturing (zone with rainfall above to 900 mm) and, high yielding (irrigated area) varieties. A range of extra-early to intermediate maturing drought tolerant OPVs have been released for commercial cultivation. Nevertheless, no drought tolerant hybrid has been released. All the hybrids released so far are intermediate to late maturing and susceptible to drought. There is a gap in breeding research on drought tolerant hybrid development as well as early maturing hybrids in Burkina Faso. However, the breeding program has maize inbred lines which could be used as sources of drought tolerance genes to improve the available germplasm. On the other side, the transgenic drought tolerance maize in Burkina Faso is not planned for now even though the country has a well established regulatory framework for GMO.

2.3 Molecular genetic diversity in maize

Maize is a model genetic organism with immense genetic diversity. Although it was first domesticated in Mexico, maize landraces are widely found across the continents (Prasanna, 2012). The diversity of maize inbred lines has been critical for diverse genetic studies including the development of linkage maps, molecular evolution and developmental genetics. A set of diverse maize inbreds has been employed to perform the first phenotype-genotype association analysis in a plant species (Thornsberry *et al*., 2001) and to estimate linkage disequilibrium in maize (Remington *et al.,* 2001; Yan *et al.,* 2009). A few studies have been presented for marker based diversity investigation focusing on diverse mixes of germplasm (Liu *et al*., 2003; Reif *et al*., 2004; Lu *et al.,* 2009; Yan *et al.,* 2009). Using SSR markers at 94 loci, Liu *et al*. (2003) studied a well represented collection of 260 lines including public lines of importance to temperate breeding and many important tropical and subtropical lines and showed that this diversity is not distributed randomly among the lines, but rather diversity is structured into five groups along breeding groups (stiff-stalk vs non-stiff-stalk) and ecological (temperate vs tropical) axes. Similarly, the amount of diversity is not same among groups, and that tropical subtropical inbreds possess greater diversity than their temperate counterparts. Yan *et al*. (2009), using SNP markers, reported similar results of higher genetic diversity in the tropical germplasm pool than that in the temperate pool. Molecular characterization of (394) CIMMYT, (282) Chinese and (94) Brazilian lines investigated with SNP markers showed that the elite lines from these diverse breeding pools have been developed with only limited utilization of genetic diversity existing in the center of origin (Lu *et al.*, 2009). Molecular genetic diversity was also studied with a specific maize germplasm including U.S. Corn Belt lines (Lu and Bernado, 2001; Gethi, 2002), European temperate lines (Reif *et al*., 2005a), Chinese temperate lines (Xie *et al.*, 2008), and tropical (Reif *et al*., 2003; Xia *et al*., 2004) and subtropical (Xia *et al.,* 2005; Laborda *et al.,* 2005) lines. Many studies on marker based genetic diversity for tropical and subtropical maize representing essentially CIMMYT germplasm using RFLP (Warburton *et al.* 2005) SSR (Xia *et al.,* 2004, 2005;Warburton *et al.,* 2002, 2008) and SNP markers (Wen *et al.,* 2011; Semagn *et al.,* 2012) for diverse purposes including assessment of diversity in maize landraces, open pollinated varieties (OPVs), and inbred lines and the structure of diversity, defining heterotic groups and identifying appropriate testers, and potential utilization of the diversity for association mapping and maize improvement have been conducted. Many results across studies showed that no clear clustering or heterotic patterns could be identified in either lowland tropical maize or subtropical, tropical mid-altitude and highland maize populations. The fact that there is no evidence for distinct population structure within CIMMYT inbred lines may not related to the level of genetic diversity but to breeding history (Lu *et al.*, 2009). This may be explained by the mixed origin of the populations used to extract those lines and the breeding program that initially emphasized developing germplasm with a wide genetic base (for development of OPVs) and only has begun to develop heterotic groups since early 1990s. Very few studies on molecular diversity have been reported for West Africa, especially IITA maize lines. Adetimirin *et al.*

(2008) analysed the diversity among 17 elite tropical inbred parents of hybrids cultivated in West and Central Africa and other lines developed in the sub-region using SSR markers. Most of diversity studies using markers with IITA germplasm (generally with a limited number less than 50) are associated with field evaluation for combining ability estimates and heterotic grouping (Menkir *et al.,* 2003; Dhliwayo *et al.,* 2009; Akaogu *et al.,* 2012; Badu-Apraku *et al.,* 2013). Menkir *et al*. (2003) found considerable genetic diversity among the inbred lines bred in Cameroon Institute of Agronomic Research (IRAD) and IITA using both AFLP and SSR markers. It has been concluded that maize breeding programmes isolated in space can play a significant role in generating divergent inbred lines implying the potential for maximizing heterosis in hybrids and synthetic varieties.

The success of a crop improvement program depends on the genetic variability and the efficiency with which this genetic variability can be manipulated. Maize breeding programs in many Africa countries obtained their genetic materials from international centres such as CIMMYT and IITA, however some of them developed their own materials. There is a gap in knowledge on the genetic variability existing in the local germplasm and their relationship with exotic germplasm. In Burkina Faso important inbred lines were developed from composite varieties with drought tolerance created by mixing different varieties from different geographical origins. It is hypothesized that the inbred lines derived from the composites would possesse high genetic variability and would be genetically distinct from other tropical and exotic temperate inbred lines. Knowledge of this diversity would be invaluable for inbred-hybrid breeding programs.

2.4 Association Mapping

Association mapping, a high-resolution method for mapping quantitative trait loci based on linkage disequilibrium, has been proposed to dissect the genetic basis of quantitative traits in plants. There are two commonly tools used for dissecting complex traits: linkage analysis and association mapping traits (Risch and Merikangas, 1996). According to Yu and Buckler (2006), linkage analysis exploits the shared inheritance of functional polymorphisms and adjacent markers within families or pedigrees of known ancestry. Linkage analysis in plants has been typically conducted with experimental populations that are derived from a bi-parental cross. Although based on the same fundamental principles of genetic recombination as linkage analysis, association mapping examines this shared inheritance for a collection of individuals often with unobserved ancestry. As the unobserved ancestry can extend thousands of generations, the shared inheritance will only persist for adjacent loci after many generations of recombination. Essentially, association mapping exploits historical and evolutionary recombination at the population level (Thornsberry *et al.*, 2001). Compared to linkage based QTL detection, association mapping addresses the relationship between genetic marker polymorphism and phenotypic variation within a population composed of diverse genotypes, ideally non-related by pedigree to each other (Truntzler *et al.*, 2012) . In order to find associations, it is necessary to have sufficient linkage disequilibrium (LD) between QTL and polymorphisms that are analyzed. Depending of the LD extent (Rafalski, 2002), two approaches are used in association studies: (i) candidate gene approach, where the objective is to validate the effect of a specific gene and ideally identify within this gene the polymorphisms underlying trait variation, or (ii) genome-wide association (GWA) approach, using markers whose function is not known *a priori* to cover the whole genome (Salvi *et al.*, 2007).

Association mapping has been useful for dissecting candidate genes associated with flowering time in maize (Thornberry *et al.*, 2001) sweetness (Whitt *et al.*, 2002), kernel composition and starch properties (Wilson *et al.*, 2004). In an association mapping study with 1229 SNPs in 540 candidate genes, Setter *et al*. (2011) identified a SNP in the maize homologue of the Arabidopsis MADS-box gene, PISTILLATA, which was significantly associated with phaseic acid in ears of well-watered plants, and a SNP in pyruvate dehydrogenase kinase, a key regulator of carbon flux in respiration, that was associated with silk sugar concentration. A SNP in an aldehyde oxidase gene was significantly associated with ABA levels in silks of water-stressed plants. In order to identify the functional genetic variations underlying drought tolerance by association analysis, Hao *et al*. (2011b) evaluated 95 maize inbred lines under well-watered and water-stressed environments. They found that 29 SNPs were associated with at least two phenotypic traits in one or more environments, which were related to drought-tolerant or drought-responsive genes; implying that these drought-tolerant SNPs could be converted into functional markers and then used for maize improvement by marker-assisted selection.

Although studies showed the potential of tropical maize for association studies (Warburton *et al*., 2008; Wen *et al*., 2011), few studies have been carried out to identify drought-related traits through association mapping in sub-Saharan Africa.

2.5 Heterosis, Heterotic patterns and Combining ability

2.5.1 Heterosis

Heterosis is the phenomenon whereby the progeny of particular crosses between inbred lines have enhanced agronomic performance relative to both parents (Ruth *et al.,* 2005). Various hypotheses explaining the phenomenon of heterosis have been reviewed (Reif *et al.,* 2005b). Although many hypotheses have been suggested, the genetical, physiological, and biochemical basis of heterosis still remains largely unexplained. The dominance (or complementation) hypothesis, the overdominance and the epistasis hypothesis are commonly invoked. The dominance hypothesis states that deleterious alleles at different loci in the two homozygous parental genomes are complemented in the heterozygous F1 hybrid while the overdominance hypothesis states that the improved performance of an F1 hybrid relative to its inbred parents is a consequence of favorable allelic interactions at heterozygous loci that outperform either homozygous state (Crow, 1997). The epistasis hypothesis attributes heterosis to epistatic interactions between non-allelic genes (Richey, 1942; Schnell and Cockerham, 1992 cited by Reif *et al.*, 2005b). When two or more genes affect the same phenotype, they are non-allelic. In general, based on parents used, two major types of estimation of heterosis are reported in the literature: 1) Mid-parent or average heterosis (MPH), which is the increased vigor of the F1 over the mean of two parents; 2) High-parent or better parent heterosis (HPH), which is the increased vigor of the F1 over the better parent (Sinha and Khana, 1975). For HPH, the term heterobeltiosis has been proposed to describe the increased performance of the hybrid over the better parent (Fonseca and Patterson, 1968). Heterosis is dependent not only on the parent combinations but also on the effect of environmental conditions and the trait under consideration. Heterosis for grain yield increased with the intensity of drought stress (Betrán *et al.*, 2003a). Welcker *et al*. (2005) reported also that mid-parent heterosis for yield was significantly higher in acid soils (32%) than in non-acid soils (20%). They concluded that the development of variety crosses between acid soil-tolerant populations could be used to increase maize yields in acid-soil cropping systems.

2.5.2 Heterotic patterns

To systematically exploit heterosis in hybrid breeding, the concept of heterotic groups and patterns was suggested. Melchinger and Gumber (1998) defined a heterotic group “as a group of related or unrelated genotypes from the same or different populations, which display similar combining ability and heterotic response when crossed with genotypes from other genetically distinct germplasm groups. By comparison, the term heterotic patterns refers to a specific pair of two heterotic groups, which express high heterosis and consequently high hybrid performance in their crosses. The concept of heterotic patterns includes the subdivision of the germplasm available in a hybrid breeding program into at least two divergent populations, which are improved with inter-population selection methods. Heterotic patterns have a strong impact in crop improvement because they predetermine, to a large extent, the type of germplasm used in a hybrid breeding program over a long period of time (Melchinger and Gumber, 1998). Sprague (1984) concluded that the single most important element of a breeding program is the recognition and utilization of heterotic patterns. This recognition both simplifies and increases the efficiency of all subsequent operations. In Tropical germplasm, several promising heterotic patterns have been described by Wellhausen (1978), Goodman (1985), and Vasal *et al*. (1999). Tuxpeño combines well with Cuban Flint, Coastal Tropical Flint (Caribbean Flint), Tuson, and ETO. Cuban Flint combines well with Tuxpeño, Tuson, Coastal Tropical Flint, and Perla. Coastal Tropical Flint combines well with Tuxpeño, Cuban Flint, and Chandelle. According to Reif *et al*. (2005b), the above mentioned heterotic patterns are seldom employed in tropical and subtropical maize breeding programs. In studies to determine the combining ability and heterotic patterns of tropical inbreds developed at CIMMYT using four line testers, Vasal *et al*. (1992a) identified and formed two divergent tropical heterotic groups (THGA and THGB). Lines showing negative SCA with Tester 1 “Pop 21” (Tuxpeño-1) and positive SCA with Tester 3 “Pop 25” (Blanco Cristalino) were classified under Tropical Heterotic Group “A”. Those showing positive SCA with Tester 1 and negative with Tester 3 were classified under Tropical Heterotic Group “B”. In a similar study in the same year using subtropical CIMMYT maize lines, Vasal *et al*. (1992b), identified and formed two divergent subtropical heterotic groups (STHGA and STHGB). Lines that had negative SCA with Tester 2 (Pop 44) and positive SCA with Tester 4 (Pop 34) were classified under Subtropical Heterotic Group “A” and those showing positive SCA with Tester 2 and negative with Tester 4 were classified under Subtropical Heterotic Group “B”. The hypothesis was that positive SCA effects between inbred lines generally indicate that lines are in opposite heterotic groups and lines in the same heterotic group tended to exhibit negative SCA effects when crossed.

Studies show that the heterotic patterns of inbred lines and populations can change depending on the test environment under which evaluation is made (Gutiérrez-gaitan *et al.*, 1986; Kim and Ajala, 1996; Vasal *et al.,* 1993). The grouping of the inbred lines is not consistent across environments due to genotype x environment interaction. Consequently, determining the stability of heterotic patterns of inbred lines under stress and non-stress conditions would be useful for the development of an efficient hybrid breeding strategy that can cater for the variable growing conditions in West African Countries (Menkir *et al.,* 2003).

There is limited information on the heterotic grouping of maize inbred lines developed in maize breeding program of Burkina Faso.

2.5.3 Combining Ability and Gene action

Combining ability of inbred lines is the ultimate factor determining future usefulness of the lines for hybrids (Hallauer and Miranda, 1988). Sprague and Tatum (1942) introduced the concepts of general combining ability (GCA) and specific combining ability (SCA). They defined GCA as the average performance of a line in hybrid combinations and SCA as those instances in which certain hybrid combinations are either better or poorer than would be expected on the average performance of the parental inbred lines included. They also emphasized that estimates of GCA and SCA are relative to and dependent on the particular set of inbred lines included in the hybrids under test, an important principle that is often forgotten. Therefore any new germplasm introduced in a breeding programme has to be tested for GCA and SCA (Hallauer and Miranda, 1988). Genetic information is obtained by different quantitative genetic methods including line × tester analysis method. This method was suggested by Kempthorne in 1957 and is used to breed both self and cross- pollination plants, as well as estimating favorable parents, crosses, and their general and specific combining ability effects (Kempthorne, 1957). The choice of an efficient breeding program for any character depnds to a large extent on knowledge of the type of gene action involved in the expression of the character. Whereas dominance gene action would favor the production of hybrids, additive gene action indicates that standard selection procedures would be effective in bringing about advantageous changes in character (Edwards *et al.*, 1975). Information on genetic variances, levels of dominance, and the importance of genetic effects have contributed to a better understanding of the gene action involved in the expression of heterosis (Wolf and Hallauer, 1997). The variance due to general combining ability (GCA) is usually considered to be an indicator of the extent of additive type of gene action, whereas specific combining ability (SCA) is taken as the measure of non- additive type of gene actions in heterosis breeding. Gene expressions and genetic variances of some traits such as yield and secondary traits in maize are influenced by stress (Bänziger and Lafitte, 1997 Bolaños and Edmeades, 1996; Ludlow and Muchow, 1990). Mode of inheritance depends on the environment under consideration. Betrán *et al*. (2003b) evaluated a 17 x 17 diallel of white-grained tropical maize inbred lines under optimal, drought and low N stress conditions and found that the type of gene action appeared to be different under drought from that under low N, with additive effects more important under drought and dominance effects more important under low N. The predominance of additive effects conditioning grain yield of tropical and subtropical maize under drought is commonly reported (Derera *et al.*, 2008; Meseka *et al.*, 2013). However, Guei and Wassom (1992) reported that non-additive genetic effects control grain yield under drought. Therefore, understanding of the genetic basis of hybrid performance under this stress is crucial to the design of appropriate breeding strategies.

Genetic information obtained from outside the country might not have direct application, because the genetic materials and environments are likely to be different. In addition, it has been clearly shown that the combining ability estimates depend on genotype and the environment implying that the CIMMYT, IITA and local lines included in this study must be definitely evaluated for their combining ability. Moreover, to make effective use of the local and exotic germplams, information about their heterotic relationships is desirable.

2.6 Heritability and Genetic associations

2.6.1 Heritability

Two types of heritability are defined depending on whether it refers to the genotypic value or breeding value, (Falconer, 1989): Broad sense heritability (ratio of genetic variance to phenotypic variance) expresses the extent to which individual phenotypes are determined by the genotypes. Narrow-sense heritability (ratio of additive variance to phenotypic variance) expresses the extent to which phenotypes are determined by the genes transmitted from the parents. It also expresses the magnitude of genotypic variance in the population, which is mainly responsible for changing the genetic composition of a population through selection. According to Hallauer and Miranda (1988), plant and ear height, and oil content had the highest narrow sense heritability stimates (h^2>70%). The h^2 value for number of ears, kernel weight, ear length and ear diameter ranged from 30 to 50%, while yield and kernel depth showed less than 30% heritability. Drought stress affects the magnitude of the heritability for grain yield and associated traits. In general, heritability for yield is low under drought because the genetic variance for yield decreases more rapidly than the environmental variance among plots with increasing stress, whereas secondary traits whose genetic variance increases under stress or are reduced less than that of yield, have relatively high heritability (Blum, 1988; Edmeades *et al.*, 1989; Ludlow and Muchow, 1990). Using a total of 3509 inbred progenies (S_1 to S_3 level) evaluated in 50 separate yield trials under two or three water regimes, Bolaños and Edmeades (1996) showed that broad-sense heritability for grain yield averaged around 0.6, but fell to values near 0.4 at very low grain yield levels. The heritability of kernels ear^{-1} and weight kernel^{-1} was around 0.60 under well-watered conditions, but also decreased with increasing stress; In contrast, the heritability for ASI and ears plant^{-1} either increased or remained fairly constant with increasing moisture stress and declining yield levels. The heritability for days to anthesis remained fairly constant across all moisture regimes. In a comparison of two maize populations, ZM601 and ZM607, for drought tolerance during flowering, Magorokosho *et al*. (2003) reported similar results for grain yield, ASI and days to anthesis, in contrast to a moderate heritability for EPP (average of 42%) under drought stress conditions. The intensity of drought and genetic variance within each tested population could explain this difference. It should be noted that heritability is a property not only of the character being studied, but also the population being sampled and the environmental circumstances to which individuals have been subjected (Dabholkar, 1992). More variable environmental conditions also reduce the magnitude of heritability while more uniform conditions increase it (Blum, 1988; Rosielle and Hamblin, 1981).

2.6.2 Genetic Association

2.6.2.1 Phenotypic and Genotypic Correlation

Phenotypic correlation involves both genetic and environmental effects. It can be directly observed from measurements of the two characters in a number of individuals in a population

(Hallauer and Miranda, 1988). Genetic correlation is the association of breeding values (i.e., additive genetic variance) of the two characters (Falconer, 1989). Both measure the extent to which the same genes or closely linked genes cause co-variation in two different characters (Hallauer and Miranda, 1988).

The main criteria for stress tolerant trait selection are the association of each trait with grain yield under stress conditions (Bänziger *et al.*, 2000; Araus *et al.*, 2002). Bolaños and Edmeades (1996) and Magorokosho *et al*. (2003) reported that grain yield under drought stress correlated with plant processes related to biomass partitioning at flowering (ASI and EPP), and was weakly associated with traits related to plant water status (leaf rolling, tassel size and leaf angle scores). In addition a strong phenotypic association was reported between harvest index and grain yield (Edmeades *et al.*, 1993; 1999), and between grain yield and grain number m^{-2} under drought (Chapman and Edmeades 1999). Bolaños and Edmeades (1996) found that, in general, phenotypic correlations between grain yield under drought and other traits were a little lower but similar in sign to the mean values of the corresponding genetic correlation, suggesting that environmental correlation for the same traits were relatively small (Falconer, 1981).

2.6.2.2 Path Coefficient Analysis

Path coefficient analysis was first described by Wright (1921, 1934) (cited by Cramer *et al.*, 1999) as a means of determining the influence of independent factors on dependent factors, while also calculating the simple correlation between pairs of independent factors. The path coefficient analysis has been used successfully to clarify interrelation between yield and several other characters of crops such as maize (Kinaci and Kinaci, 2001; Sreckov *et al.*, 2011), sugarcane (Kang *et al.*, 1983), peanut (Sadeghi and Niyaki, 2012) bread wheat (Farshadfar *et al.*, 2012). The nature of association between grain yield and its components determine the appropriate traits to be used in indirect selection for improvement in grain yield. Correlation studies provide reliable information on nature, extent and direction of selection (Ajibade, 1998). However, it may not give satisfactory results because its analytical resolution is limited to identification of mutual associations among parameters (Azeez and Morakinyo, 2009). The information obtained from the correlations coefficient can be enhanced by partitioning it into direct and indirect effects for a set of a pair of cause-effect inter-relationships (Kang *et al.*, 1983). Such information provides realistic basis for allocation of appropriate weight-age to various yield components (Rafiq *et al.*, 2010). The cause-effect relationship was studied in maize by Arias *et al*. (1999) for ear weight (the principal trait), plant and ear height, the ratio of ear height/plant height, number of kernel rows and kernels per row on each ear. The direct and indirect effects on ear weight of plant and ear height and its ratio varied according to the progeny type. Among the other traits, number of kernel rows showed only a small positive indirect effect via ear diameter for all progeny types and populations, while the number of kernels per row showed high positive direct effect. According to Ahmad and Saleem (2003) the ratio of growing degree days to reproductive phase and vegetative (GDD R/V) phase had the maximum positive direct contribution to grain yield per plant followed by growing degree days to tasseling and growing degree days to maturity (GDDMT), indicating the importance of these traits for selecting high yielding genotypes in maize. On the other hand, growing degree days to reproductive phase (GDDREP) had the negative direct effect followed by growing degree days to silking (GDDSL). In addition the authors indicated that if selection is made through plant and ear heights which presented negative direct and small direct effects on grain yield respectively, then the traits like GDD R/V GDDMT, GDDSL and 1000- kernel weight should also be considered simultaneously as indirect effects through them if their effects were high and positive. Gautam *et al*. (1999) found that grain yield was positively correlated with number of grain rows, 1000-grain weight, shelling percentage, plant and ear heights. The direct effects of plant and ear heights towards grain yield were small, as was that of days to silking, indicating the possibility of developing high yielding plant types with short plant height, medium ear placement and early maturity. However the positive direct effect of grain yield on plant height (Shakoor *et al*., 2007), ear height (Akbar *et al*., 2008; Sreckov *et al*., 2011) was reported. The direct and indirect effects of different quantitative traits on grain yield were studied in 90 hybrids by Geetha and Jayaraman (2000) who reported that number of grains per row exerted a maximum direct effect on grain yield, indicating that selection for number of grains per row will be highly effective for improvement of grain yield. A similar result was reported for number of grains per ear and per row (Ojo *et al*., 2006). Khalily *et al*. (2010) studied the interrelationship between grain yield and its components in eight medium maturing hybrids of maize under different levels of drought stress at differenet stages of plant growth and developmeng (the vegetative, reproductive and grain filling phases). The path analysis revealed that the most important direct effects on grain yield were number of kernels/row, ear length, ear girth and kernel rows/ear thus suggesting that these should be used as target traits for tailoring an ideal plant type for higher yield of maize exposed to drought stress at vegetative stage. Based on the results the authors recommended anthesis to silking interval, days to silk emergence, stay green characteristic and kernel number per row as major traits for selecting drought tolerant hybrid varieties of maize. Khalili *et al*. (2013 also) reported 100-grain weight, total number of grains per ear as target traits to improve maize grain yield under drought stress based on the path analysis.

Estimates of heritability and secondary traits on grain yield of genetic materials in evaluation is needed to determine the appropriate traits to be used in indirect selection for improvement in grain yield. Path coefficient analysis has been successfully used to elucidate the nature of association between yield and its associated traits in partitioning the correlation into direct and indirect effects. However, the exploitation of this information in integrating into the selection index for selecting drought tolerant varieties was not reported.

CHAPTER THREE

3.0 Assessment of farmers' preferences and constraints to maize production in Burkina Faso

3.1 Introduction

Food security exists when all people, at all times, have access to food available in quantity and quality. This is closely linked with issues of poverty, income generation and health. Burkina Faso is a poor, landlocked, sub-Saharan country of 15 million inhabitants. Agricultureaccount for approximately 80% of the working population. In Burkina Faso, agriculture has experienced remarkable growth in recent years. This growth is due partly to the increase in area planted and, secondly, to improved crop yields (DGPSA, 2005). Despite the potential of the agricultural sector, a significant proportion of the population cannot meet its food needs. Burkina Faso, like the other countries of the Sahel, is characterized by climate variability and lack of economic diversity. Agricultural production, in particular, is dependent on climatic and soil conditions, but also technical, economic and social situation of famers. For agricultural activities practiced mostly under rainfed conditions, variation in rainfall or invasion of locusts, often have catastrophic effects on production, resulting in declining incomes that can lead to serious food crises (Egg and Gabas, 1998). In addition to environmental constraints, the low yield of crops is also associated with low adoption of productivity improving technologies such as improved seed, which many farmers ignore or do not have access to (Hassan, 1998; Achieng *et al.,* 1999 cited by Odendo *et al*., 2001). Farmers' low adoption of technologies developed by research institutions shows the need for client-orientation in research and development. A farmer deals with a variable environment and has multiple production objectives that will affect his or her choice of crops and selection of genotypes. Next to yield, which, in formal breeding programs, is by far the most important objective, yield stability, adaptation to production techniques and conditions, and various consumption preferences are desired. This range of objectives often results in the use of a large number of varieties by individual farmers. In INERA's maize breeding program, efforts are being made to reduce the communication gap between breeders and famers by using a participatory varietal selection process. However, the selected sites are close to the research centers and not diversified. Fewer sites are used in more distant and less accessible zones. To have a true picture of farmers' preferences, there is a need to involve other sites.

Farmers' requirements have to be identified first so that they can be given more appropriate genetic materials to test (Nkongolo *et al.,* 2008). This can be done by using several methods, either separately or in combination. The methods include participatory rural appraisal (PRA), the examination of farmers' crops around harvest time, and the pre-selection, by farmers, of varieties from trials of many entries, grown either on a research station or on farm. Participatory research allows incorporation of farmers' indigenous technical knowledge, identification of farmers' criteria and prioritization of the research agenda.

Generally, three main factors are available for explaining adoption decisions, the economic constraints, the innovation-diffusion and the "adopter perception" (Adesina and Zinnah, 1993). However, Dankyi *et al.* (2005) identified ecological zones in which famers' farm serve as an explanatory factor affecting the adoption of improved technologies. They argued that famers in different ecological zones may adopt improved technologies differently because of the environment in which they live. If technologies developed are not compatible with the conditions prevailing in the ecological zones, adoption may be low. Thus, the type of improved crop varieties and other technologies developed should be in response to conditions prevailing in the ecological zones.

Area geographic characteristics can be integrated in the analysis of farmers' preferences and selection criteria by combining PRA data into Global Information System (GIS) framework. De Groote *et al.* (2004) provided a systematic approach to integrate PRA data into GIS software and applied it to determine and analyze farmers' demand for insect resistant varieties in Kenya. Thus, in integrating farmers' concerns and conditions into agricultural research, it is hoped that research would develop technologies that become widely adopted, resulting in more productive, stable, equitable and sustainable agricultural systems.

The objectives of this study were (i) to determine the type of maize varieties grown by farmers grow, farmers' preferences and constraints to maize production, (ii) combine PRA data into a GIS framework to determine and analyze farmers' perception and constraints to maize production and (iii) identify key explanatory factors affecting adoption of improved maize technologies.

3.2 Methodology

3.2.1 The study area

The study was conducted in two agro-ecological zones where maize is extensively cultivated, North-Sudan and South Sudan zones. Burkina Faso has not yet established an agro-ecological zoning in the strict meaning of the term. Photo-geographical zones defined by Monod (1957) and Guinko (1984) according to floristic and climatic characteristics take the place of agro-ecological zones. Figure 3.1 shows the four zones with their characteristics summarized in Table 3.1

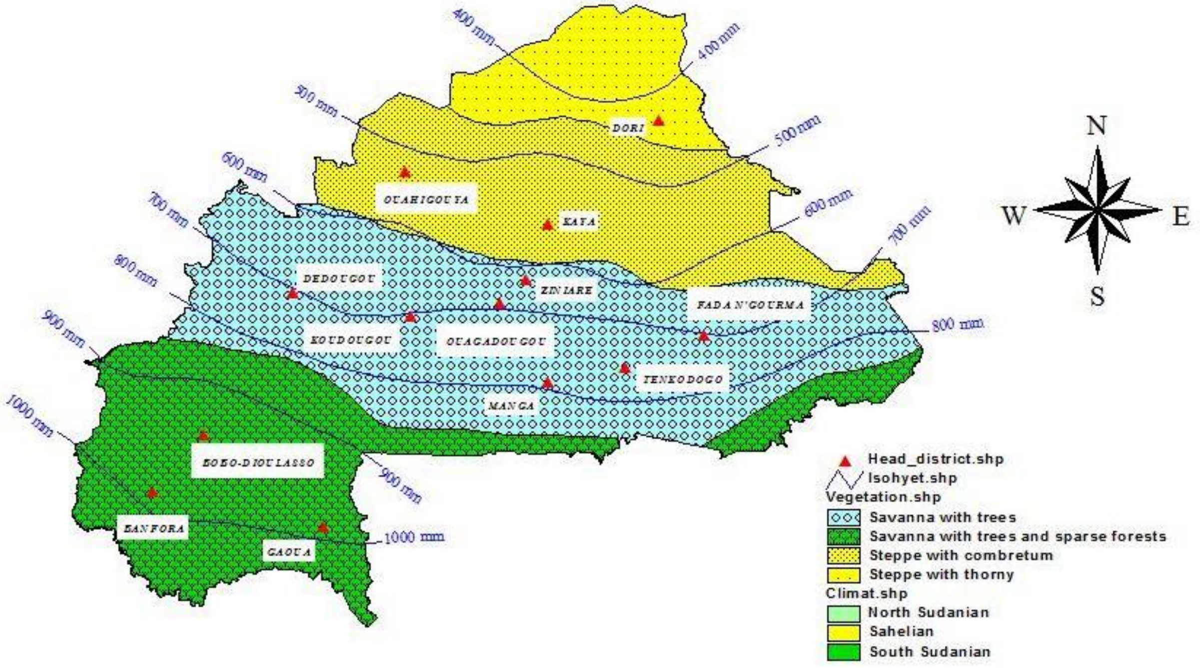


Figure 3. 1. Map of the agro-ecological zones of Burkina Faso (IGB database, 2012)

Table 3. 1. General characteristics of Burkina Faso's agro-ecological zones.

Zone	**Annual rainfall (mm).**	**Land cover**
Sahel (13.4 %)	< 400	Steppe with thorny bushes and annual grasses
		Pastoral zone evolving to agropastoralism , mainly pastoral at present Subsistence farming based on millet, sorghum and cowpea
		Transhumant herding
Sub-Sahel (15.3 %)	400 to 700	Steppe with combretum and annual grasses
		Agropastoral, mainly agricultural zone
		High population density and land fully occupied
		Subsistence farming based on millet, sorghum and cowpea
		Transhumant herding and sedentary agropastoral agriculture
North-Sudan (38.9 %)	700 to 900	Savannas with trees or shrubs
		Agropastoral, mainly agricultural zone
		High human and livestock population density
		Cotton area with agriculture based on
		sorghum , millet, cowpea and groundnut
		Transhumant pastoralism and sedentary village stock raising
Sud-Sudan (34.2 %)	900 to 1200	Savanna with trees or shrubs, sparse forests
		Agricultural area characterized by perennial crops (mangos, citrus, cashew etc.) cotton growing, yam s and cereals (sorghum, millet and maize.
		Used by transhumant in the dry season with sometimes mortal conflicts between pastoralists and farmers.
		Sedentary village stock rearing of taurin cattle.

Percentage of national territory in parenthesis

3.2.2. Sampling Procedures

Multi-stage sampling techniques were adopted to select the study sites and to capture maximum variability in agro-ecological and socio-economic environments. The North-Sudan and South-Sudan agro-ecological zones were selected to represent the two major maize production zones in Burkina Faso. Four districts from North-Sudan and three districts from the South-Sudan zones were selected to capture the variability in the zones. Eighty (80) farmers were randomly selected across districts in each zone for semi-structure surveys. Additionally, four focus group discussions were held, one in North –Sudan zone and three in South-Sudan zone.

3.2.3. Data Collection

Primary data were collected through both formal household surveys and the informal Participatory Rural Appraisal (PRA) tools. Contact personnel, local extension staff, councilors, school headmasters or village headmen facilitated the survey by creating a good rapport with local people, mobilizing farmers for the focus group discussions and providing lists of farmers to be sampled for the formal surveys. The PRA involved four Focus Group Discussions (FGD) and interviews with key informants such as experienced farmers, traders, school headmasters, councilors and agricultural extension staff. The technique employed consisted of problem listing and analysis. A checklist was designed to guide the discussions and to provide the group sufficient opportunity to bring up their own issues. The checklist was structured in three main parts: identification of maize varieties grown, farmers’ preferences for maize varieties and constraints to maize production. Throughout the discussions, a facilitator guided the process, while enumerators concentrated on taking notes. A pilot study was conducted in *Soumousso* village involving 38 households (28 men and 10 women) to assess the suitability of the checklist designed for the focus group discussions (FGD) and questionnaires for formal survey. The field research team comprised a principal investigator, an enumerator and a local facilitator. The medium for discussion was the local dialect which is widely spoken in the area. At least one of the members of the research team spoke the local dialect thus, whenever necessary, translation was provided. To eliminate gender dominance in discussions at *Soumousso*, separate discussions were held with men and women farmers. This was necessary because in the village's culture, men are dominant over women and women would not speak freely in the presence of men.

Both formal and informal approaches were employed in data collection in order to enhance precision and value. Data generated were compared and the final synthesis of the information is presented.

3.2.4 Data Analysis

Statistical analysis of both quantitative and qualitative data were performed using Sphinx plus version 4.5 and SPSS (Release 16.0.0) computer package. Sphinx-plus was used to draw up questionnaires and perform descriptive statistics. Analysis of variance was computed with SPSS for data collected using nonparametic test; Mann-Whitney U test was used for agro-ecological zones and Kruskal-Wallis H Test for districts.

3.2.5 Incorporating PRAs into Geographic information system (GIS) : Procedure

Information from PRAs can easily be incorporated into a GIS framework, providing a systematic approach and some organization (De Groote *et al*., 2004). Only PRA results from formal surveys were used in this analysis. The information was structured in a matrix. The data from each district formed a line or observation. The information from each district was structured over different columns or variables. Then, all information was geo-referenced, meaning that, for each line, the coordinates (latitude and longitude) of the district were entered as two separate variables or columns in the database. These coordinates were measured with a Global Positioning System (GPS) device. A district is composed of regions; the coordinates of the region considered as the head of this district were used to represent the whole district. Country maps, vegetation and climate databases were obtained from Geographic Institute of Burkina (IGB). Data were analyzed in Arcview GIS version 3.3 software.

Three topics were analyzed: traits of preference, constraints to production, and biodiversity. The variables used in the GIS analysis are presented in Table 3.2. Different types of variables were used for the different analysis.

Table 3. 2. Variables used in the GIS analysis

Topic	**Variable**	**Type**	**Definition**
Importance of traits of preference	Yield	Derived Score (from ranking)	3=top score, if ranked 1st; 2=if ranked 2nd; 1=if ranked 3rd
	Early maturity	Derived Score	same as above
	Drought tolerance	Derived Score	same as above
Importance of constraints to maize production	Drought	Score	0 = Not Important; 1 = Slightly Important; 2 = Moderately Important; 3 = Very Important; 4 = Extremely Important
	Striga	Score	same as above
	Termite	Score	same as above
Biodiversity in maize	White short cycle	Count	Total number of varieties as distinguished by the farmers
	White long cycle	Count	same as above
	Yellow short cycle	Count	same as above
	Yellow long cycle	Count	same as above

3.3 Results

3.3.1 Maize varieties grown

Farmers grow both local and improved varieties to meet their multiple objectives in maize farming (Table 3.3 and 4). The names of the maize varieties are often descriptive, referring to certain key identifiable characteristics (Table 3.3**),** especially grain color and maturity. White early and intermediate varieties and yellow early and extra-early varieties are mostly grown, white extra-early and yellow intermediate varieties are grown less frequently and late (yellow and white) varieties are not commonly grown.

Table 3. 3. Farmers' description of maize varieties grown

Sites	**Varieties**	**Physical characteristics**
Soumoussou	Local varieties	Yellow maize: 70 days to maturity; short plant height. White maize: 4 months to maturity; high plant height; long cob but small grains size. *Sissebokaba* : multiple grain color; 70 days to maturity and small grain size
	Massongo*	White, high yield, high plant height ; 3 months to maturity, needs a lot of fertilizer
	SR 21	White, short cob, large grain size; good standability ; needs less input
	SR 22	Leaves scrape skin, produces higher flour density than SR 22
	Barka	White maize; 70 days of maturity; normal plant height ; long cobs with small grain size, recently introduced to the region
	KPB	Recently introduced to the region
Orodara	Local varieties	White and Yellow maize
Moussomourou	Local varieties	High plant height and late maturity
	Obatanpa*	High yield; 65 days to maturity; high plant height; short cobs
	SR22	High yield; leaves are large and numerous; large grain size with short cobs; high flour density
	Massongo	High flour density but less than SR 22
Koudougou	Massongo	A thin stem ; grains are small; high yield
	Barka	Early maturing; low yield

* Obatanpa and Massongo are different names of the same variety. Obatanpa is the original name as introduced from Ghana and Massongo is the corresponding meaning but in a dialect of Burkina Faso.

All the varieties are grown in both northern and southern zones but, in general, early maturing varieties are grown in the northern zone (short rainy seasons) while late varieties are planted in the southern zone (long rain season) Figure 3.2.

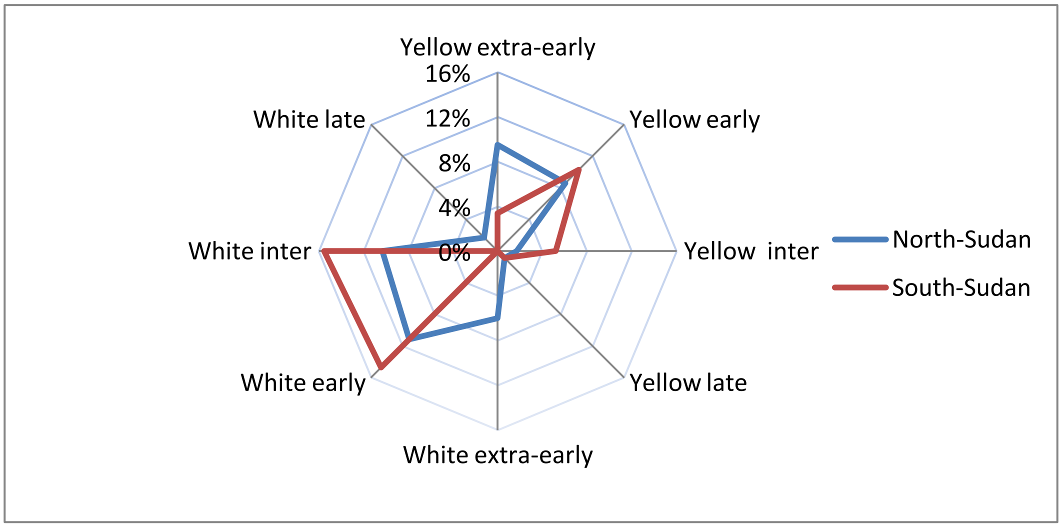


Figure 3. 2. Distribution of maize varieties grown in North and South –Sudan zones.

Sixty-three (63) % of the respondents predominantly grow varieties that they don't know by name (Table 3.4). These are local varieties or recycled seed from improved varieties. However, 37.1 % were able to name their varieties.

Table 3. 4. Maize varieties grown

Variety	Frequency	Percentage
NoID	112	62.92
Obatanpa	22	12.36
SR22	10	5.62
SR21	10	5.62
Wari	8	4.49
Barka	8	4.49
Espoir	4	2.25
KPJ	2	1.12
KPB	2	1.12
Total	178	100

NoID : name of the variety is unknown by farmer

To analyze maize biodiversity using GIS, simple counts of varieties were used. The total number of varieties (combination of grain color and maturity) varied from 3 to 7 represented by the size of the circle in each district (Figure 3.3).

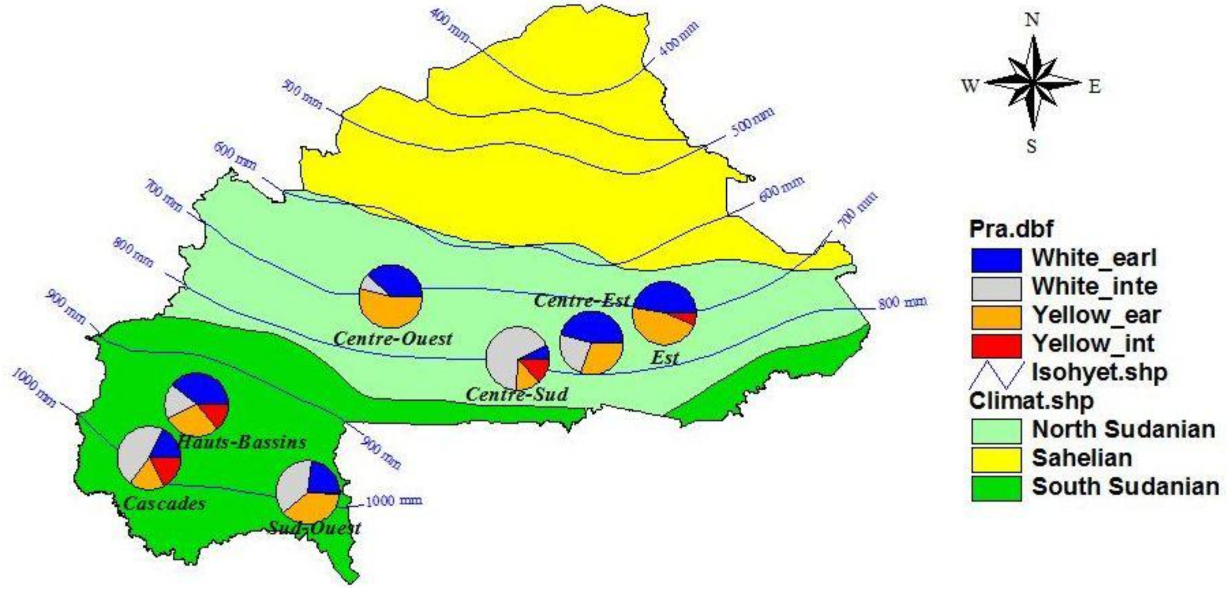


Figure 3. 3. Biodiversity in maize

Late and early maize is found in the two zones but early maize predominates. The analysis of varieties in terms of maturity period shows that all districts have a number of early varieties except for two districts and the late varieties are important only in moist zones in the South.

3.3.2 Constraints to maize production

Prioritization of the constraints was based on severity and frequency of occurrence of the constraint. The most important constraints mentioned by farmers across sites are unreliable rainfall (4 sites) followed by *Striga* and weeds (3 sites) and termites (3 sites) (Table 3.5). In addition, high cost or lack of seed and fertilizer (3 sites) poor extension service (3 sites) and lack of tools (3 sites) are also important. Lack of market was mentioned in *Soumousso* and *Moussomourou* as an important constraint.

Table 3. 5. Constraints to maize production recorded in FGD

List of constraints summarized by site
SOUMOUSSO Poor extension service (lack of training); Unreliable rainfall; Lack of farm tools; Wind; Liquidity problems; Striga and weed problems; Lack of market; Termites; Bird damage; Storage pests ; Low soil fertility.
ORODARA Poor extension service (lack of training); Unreliable rainfall; Lack of farm tools; Termites; High price of fertilizer and seed; Stem borer; late maturity of varieties
MOUSSOMOUROU Lack of farm tools; Unreliable rainfall; Liquidity problems; Striga and weed problems; Lack of market; Termites; High price of fertilizer and seed; late maturity of varieties; MSV.
KOUDOUGOU High price of herbicide and fertilizer; Unreliable rainfall; lack of seed; Stem borer; Poor extension services; Striga problem; Lack of seed; Fear of farmers that maize cannot produce high yield in dry areas.

In the matrix of constraints, constraints were associated with a particular variety (Table 3.6). The varieties SR21, Massongo, Obatanpa and local white maize varieties grown at *Orodara* are susceptible to drought. Dehulling of SR22 and local white maize grains pose problems to famers at *Moussomourou* and *Orodara.*

Table 3. 6. Matrix of constraints

SITE	VARIETY	CONSTRAINTS
SOUMOUSSO	Massongo	Susceptible to drought and weed
	SR 21	Susceptible to drought and weed
ORODARA	White maize	late in maturing Susceptible to drought Grains are easily broken
MOUSSOMOUROU	SR 22	Farmers (particularly women) found the grains of this variety difficult to dehull
	Massongo	Susceptible to drought and weed
	Obantapa	Susceptible to drought and weed; thin stem; lodging resistant; suscetiple to storage pests

Results from formal surveys showed no significant differences in ranking production constraints between agro-ecological zones except for seed cost, soil fertility, stem borer, maize streak virus and field insect pests (Table 3.7). Overall results indicated that the most important constraint was poor cash flow while the quality of seed was perceived as acceptable.

Table 3. 7. Mean rank for perceived production constraints in formal survey

	Agro-ecological zone			**Mann-**	**Asymp. Sig.**
Constraints	North-Sudan	South-Sudan	**Overall**	**Whitney U**	**(2-tailed)**
Liquidity problems	2.47	2.95	2.71	623.00	0.08
Lack of farm tools	2.73	2.60	2.66	769.00	0.76
Poor extension services	2.43	1.92	2.18	663.00	0.17
Unreliable rainfall	1.65	2.18	1.91	670.50	0.20
High price of seed	0.55	2.80	1.67	475.50	0.00
Striga weed	1.32	2.02	1.67	670.50	0.20
Termites	1.32	1.98	1.65	650.50	0.13
Low soil fertility	0.25	1.65	0.95	343.50	0.00
Stemborer	0.58	1.30	0.94	635.00	0.07
Labor shortage	0.77	0.78	0.78	704.50	0.25
Theft	0.58	0.92	0.75	660.00	0.09
Maize streak	0.22	1.05	0.64	595.50	0.02
Field insect pests	0.03	0.72	0.37	635.50	0.01
Poor quality of seed	0.28	0.32	0.30	787.50	0.84

Constraint with highest mean rank is the most important

For the GIS analysis, the three main constraints were: Drought, *Striga* and termites. The mean score of importance in each district was used. The data were geo-referenced and the results are presented in Figure 3.3. By analyzing the map in the two zones, some very important observations could be made. First, drought, Striga and termites are great threats to maize production. All the districts surveyed face these major constraints. In the dry areas (North-sudan zone), drought is relatively more important than the two other constraints. However in *Est* district, termite attack is the biggest problem. The importance of drought, *Striga* and termites is the same across the different districts in South-Sudan zone.

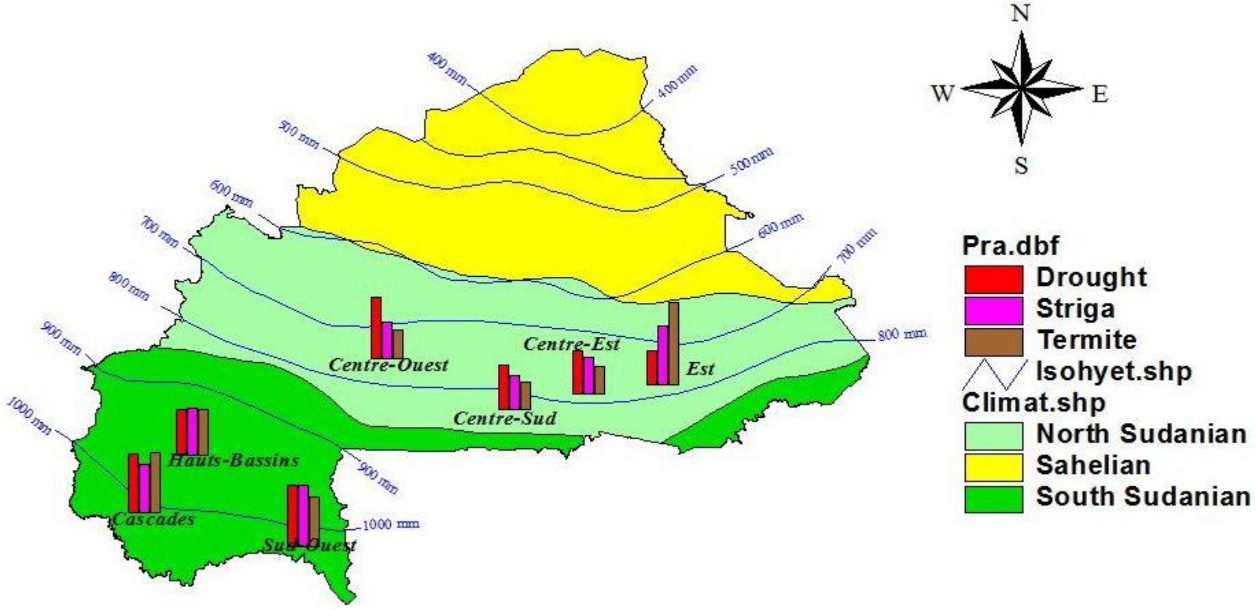


Figure 3. 4. Importance of constraints to maize production

3.3.3 Farmers' suggestions to boost maize production in the country

The focus group discussions across sites identified that farmers think that their maize production could be boosted by providing them with farm tools, reducing fertilizer and seed prices and providing training to farmers (Table 3.8).

Table 3. 8. Farmers suggestion in FGD

Site	Suggestions
Soumousso	Support in farm tools acquisition Provide training to farmers Promote farmers associations
Orodara	Reduce fertilizer prices; Provide support at the right time
Moussomourou	Reduce fertilizer and seed prices; Provide subsidy for farm tools and fertilizer; Fix an official price in the market and control the fluctuation;
Koudougou	Government should promote maize production and Visit farmers in the field to motivate them

Formal surveys indicated that training farmers and providing support (farm tools and loans) were the most important ways to solve problems and increase maize production. However, in the South-Sudan zone, farmers ranked tolerant varieties first followed by reduction of fertilizer price as the most important solutions (Table 3.9).

Table 3. 9. Mean rank for perceived solution in formal survey

Solutions	**Agro-ecological zone**		**Overall**	**Mann-Whitney U**	**Asymp. Sig. (2-tailed)**
	North-Sudan	South-Sudan			
Training famers	1.70	2.04	1.84	286.50	0.10
Support/Funding	1.95	1.95	1.95	218.50	0.96
Resistant varieties	2.40	1.88	2.00	27.50	0.21
Reduce seed price	2.00	2.13	2.08	58.00	0.70
Reduce fertilizers prices	2.24	1.91	2.09	481.50	0.11
Produce good quality of seed	2.00	2.30	2.12	59.00	0.21

Solution with smallest mean rank is the most important

3.3.4 Farmers' choice of varieties.

The pilot study showed (data not presented) that farmers tend to mix up criteria used to select maize varieties and traits they would prefer to be incorporated into their varieties. As a result, the questionnaire was reviewed and adapted, thus farmers' preferences are presented under two headings: criteria used to choose what variety to grow (an open question) and traits of preferences.

3.3.4.1 Selection criteria

In all the FGD sites, farmers use many but similar criteria in selecting the maize varieties they grow. The most important criteria across the sites were high yield, early maturing, and tolerance to drought (Table 3.10).

In formal surveys, the three main criteria mentioned by famers (an open question) were, in order: the grain color, high yield and grain size (Figure 3.5).

Table 3. 10. Farmers' preferences in choice of varieties in FGD

Sites	Criteria of preference	Sites	Criteria of preference
Soumousso	High yield	Moussomourou	High yield
	Early maturing		Early maturing
	Drought tolerance		Drought tolerance
	Plant height (Medium)		Weed tolerance
Orodara	High yield	Koudougou	High yield
	Early maturing		Early maturing
	Drought tolerance		

The grain color is very important for farmers in selecting the maize varieties they grow. Preferred color is different depending on the region and/or farmer. White maize is grown for sale while yellow maize is grown for family consumption. White maize is believed to be higher yielding than yellow maize. In some regions, yellow maize is reputed to have good taste whereas, in other regions, it is believed to cause a stomach disorder.

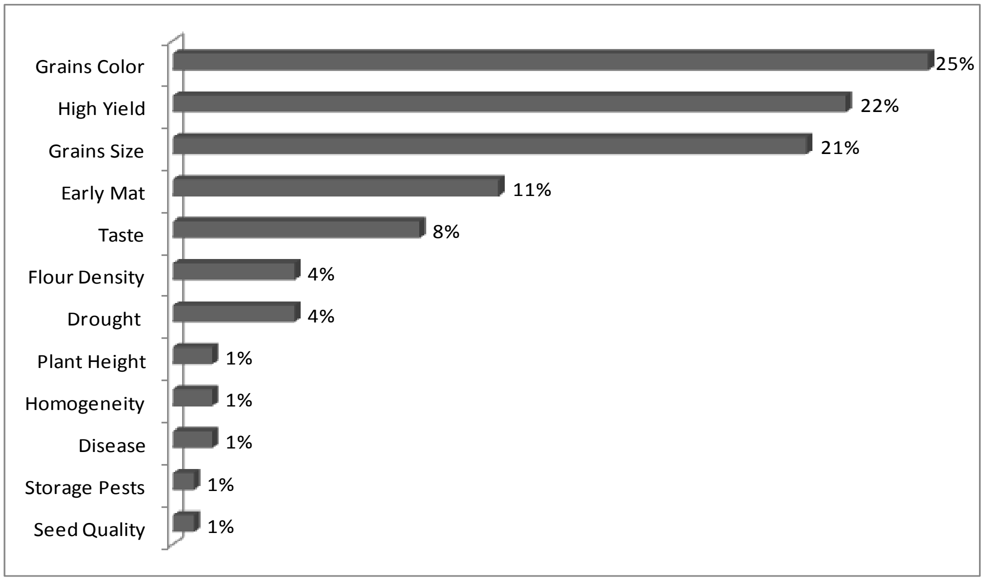


Figure 3. 5. Selection criteria

3.3.4.2 Traits of preference

Farmers were asked to select and rank three traits out of nine that they would prefer breeders to incorporate into the existing cultivars. Results indicated that most farmers would prefer to have their varieties to be high yielding, early maturing and tolerant to drought. Except for high flour density, farmers found no significant differences in ranking of cultivar traits of preference between agro-ecological zones (Table 3.11).

Table 3. 11. Mean rank values for preferred traits from formal survey

Traits	Agro-ecological zone		Overall	Mann-Whitney U	Asymp. Sig. (2-tailed)
	North-Sudan	South-Sudan			
High yield	1.41	1.63	1.50	357.50	0.35
Early maturity	1.91	1.68	1.83	255.50	0.14
Drought tolerant	1.89	1.89	1.89	121.00	0.98
Tolerance to Striga	1.75	2.50	2.17	21.00	0.07
Large grain size	2.00	2.25	2.22	3.00	0.67
Tolerance to diseases	3.00	2.27	2.38	4.00	0.12
Taste	2.63	2.00	2.56	1.50	0.25
Resistance to storage pests	2.67	2.54	2.60	69.00	0.51
High flour density	2.92	2.14	2.63	20.50	0.02

Trait with smallest mean rank is the most important

For ease of presentation, only the three most important traits, based on the overall means, are presented in GIS analysis: yield, early maturity and drought tolerance.

The GIS analysis of these traits used the scores derived from ranking in which farmers indicated their preferences (from 3 for first choice, to 1 for the third choice). By geo-referencing the data, a map was constructed and presented in Figure 3.6.

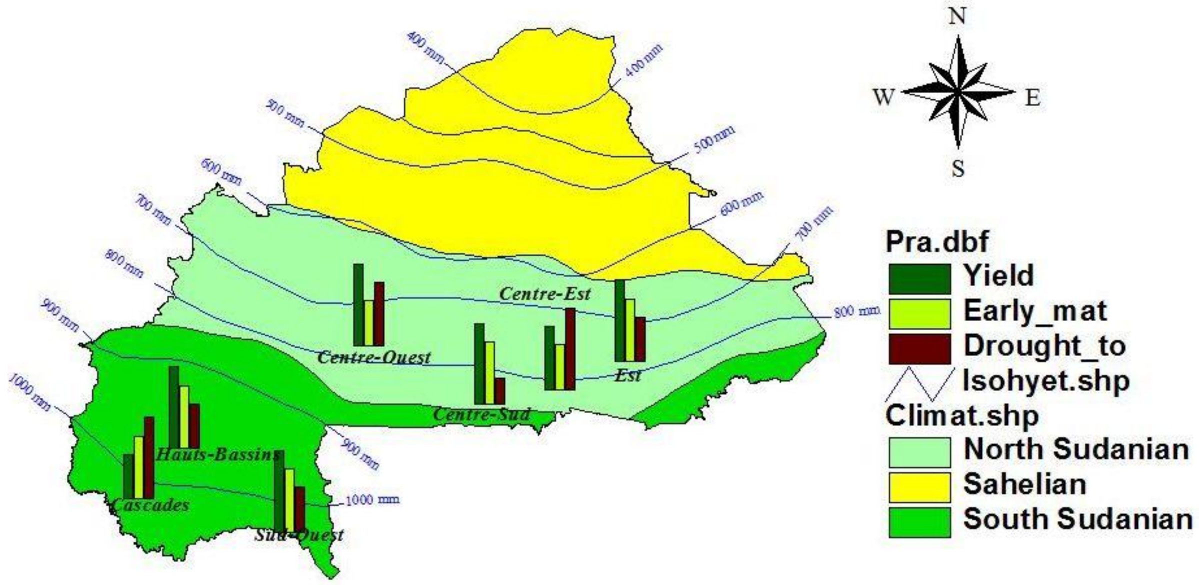


Figure 3. 6. Importance of traits of preference

The results showed that yield is almost universally the first preferred trait except in two districts *Cascade* and *Centre-Est* located, in South-Sudan zone (rainfall > 1000mm) and in North-Sudan zone (rainfall 600 -900mm), respectively. In the South-Sudan zone (rainfall > 1000mm), early maturity is generally the second trait preferred by famers. In the North-Sudan zone (rainfall 600 -900mm) the ranking of early maturity and drought tolerance shift between second and third preferred trait depending on the district. Drought tolerance was considered of minor importance only in the *Centre-sud* district .

3.4 Discussion

3.4.1 Maize varieties grown.

Both local and improved varieties are grown in Burkina Faso. However, local maize varieties are the most predominant. It was noted that only 15 out of 32 released improved varieties are produced by seed growers. Many varieties were named by the breeders in local language with the aim of facilitating the adoption; for instance variety “wari” means “money” in Dioula. Most of the varieties which were not named in that way are hard to pronounce by illiterate farmers; for example in the focus group discussion sessions, many farmers could not name correctly the following varieties they grow *SR 21; KPJ* and *KPB*. In some instances, the same local varieties are known by different names and different improved varieties had a common name, depending on the area or language. The names of the maize varieties are mainly descriptive as they refer to certain key identifiable characteristics. This result was consistent with that reported by Sanou (1996) who showed that famers name maize varieties in their own dialect using one or more distinctive characteristics. For instance, *kaba gè* (white maize in dioula), *Vagaka pènin* (yellow maize in bobo) and *Kaman gnanga* (late maturing maize in mooré). The consistency in the two studies shows that, after 15 years, farmers still name their varieties by key characteristics and not the name given by the breeder. White maize varieties are mostly preferred and the early maturing varieties are largely grown. However, the notion of early maturity has to be taken with caution because famers and researchers do not understand it in the same way. In general, farmers define the cycle (from planting to harvest) in months (2, 3 or 4 months) while maize breeding program set a range of days to define a cycle of a particular variety (Sanou, 2006): extra-early (70-84 days), early (85-94 days), intermediate (95-110 days) and late (more than 110 days). Farmers do not classify a variety of three months maturity as an early variety whereas breeder assign it in early maturing group. The popularity of early varieties has implications for breeding. Breeding programs should consider improving early varieties with a wide adaptation, instead of trying to develop these varieties only for the dry areas. The analysis of the grain color of the varieties shows that farmers will adopt new varieties if they fit their preferences regardless of grain color. The number of late maturing yellow varieties in many districts is also very revealing. Maize breeding programs have released only one late yellow maturing variety; hence, famers will more likely adopt new late maturing varieties.

3.4.2 Constraints to maize production.

Drought is a major abiotic constraint affecting maize production in Burkina Faso. Climate instability has resulted in variation of the intra-and inter-annual rainfall and made drought a recurrent problem This was acknowledged by famers in the two agro-ecological zones. Drought is characterized by an unequal distribution of rainfall in both time and space. Results show significant difference in drought ranking between the two zones. In South-Sudan zone (annual rainfall is above 900 mm), drought is perceived to be more important than in the north-Sudan zone (annual rains is between 600 – 900 mm) because, during the long season (South-Sudan zone), two frequent drought periods are generally observed. One is at seedling stage, during the period that many famers plant when the first rains fall, but, unfortunately, the rains may stop after one to two weeks. The second drought occurs during the grain filling stage. In short season areas (north-Sudan zone), plants are more subjected to drought just after planting. The matrix of constraints indicated that farmers found the following maize varieties drought susceptible, *massongo, obatanpa* and *SR 21*. Though these improved varieties, preferred by farmers, have high yield potential, they were not improved for drought tolerance (Sanou, 2006). The adoption of these varieties by farmers will be substantially increased if they are improved for drought tolerance. Maize varieties, *Barka* and *Wari*, tolerant to drought, extra-early and early maturity, respectively, (Sanou, 2006) are generally grown in North-sudan zone. This could explain why drought is perceived as being less important in this area compared to the South-Sudan zone where late maturing maize varieties are grown but no drought tolerant improved varieties have been released. Low soil fertility is another abiotic constraint that farmers in Burkina Faso face.

There are highly significant differences across agro-ecological zones for low soil fertility. In Southern sudan zone, the problem is perceived to be more important than in Northern sudan zone. In the North-Sudan zone, maize is mainly cultivated around houses, dams and rivers where the soil is more fertile, while, in South-sudan zone, even though maize is grown around the house, most of the production is done in fields far from the village and, due to the limited resources, few inputs are used. Recurrent drought and declining of soil fertility, particularly nitrogen, imply that breeders should develop drought- and low N-tolerant maize varieties.

Farmers ranked *Striga* as the major biotic constraints to maize production in both agro-ecological zones. *Striga hermonthica* is a root parasite of grasses occurring throughout Burkina Faso. It is an important biotic constraint to maize production in the country and is the weed that farmers fear most. *Striga* became an important constraint to maize production recently due to increasing maize cultivation on land initially occupied by sorghum and millet. Control methods available for farmers are limited. Due to limited resources, chemical control and normal fertilization are not affordable by small scale farmers and there are no tolerant varieties released by research. Hand pulling and crop rotation are the only control methods accessible to small scale farmers. *Striga* control methods developed by research are often unknown and unexploited by farmers. A project was initiated to extend the technologies and enhance the adoption of integrated *Striga* control methods by farmers (Ouedraogo *et al*., 2008). In this project, farmers' field schools were used to train farmers on *Striga* biology and integrated control methods. *Striga*, which was not a major constraint to maize production in Burkina Faso 20 years ago and was limited to the dry areas (North-sudan), appears now to be a major constraint all over maize production zones and a big challenge for maize breeding program. The implications are quite clear, demand for resistant

Striga varieties is real. Breeding programs should develop new varieties tolerant to *Striga* and/or improve existing adapted varieties for *Striga* resistance.

Attack by termites in farmers' fields is another major problem to which less attention is paid by research. Farmers indicated that they adopted a range of solutions without realizing a substantial impact. The problem needs to be addressed in the two zones (North and South sudan) using an integrated pest management approach.

Among the constraints which cannot be addressed with improved or tolerant varieties, famers rank poor cash flow problems, inadequate farm tools and poor extension service as important. Agriculture in Burkina Faso is mostly dominated by traditional farming, characterized by the use of "daba" (an African hoe) and animal traction. Farming knowledge is learned in the family from generation to generation and most of the time farmers do not have access to modern knowledge and new technologies developed by research. As a result Governments, NGOs, and Research Centres have adopted strategies to teach farmers about farming systems. Different methodologies have been developed: formal training sessions, field demonstrations, broadcasts in radio and TV and booklets in official (French) and local languages. However, the results of the present study indicate that a lot needs to be done to reach farmers, especially small-scale famers in the remote villages .

3.4.3 Farmers' suggestions for increasing maize production

Farmers think that maize production could be increased by providing training and support (farm tools and loans) to them. This suggestion proposed by farmers could be based on the experience of farmers in cotton production systems put in place in Burkina Faso. Cotton accounts for 60% of the income of the country from exports. Due to this importance, multiple stakeholders take part in cotton production. Farmers receive supports in the form of inputs, loans from the bank, advice, and training in cotton production and marketing. Due to the success of this organization, farmers believe maize production could be improved with such organizations. Many farmers mentioned this during the focus group discussions and interviews. In the light of this, itmay be concluded that the type of organization promoting the production of a particular crop and the number of contacts of extension service with famers will substantially influence the adoption of the crop by famers.

In general, famers perceived maize production constraints related to breeding action less important than the general constraints not calling for a breeding action. This explains the ranking of the tolerant varieties in the third position as a solution proposed alleviating maize production problems. Throughout the focus group discussions and interviews with farmers, it was clearly shown that most of them, particularly the illiterate farmers, are not aware that the research could improve maize varieties to tolerate the abiotc and biotic constraints they are facing. Famers receive improved seed from Government services, NGOs or buy it from middlemen at the market and none of these stakeholders explain to famers the potential of the varieties to reduce abiotic and biotic stresses effects. These observations imply that breeders, seed growers, middlemen, Government services and NGOs should work in collaboration to teach farmers about the characteristics and potentials of the variety received or purchased. This approach will include all the stakeholders in maize production (farmers, famers association, research, government, NGO, school etc). A platform gathering these stakeholders could be organized at the local level and national levels.

3.4.4 Farmers’ choice of varieties

The grain color is the first criterion used by farmers in selecting maize varieties. This does not mean farmers are interested only in the color, rather it reflects that key characteristics, especially grain color and cycle of maturity, used to identify maize varieties. Grain color represents, in the eyes of farmers, some features, especially yield, of a particular variety. Grain size is an important criterion for varietal selection. In this regard, farmers always select seed with the large grains size to grow. The results showed farmers, in all the FGD sites but one, could not identify the improved varieties they grow. A farmer in FGD at *koudougou* identified successfully *Barka* and *Massongo* grains among grains of eight varieties proposed. He has grown these varieties for several years. The lack of identification in other sites could be explained by the recent use of these varieties by farmers. They indicated they have grown improved varieties for about five years. High yield is generally the first trait desired by farmers, followed by early maturity which farmers prefer to have quick food for their families and to avoid drought. The GIS analysis showed that the early maturing and drought tolerant varieties are demanded in wet zones as well as in dry zones. These results have important implications for breeding: early maturing and drought tolerant varieties should receive more weight in the breeding for all the agro-ecological zones and not only for the dry areas.

3.5 Conclusion

This study characterized maize varieties that farmers grow, examined famers' preferences and identified constraints the farmers face in maize farming in the two agro-ecological zones of Burkina Faso. It integrated the analysis of PRA data into a GIS framework and identified implications for breeding and factors affecting adoption of improved maize varieties.

Farmers grow both local and improved varieties to meet their multiple objectives in maize farming. In general, famers prefer to use grain color and maturity period to name maize varieties they grow. All the different varieties are grown in both northern and southern zones but, in general, early maturing maize varieties are grown in northern zones (short rainy seasons) while late maturing varieties are planted in southern zones (long rainy season).

The results indicated that farmers would prefer to have their varieties be high yielding, early maturing and tolerant to drought. Next to yield, which for farmers is by far the most important objective, early maturity and drought tolerant varieties are viewed by farmers as a way to ensure early provision of food to the households to alleviate hunger and to cope with the unreliable rainfall. Maize production in the two zones is constrained by a variety of related factors, the extent of contribution of each factor varies across the districts. The main constraints are poor cash flow, low soil fertility, and poor extension services. The most important constraints related to breeding, in decreasing order of importance, are drought, *Striga* and termites. To cope with financial constraints, farmers recycle the varieties for a long period of time and do not apply or apply low rates of fertilizers and pesticides in maize fields.

The analysis of farmers' preferences and constraints to production from the PRA data was improved by a GIS framework. The maps allow visual comparison of all districts at a glance and variables are represented with geographic characteristics leading to synthesis without loss of detail. The maps are easy to understand and the information is relevant to the breeders. Most importantly, the geographic characteristics can be replaced by any other relevant feature that will help to understand farmers' preferences dynamics for instance social characteristics database.

Few studies have been conducted on models on Burkina Faso agriculture and technology adoption. This study identified organization of maize production, agro-ecological zones where farmers cultivate maize and access to extension services as key factors in adoption decision of new maize variety. It is expected that future studies will include these factors as some of the explanatory variables in adoption models.

CHAPTER FOUR

4.0 Genetic characterization of maize inbred lines with SNP Markers

4. 1.Introduction

Genetic diversity in agricultural populations provides the capacity to meet changing environments and market requirements (Emigh, 1980). In crop breeding, genetic diversity is very important for an analysis of genetic variability in cultivars (Smith, 1984), the identification of diverse parental combinations to create segregating progenies with maximum genetic variability for further selection (Barrett and Kidwell, 1998), and the introgression of desirable genes from diverse germplasm into the available genetic base (Thompson *et al.*, 1998).

Molecular marker analysis provide an important approach for estimating genetic relationships. RFLP, SSR and SNP markers have been used to study genetic diversity in maize. Warburton *et al*. (2005) characterized 218 elite CIMMYT maize inbred lines using RFLP markers and suggested the use of molecular markers and cross performance information to refine heterotic groups and select representative testers. SSR markers have been used to characterize the genetic structure and diversity of 260 important tropical and temperate maize inbreds (Liu *et al.* 2003), investigate genetic diversity in CIMMYT lowland tropical (Xia *et al.,* 2004) and mid-altitude, highland subtropical (Xia *et al.*, 2005) inbred lines. Population structure and patterns of relationships of 770 inbred lines representing both temperate and tropical/subtropical maize germplasm (Lu *et al.*, 2009) and 450 maize inbred lines from CIMMYT breeding programs in Zimbabwe and Kenya (Semagn *et al*., 2012) have been investigated using SNP markers.

A very large number of SNP markers are now available in maize, many of which have been developed from the DNA sequence of known genes. For this reason, SNP markers are now the assay of choice for a variety of tasks in maize improvement including genetic diversity analysis (Lu *et al.*, 2009).

CIMMYT and IITA are the source of maize breeding materials for a significant portion of Africa. CIMMYT and IITA inbred lines and OPVs are bred to contain considerable diversity and are then taken by National Agriculture Research Programs and selected for further adaptation in their own particular environment(s). Maize germplasm in Burkina Agricultural Research Institute (INERA) includes different materials from CIMMYT and IITA. The hybrid maize breeding program at INERA was established in the late 1980s using top-crossing as a major method for estimating general combining ability but little progress was made because a lot of effort was devoted to OPVs development, which were more suitable to the majority of farmers. However, INERA initiated a new inbred-hybrid breeding program in 1991. INERA inbred lines are essentially derived from OPVs, which have the advantage of being both environmentally adapted and adopted by farmers. Several studies have addressed the relative performance *per se* and in top-cross combination of the newly developed inbred lines but they have not yet been characterized at the molecular level. Knowledge of molecular genetic diversity among the maize inbred lines developed for the national breeding program will provide guidance to the breeder on how to use more efficiently the local germplasm. A better understanding of how the local lines are related to lines from different sources may be useful for on the incorporation of exotic germplam into the existing breeding program.

The objectives of this study were to (1) analyze the genetic diversity in a diverse set of maize inbred lines; (2) determine the level of genetic diversity in INERA inbred lines and patterns of relationships of these inbred lines developed from two sources; and (3) explore the genetic differences between local and exotic germplasms.

4. 2. Materials and Methods

4. 2.1 Plant Materials

A total of 100 maize lines representing INERA, CIMMYT, IITA and Temperate germplasm was chosen for molecular characterization. CIMMYT and IITA lines were chosen due to their resistance/ tolerance to biotic and/or abiotic stresses and INERA lines representing a sample of advanced lines selected for morphological characteristics and disease tolerance. The 100 lines included 59 lines from INERA, 16 lines from CIMMYT/Zimbabwe, 15 lines from IITA, and 10 temperate lines obtained from INRA, France. The Temperate lines represented European and U.S elite inbred lines. The INERA germplasm included 3 Open Pollinated varieties (FBC6, ESPOIR and FBMS1), 34 and 20 lines extracted from FBC6 and ESPOIR, respectively (thereafter called Subset A and B, respectively). FBC6 has a mixed genetic background, It was developed from a mixture of 8 varieties (DMRESR-Y and TZESR-Y C2 from IITA ; ROD4, ROD12 and « Révolution précoce » from CIRAD/IRAT/Réunion and FBC4, Maka and IRAT217 from INERA). ESPOIR was developed from Population 66 SR of CIMMYT/IITA using different cycles of recurrent selection. FBML10 was derived from TZI35 with a more uniform grain color. The list of the lines together with kernel color, environmental adaptation and reactions to stresses (where available) are listed in Appendix Table S1 and summarized in Table 4.1.

Table 4. 1. Summary of characeristics of 96 maize inbred lines used in the study.

	No	Color		Drought			*Striga hemonthica*			
		W	Y	T	S	Unknown	R	T	S	Unknown
INERA	59	15	44			59				59
CIMMYT	16	16	0	12	2	2				16
IITA	15	0	15	11	1	3	10	1		4
U.S	6	0	6			6				6
European	4	0	6			4				4
Total	100	31	71	23	3	74	10	1	0	89

Y yellow kernel color, W white kernel color, R resistant, T tolerant, S susceptible

4. 2.2 SNP genotyping

Genomic DNA for each sample was extracted from seedling leaves (at V3 to V5 stage) using a magnetic bead and Klearcall extraction buffers (protocol http://www.lgcgenomics.com/nucleic-acid-extraction/kits/). They were then genotyped by Kbiosciences (Hoddesdon Herts, UK) using their Kompetitive Allele-Specific PCR Single-Nucleotide Polymorphism (SNP) genotyping system (KASP). The KASP assay uses a technique based on allele specific oligo extension and fluorescence resonance energy transfer (FRET) for signal generation. The fluorescent reporting system is comprised of four single-labeled oligonucleotides that hybridize to one another in free solution to form a fluorescent quenched pair which upon introduction of complementary sequences generates a measurable signal. Complete details on the principle and procedure of the assay are available at http://www.kbioscience.co.uk/reagents/KASP_manual.pdf. All the SNP markers used in this study were developed by CIMMYT and validated in KASP system. A detailed list of SNPs used can be seen in Appendices Table S2

4. 2.3 Statistical Analysis

4. 2.3.1 *Genetic diversity analysis*

Summary statistics, including the minor allele frequency (MAF), gene diversity (H, unbiased estimate), observed heterozygosity, and PIC value, were calculated using PowerMarker software (Liu and Muse 2005). The polymorphism information content (PIC) value, described by Botstein *et al.* (1980), was used to refer to the relative value of each marker with respect to the amount of polymorphism revealed. It is estimated as:

$$\widehat{PIC}_l = 1 - \sum_{u=1}^{k} \tilde{p}_{lu}^{\,2} - \sum_{u=1}^{k-1} \sum_{v=u+1}^{k} 2\tilde{p}_{lu}^{2}\tilde{p}_{lv}^{2}$$

Where P_{lu} is the frequency of the *u*th allele, and P_{lv} is the frequency of the *v*th allele. Heterozygosity and unbiased gene diversity were calculated to quantify the genetic variation in the maize lines sampled. The heterozygosity value reflects the proportion of heterozygous loci detected in a single maize line and its estimated as :

$$\hat{H}_l = 1 - \sum_{u=1}^{k} \tilde{P}_{luu}$$

where P*lu* is the frequencies of the *u*th alleles for marker *u* and the summation extends over k alleles. The gene diversity was defined as the probability that two alleles randomly chosen from the test sample are different. The unbiased estimator of gene diversity at the *u*th locus is

$$\hat{D}_l = (1 - \sum_{u=1}^{k} \tilde{p}_{lu}{}^{2}) / (1 - \frac{1+f}{n})$$

Where P*lu* is the frequency of the *u*th allele, *f* is the inbreeding coefficient, and *n* is the sample size. Allele frequency was calculated for each locus across each of the four distinct sets of maize germplasm : INERA, CIMMYT, IITA and Temperate. Difference in allele frequency between local germplasm and each of exotic material was calculated and statistical significance of differences in allele frequency was based on the P value from Fisher's exact test (Fisher, 1922).

4. 2.3.2 *Genetic distance and relative kinship*

The genetic distance between genotypes was computed using the Roger's genetic distance (1972) with Powermarker software. Genetic distance is defined as :

$$D_R = \frac{1}{m} \sum_{j}^{m} \sqrt{\frac{1}{2} \sum_{i}^{a_j} (p_{ij} - q_{ij})^2}$$

where *p* and *q* are the frequencies of *i*th allele at the *j*th locus in populations X and Y respectively, while a_j is the number of alleles at the *j*th locus, and *m* is the number of loci examined. Genetic distance was calculated between pairwise comparison of all the lines and all the lines bred only in INERA. In addition genetic distance between pairs of inbred lines in each germplasm set was estimated.

SPAGeDi software (Hardy and Vekemans, 2002) was used to obtain the kinship matrix between lines using Loiselle method (Loiselle *et al*. 1995). Loiselle's estimator is expected to be unbiased with respect to allelic frequencies (Hardy and Vekemans, 2002). The relative kinship reflects the approximate degree of identity between two given individuals. Negative values between two individuals (indicating less relationship than expected between two random individuals) were changed to zero. The relative kinship coefficients were estimated between pairs of the entire germplasm set and INERA germplasm set.

4. 2.3.3 *Grouping of lines*

Three multivariate analysis including cluster analysis, principal component analysis and model-based population structure analysis were employed to subdivide inbred lines into genetic subgroups. A dendrogram was constructed, in cluster analysis, from the genetic distance matrix using the neighbor-joining algorithm (Saitou and Nei, 1987) with PowerMarker and the resulting trees were visualized using MEGA version 5.2.2 (Tamura *et al.* 2011). Principal component analysis (PCA) were conducted using TASSEL 4.1.29 software (Bradbury *et al*. 2007). To infer the structure of the population, the software STRUCTURE (Pritchard *et al*. 2000) was used. The dataset was tested for a number of subpopulations ranging from k = 1–12. Three runs for each k value were performed using the admixture model and correlated allele frequencies (Falush *et al*. 2003). The burn- in length and iterations were all set to 500,000. To infer the most likely number of groups within the population, the Evanno transformation method (Evanno *et al*. 2005) was used on the STRUCTURE outputs. In the model-based method, membership coefficients (Q values) for each inbred line were estimated to have its memberships in multiple subgroups. Inbred lines with membership probabilities ≥ 0.60 were assigned to the corresponding subgroup and lines with membership probabilities <0.60 were assigned to a mixed subgroup.

4. 2.3.4 *Linkage disequilibrium (LD) analysis*

Linkage disequilibrium between pairwise polymorphisms identified on the sequences was studied using the TASSEL 4.1.29 software. LD measurement parameter r^2, which is the squared correlation between alleles at two loci, was used to estimate LD between SNPs with MAF >0.05 on each chromosome for the entire germplasm set. In order to see the extent of LD in different subpopulations, r^2 was also calculated separately for the INERA and tropical germplasm, which represent all the INERA, CIMMYT and IITA lines included in this study.

4. 3. Results

4. 3.1 Descriptive summary and statistics of 1 237 SNPs in the assay

Of the 1237 SNPs in the KASP assay, 1151 (93%) were successfully called in the 100 lines. SNP markers that were monomorphic (75 SNPs) or had missing data points more than 20% (19 SNPs) in the diversity panel of inbred lines were removed from further analysis. As a result, a total of 1057 SNPs (91.83%) was called successfully with high quality. Of the 100 maize lines, 4 lines were deleted from the next statistical analysis due to missing data called ≥20%. Among the 1057 informative SNPs, base changes involved A/G (673), T/C (11), A/C (176), G/T(4), A/T (71), C/G (122). Base transition A/G and T/C variations accounted for more than 50% of the polymorphic SNPs in lines, and base transversions of other types accounted for the remainder (Table 4.2). A detail list of these informative SNP loci including chromosome, base change, minor allele frequency (MAF), heterozygosity, gene diversity, and PIC is presented in Appendice Table S2. Of the 1057 SNPs, 55.91% (591 of 1 057) had a MAF >0.2 and were selected as markers with normal allele frequencies. Approximately 25.26% SNPs (267 of 1057) had a MAF ≤0.1, and 10.12% (107 of 1 057) had a MAF ≤0.05. In addition, 88 (8.33%) showed almost equal allele frequencies (with MAF close to 0.5) for two alternative alleles (Figure 4.1).

Table 4. 2. Distribution and allelic frequency of 1 057 informative single nucleotide polymorphisms

		Minor Allele Frequency			SNP variation types					
Chromosome	SNP no	<=0.05	<=0.1	<=0.2	AC	AG	AT	CT	CG	GT
1	173	19	43	81	30	109	13	2	19	0
2	114	14	27	46	20	72	9	1	11	1
3	119	24	33	50	18	76	10	0	15	0
4	115	10	35	59	18	71	7	2	17	0
5	133	12	41	65	21	87	12	0	12	1
6	84	6	17	35	14	55	8	0	7	0
7	64	4	17	30	10	40	0	2	12	0
8	97	5	18	35	24	58	2	1	10	2
9	73	5	19	32	11	49	2	3	8	0
10	69	8	15	25	9	45	6	0	9	0
unknown	16	0	2	8	1	11	2	0	2	0
Total	1057	107	267	466	176	673	71	11	122	4

The average PIC was 0.256, ranging from 0.02 to 0.375 with a peak distribution between 0.350 and 0.375 (Figure 4.1). In the present study, the unbiased gene diversity had a range of 0.020 to 0.496 and an average value of 0.314. The average heterozygosity of each line was 3.8%, this is within expected ranges of normal level of residual heterozygosity in inbred lines of maize. Among all the germplasm, the temperate set showed the lowest heterozygosity with an average of 0.75% ranging from 0 to 1.2 % (Table 4.3).

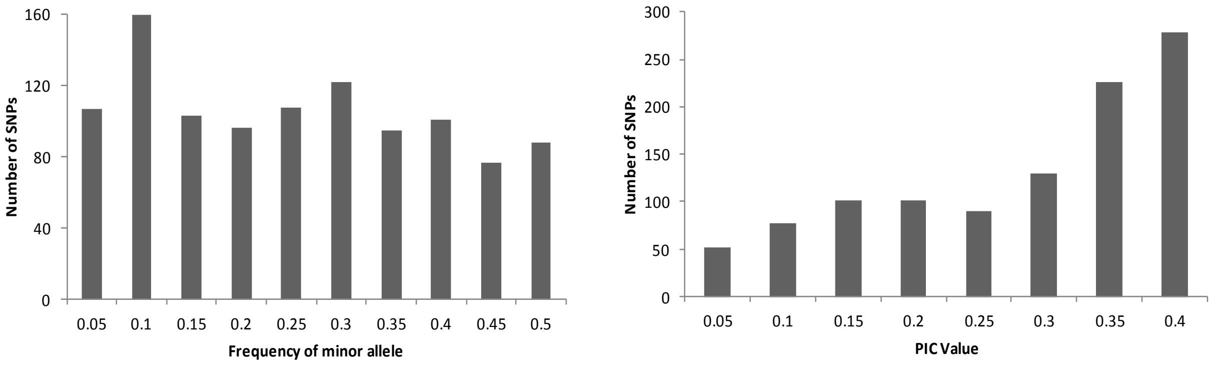


Figure 4. 1. Frequency distribution of minor allele and Polymorphic information content (PIC) among 96 maize inbred lines based on 1057 single nucleotide polymorphisms (SNPs).

On the other hand, eight lines including 5 INERA (ELN431251, ELN43453, ERL24212, ERL257118 and ERL26411) 2 CIMMYT (VL054881 and VL057903) and 1 IITA (TZEI8) line had more than 10% heterozygosity. These need further selfing to reduce the residual heterozygosity. The heterozygosity rate of the 96 inbred lines is provided in Appendices Table S1. Heterozygosity rate of each SNP across all the inbred lines ranged from 0 to 63.6% with an average of 4.1%.

Table 4. 3. Inbred lines heterozygosity rate for each germplasm set

Germplasm	**Number of lines**	**Heterozygosity**		
		Minimum	Maximum	Average
CIMMYT	16	0.002838	0.199622	0.037666
IITA	15	0.002838	0.124882	0.035509
INERA	55	0.008515	0.220435	0.044104
Temperate	10	0.000946	0.012299	0.007474
All lines	96	0.000946	0.220435	0.037873

As the quality of markers have an effect on diversity estimation, we identified 580 high quality SNPs out of 1057 based on two factors : normal MAF (> 0.2), and high PIC values (> 0.25). In addition, to understand the influence of the two subsets in INERA germplasm on genetic diversity, the parameters of genetic diversity were estimated for each subset. The SNPs subset selected and entire germplasm were compared with the entire counterparts for PIC and gene diversity (Table 4. 4).

Using all 1057 informative SNPs and 96 inbred lines, temperate germplasm was found to show the highest average PIC value and gene diversity, followed by INERA germplasm for PIC value and by CIMMYT germplasm for gene diversity, whereas the IITA germplasm showed the lowest PIC value and gene diversity (Table 4. 4).

Table 4. 4. PIC and gene diversity as revealed by 580 SNPs selected compared to their entire counterparts

	MAF	Gene Diversity	Heterozygosity	PIC
1057 SNPs				
Temperate	0.256	0.302	0.008	0.268
IITA	0.202	0.252	0.038	0.218
CIMMYT	0.223	0.278	0.039	0.238
INERA	0.211	0.282	0.048	0.232
Subset A	0.199	0.263	0.044	0.219
Subset B	0.205	0.263	0.055	0.222
580 High quality markers				
Temperate	0.306	0.348	0.01	0.305
IITA	0.287	0.348	0.053	0.295
CIMMYT	0.314	0.372	0.054	0.311
INERA	0.321	0.408	0.071	0.326
Subset A	0.305	0.383	0.067	0.312
Subset B	0.302	0.37	0.079	0.307

On the other hand, using 580 high-quality markers, INERA germplasm was found to show the highest average PIC value and gene diversity, followed by CIMMYT germplasm, while the IITA germplasm still showed the lowest (Table 4. 4). The order of genetic diversity in different germplasm sets changed between the 1057 SNPs and the 580 high quality markers selected, however there was a significant increase in the estimates of PIC and gene diversity for all germplasm collections. Compared to the entire set of INERA germplasm, the estimates for PIC and gene diversity decreased in the two subsets. In subset A (FBC6 derived lines), the order of genetic diversity in different germplasm collections did not change whereas with subset B (ESPOIR derived lines) the order changed, indicating that the level of genetic diversity in subset A was slightly higher than that in subset B. However, the PIC value and gene diversity in each subset of INERA germplasm were not significantly different from the entire INERA germplasm set. This could be attributed to the fact that the two subsets share a common genetic background. In all cases, the IITA maize lines tested in this study appear to have relatively narrow genetic base as revealed by their estimates for both PIC and gene diversity.

4. 3.2 Population structure and Relative kinship

An admixture model-based clustering method in STRUCTURE was implemented to infer population structure for all 96 tested lines and it was run for the number of fixed subgroups k from 1 to 12. The likelihood (Ln) value of this analysis is shown in Figure 4.2. Likelihood increases continuously with no obvious inflection point. This could imply that the lines included in the analysis were very diverse as well as highly mixed, however the estimated Ln probability of data value for each given Pritchard's K (the supposed number of subpopulations based on the model) increased sharply when K < 5, and the increasing trend became more moderate for K >5. In addition, the Evanno criterion supported the choice of k = 5 as the highest level of structure, so that five genetically distinct subgroups can be claimed. Three of these groups (Groups 3, 4, and 5) included all INERA maize inbred lines and the other two groups (1 and 2) included maize lines predominantly from Temperate in group 1 and exclusively from IITA for group 2.

The five groups (1,2,3,4 and 5) are named as Temperate, IITA, INERA-1,INERA-2 and INERA-3 respectively (Figure 4. 3).

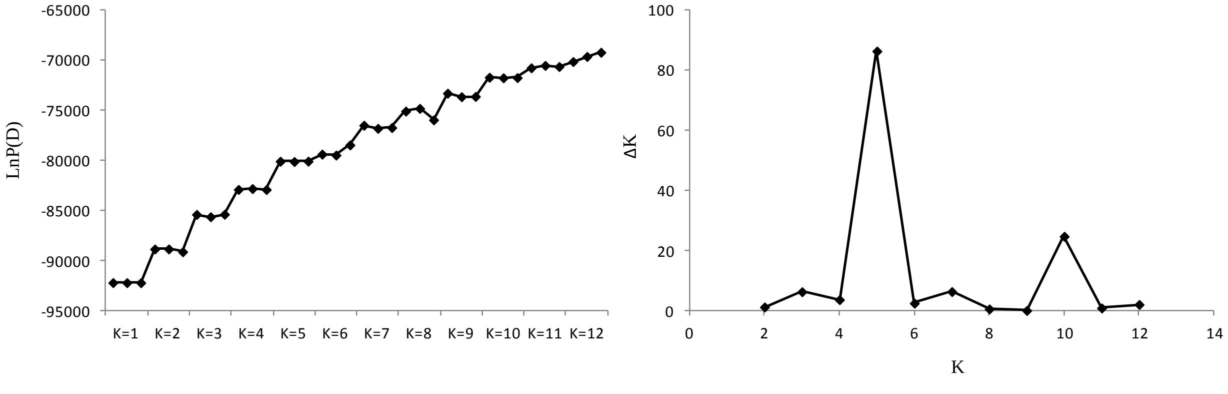


Figure 4. 2. Analysis of the population structure of 96 maize inbred lines. Changing trends of estimated Ln probability of data (LnP (D)) and Pritchard's K (ΔK) over three repeats at each K value in the STRUCTURE analysis are shown.

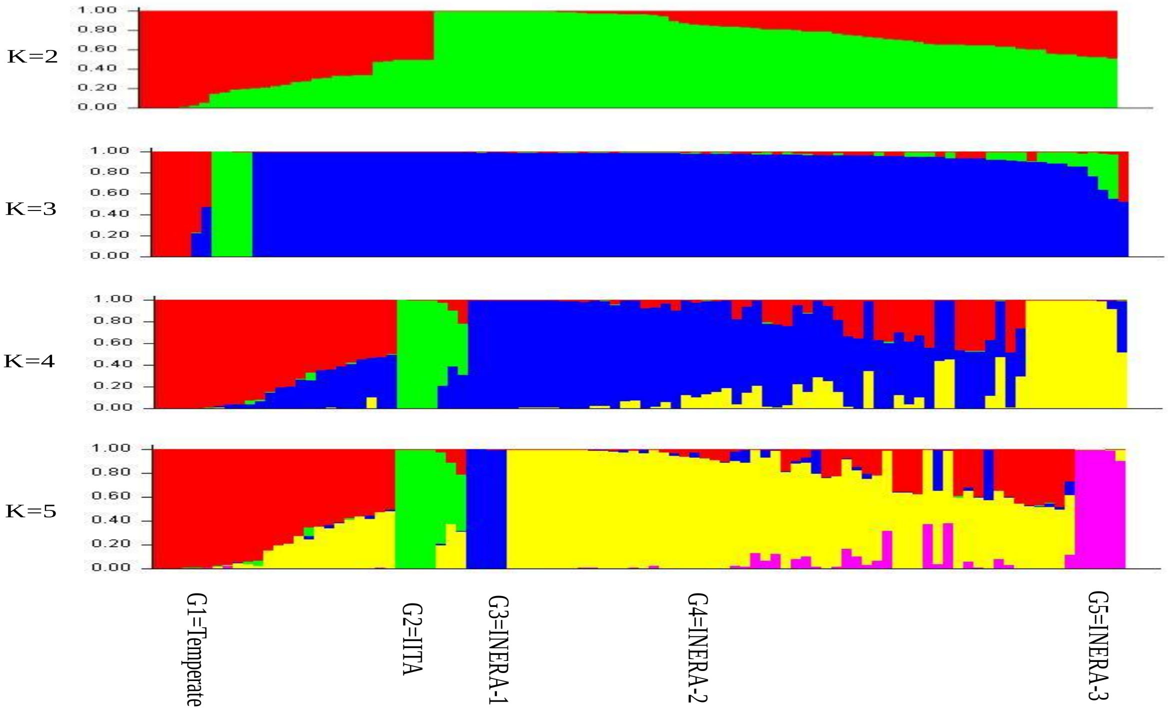


Figure 4. 3. Population structure of the 96 maize lines shown as membership coefficients (Q values). Each vertical bar represents one maize line, which is partitioned into up to k colored segments. Color codes are as follows: Temperate, *red*; IITA, *green*; INERA-1, *blue*; INERA-2, *yellow*; INERA-3, *purple.*

Group 1 (named temperate) consisted of 10 temperate lines, 7 CIMMYT lines and 1 IITA line. The temperate lines belong to different heterotic groups (BSSS, Lancaster, European group). In addition, the line VL05616 from CIMMYT, included in this group, was also classified with temperate lines particularly with Lancaster heterotic group in a previous study (Lu *et al*., 2009). Temperate line (FR812) constitutes 50% of its pedigree. Group 2, IITA, contained only 5 IITA inbred lines. Group 4, also named INERA-2, was the largest group containing 41 INERA inbred lines, of which 23 were extracted from FBC6, 17 from ESPOIR, and 1 line (FBML10) selected from TZI35 in INERA. In addition, 3 other IITA inbred lines belong to this group. The group 3, INERA-1, and the group 5, INERA-3, contained 4 and 5 INERA inbred lines respectively, that are closely related in pedigree. In addition to the inbred lines that were clearly assigned with probability ≥ 0.60 to a single group (population), 19 inbred lines (19.8% of the total) could not be clearly assigned to any of these groups. These lines, called mixed group, include 8 lines from CIMMYT, 6 from IITA and 5 from INERA. Out of six testers used in this study belonging to heterotic groups A and B, only TZEI 17 (heterotic group A) was assigned to one group (INERA-2). The remaining testers (one from heterotic group A and 4 from group B) were included in the mixed group. Inbred lines with proportional memberships in the model-based groups are presented in Table 4.5.

Relative kinship

The relative kinship reflects the approximate degree of identity between two given individuals. Relative kinship coefficients between pairs of lines varied from 0 to 1.55, with an overall average of 0.039, and 61.3% of the pairwise kinship estimates had a value of zero (Figure 4. 4), indicating that the lines were unrelated. Kinship analysis of INERA germplasm showed the lack of redundant lines among the germplasm since kinship coefficients for approximately 64% of the pairs of lines had a value of zero.

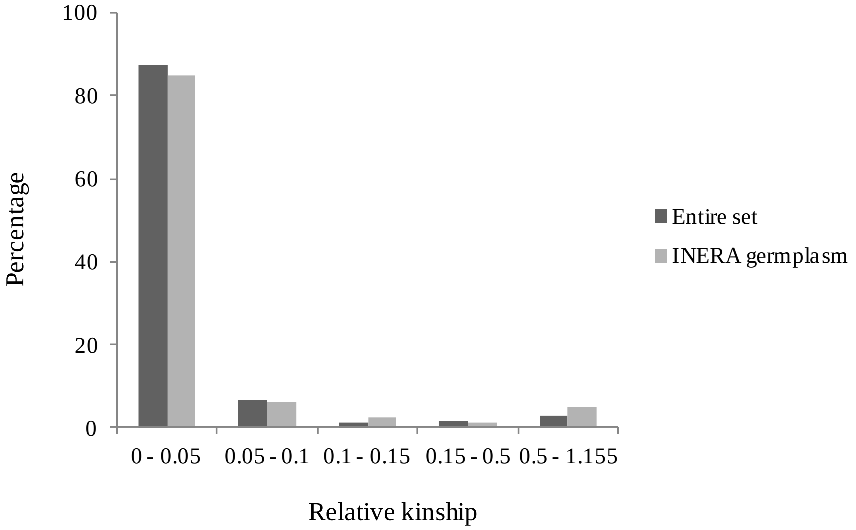


Figure 4. 4. Distribution of pairwise relative kinship values for 96 (entire set) and 54 (INERA) maize inbred lines.

Table 4. 5. Inbred lines with their proportional memberships in the model-based subgroups determined by STRUCTURE

Group	Line	Temperate	IITA	INERA-1	INERA-2	INERA-3
Group1	B73	0.989	0.001	0.000	0.001	0.009
Group1	C103	0.998	0.002	0.000	0.001	0.000
Group1	DE811	0.997	0.001	0.000	0.001	0.001
Group1	EP1	0.978	0.001	0.000	0.019	0.001
Group1	F2	0.966	0.004	0.001	0.006	0.024
Group1	F252	0.991	0.006	0.000	0.003	0.000
Group1	F7	0.992	0.002	0.001	0.002	0.003
Group1	Mo17	0.996	0.003	0.000	0.000	0.000
Group1	OH43	0.932	0.044	0.000	0.023	0.002
Group1	TZEI146	0.654	0.074	0.026	0.246	0.000
Group1	VL0511247	0.630	0.000	0.028	0.341	0.001
Group1	VL05616	0.950	0.000	0.001	0.049	0.000
Group1	VL057903	0.724	0.002	0.001	0.271	0.001
Group1	VL057967	0.614	0.000	0.003	0.382	0.001
Group1	VL058014	0.800	0.000	0.001	0.198	0.000
Group1	VL058025	0.788	0.000	0.002	0.209	0.001
Group1	VL058589	0.645	0.000	0.003	0.351	0.002
Group1	VL081466	0.848	0.001	0.001	0.149	0.002
Group1	W182E	0.936	0.025	0.000	0.034	0.005
Group2	TZEI148	0.001	0.999	0.000	0.001	0.000
Group2	TZEI158	0.001	0.998	0.000	0.001	0.000
Group2	TZEI161	0.003	0.990	0.000	0.005	0.002
Group2	TZEI177	0.027	0.765	0.015	0.193	0.000
Group2	TZEI23	0.001	0.999	0.000	0.001	0.000
Group3	ELN42441	0.000	0.000	0.997	0.002	0.000
Group3	ELN42442	0.001	0.000	0.994	0.003	0.001
Group3	ELN42444	0.000	0.000	0.998	0.001	0.000
Group3	ELN42445	0.001	0.000	0.996	0.002	0.001
Group4	ELN39111	0.067	0.000	0.046	0.783	0.103
Group4	ELN39381	0.002	0.000	0.001	0.995	0.001
Group4	ELN39382	0.007	0.000	0.024	0.968	0.001
Group4	ELN39391	0.028	0.000	0.043	0.927	0.003
Group4	ELN39392	0.030	0.000	0.026	0.943	0.002
Group4	ELN39427	0.059	0.002	0.002	0.935	0.002
Group4	ELN401172	0.075	0.000	0.009	0.750	0.166
Group4	ELN402213	0.006	0.000	0.001	0.967	0.027
Group4	ELN40441	0.002	0.000	0.001	0.866	0.132
Group4	ELN40791	0.002	0.000	0.010	0.981	0.007
Group4	ELN40823	0.003	0.000	0.202	0.779	0.016
Group4	ELN40941	0.002	0.000	0.006	0.982	0.009
Group4	ELN41271	0.006	0.000	0.002	0.608	0.384
Group4	ELN41272	0.012	0.000	0.001	0.672	0.315
Group4	ELN41273	0.003	0.000	0.002	0.620	0.375
Group4	ELN424103	0.007	0.000	0.342	0.614	0.037
Group4	ELN431251	0.075	0.001	0.002	0.917	0.005
Group4	ELN43453	0.086	0.000	0.001	0.910	0.003
Group4	ELN443101	0.002	0.000	0.064	0.864	0.070
Group4	ELN45111	0.098	0.000	0.020	0.800	0.082

Continued

Group	Line	Temperate	IITA	INERA-1	INERA-2	INERA-3
Group4	ELN45272	0.184	0.000	0.007	0.804	0.005
Group4	ELN47132	0.002	0.000	0.109	0.870	0.020
Group4	ELN48392	0.204	0.000	0.044	0.721	0.030
Group4	ERL21811	0.148	0.001	0.025	0.722	0.104
Group4	ERL221712	0.220	0.000	0.001	0.708	0.071
Group4	ERL221813	0.234	0.000	0.003	0.758	0.004
Group4	ERL227213	0.012	0.000	0.001	0.973	0.014
Group4	ERL23025	0.014	0.000	0.002	0.981	0.002
Group4	ERL230212	0.003	0.000	0.004	0.990	0.003
Group4	ERL24211	0.017	0.000	0.083	0.872	0.028
Group4	ERL242152	0.001	0.000	0.001	0.997	0.001
Group4	ERL242153	0.001	0.000	0.001	0.997	0.001
Group4	ERL242154	0.001	0.000	0.001	0.997	0.001
Group4	ERL24212	0.005	0.001	0.002	0.864	0.127
Group4	ERL251323	0.005	0.000	0.002	0.989	0.003
Group4	ERL251352	0.002	0.000	0.021	0.975	0.002
Group4	ERL257112	0.222	0.002	0.002	0.757	0.018
Group4	ERL257118	0.004	0.000	0.001	0.987	0.008
Group4	ERL263222	0.004	0.000	0.001	0.993	0.002
Group4	ERL264411	0.027	0.000	0.001	0.967	0.005
Group4	FBML10	0.352	0.000	0.006	0.639	0.004
Group4	TZEI16	0.376	0.001	0.001	0.621	0.000
Group4	TZEI17	0.102	0.002	0.008	0.887	0.000
Group4	TZI3	0.354	0.003	0.007	0.636	0.000
Group5	ELN41111	0.001	0.000	0.002	0.008	0.990
Group5	ELN41112	0.001	0.000	0.003	0.096	0.900
Group5	ELN41113	0.000	0.000	0.000	0.002	0.997
Group5	ELN41114	0.000	0.000	0.000	0.001	0.998
Group5	ELN41115	0.000	0.000	0.000	0.001	0.998
Mixed group	CML444	0.502	0.001	0.018	0.479	0.000
Mixed group	ELN424101	0.003	0.000	0.419	0.573	0.004
Mixed group	ELN43574	0.267	0.000	0.117	0.495	0.120
Mixed group	ERL222722	0.344	0.000	0.003	0.568	0.085
Mixed group	ERL225132	0.320	0.000	0.025	0.591	0.064
Mixed group	ERL24132	0.395	0.000	0.008	0.586	0.010
Mixed group	T02058	0.484	0.001	0.017	0.497	0.001
Mixed group	TZEI10	0.572	0.014	0.001	0.412	0.000
Mixed group	TZEI124	0.470	0.007	0.004	0.519	0.000
Mixed group	TZEI149	0.214	0.471	0.003	0.312	0.000
Mixed group	TZEI8	0.109	0.513	0.001	0.378	0.000
Mixed group	TZI18	0.447	0.003	0.030	0.519	0.000
Mixed group	TZI35	0.389	0.016	0.001	0.594	0.000
Mixed group	VL0511298	0.393	0.000	0.013	0.564	0.030
Mixed group	VL0512593	0.525	0.000	0.001	0.466	0.008
Mixed group	VL054794	0.558	0.000	0.001	0.437	0.003
Mixed group	VL054881	0.470	0.001	0.001	0.526	0.002
Mixed group	VL05615	0.551	0.000	0.029	0.415	0.004
Mixed group	VL081464	0.451	0.000	0.002	0.547	0.000

4. 3.3 Cluster analysis

In order to gain further insight into the genetic diversity among different groups of the maize inbreds, a neighbor-joining tree based on Roger’s genetic distance was constructed. The 96 tested lines were classified into three major groups (Figure 4. 5). In the first major group composed largely of INERA lines, FBC6 derived lines were grouped together with 2 lines from CIMMYT and 3 lines from IITA. The second major group was divided into two subgroups : 21 INERA lines, representing ESPOIR derived lines and 1 CIMMYT line in first subgroup and the 10 temperate lines plus 2 CIMMYT and 2 IITA lines in the second subgroup together with another group representing 8 IITA lines. The third major group, represented mostly CIMMYT germplasm and contained 11 lines from CIMMYT and 2 lines from IITA.

Each major group included 2 testers with well-known heterotic grouping. Testers VL0511298 and TZEI 17 (Group A) were clustered in the first major group containing mostly lines from INERA (FBC6 derived). The testers, TZEI 10 and TZEI 23, belonging to heterotic group B, were placed in the second major group and testers VL054881 and T02058 from heterotic group B, were included in the third major group. All the groups identified by the STRUCTURE analysis were also identified by the cluster analysis except the group 3 (named as INERA-2. Futhermore, some inbred lines assigned to some groups by the structure analysis were placed in different groups by the cluster analysis. In addition, the third cluster group was mainly composed of lines assigned to the mixed group by structure analysis.

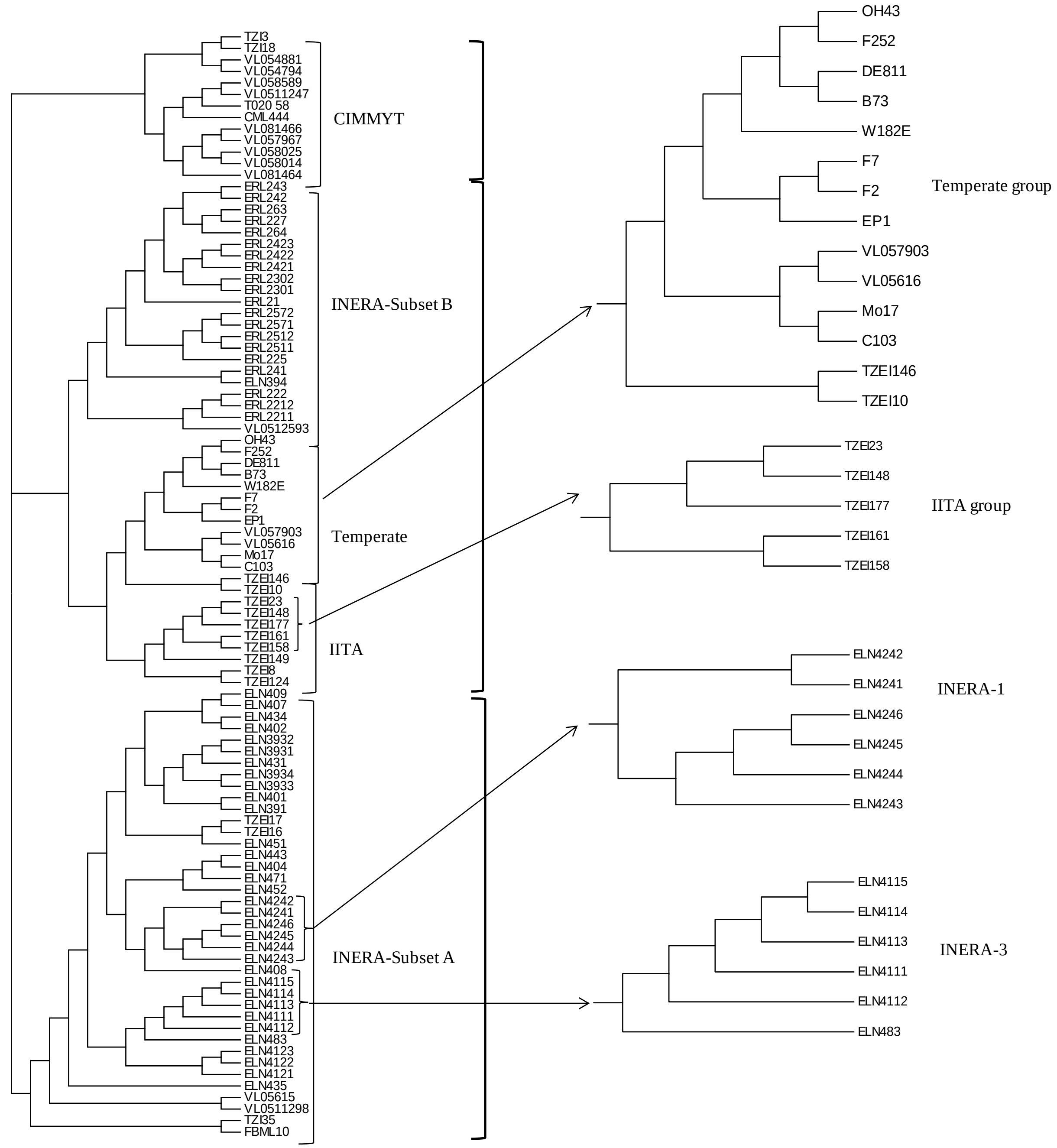


Figure 4. 5. Neighbor-joining (NJ) tree for the 96 maize inbred lines based on Roger's genetic distance. A sectional tree representing Temperate, IITA, INERA-1 and INERA-3 groups identified in the structure analysis are shown in the right. Inbreds codes are presented in Table S1 (Appendices).

4. 3.4 Principal component analysis

Principal Component Analysis (PCA) has been proposed as an alternative to STRUCTURE software for studying population structure of genotypic data (Patterson *et al.*, 2006). Principal Component Analysis results were consistent with those of the structure analysis. PCA on the entire set of 96 inbred lines (Figure 4.6) showed a clear separation of the 5 groups identified in structure analysis. The second PC separated group 1 (temperate) from the 4 other groups. Group 2 (IITA) was well separated from the 3 other groups (INERA-1, INERA-2 and INERA-3) by the first PC.

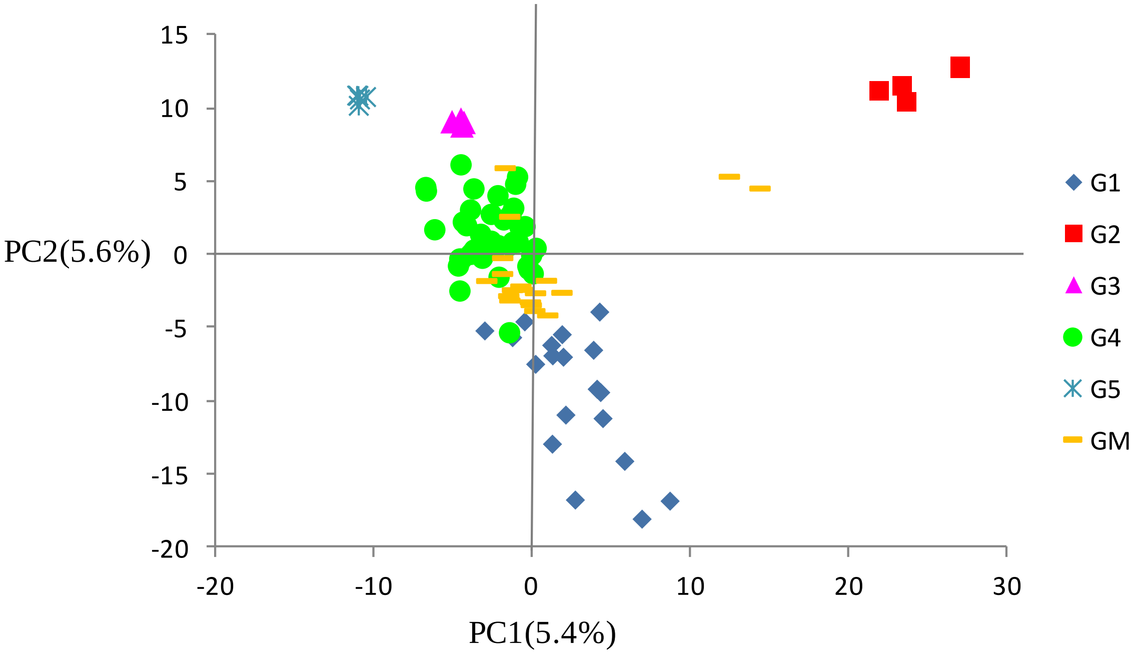


Figure 4. 6. Principal component analysis of the entire set of maize lines.
G1,G2,G3,G4,G5 and GM refer to groups 1,2,3,4, 5 and mixed group respectively in STRUCTURE.

Principal component analysis (PCA) classified the INERA inbred lines into four distinct groups (Figure 4.7.a), which included the two groups (INERA-1, INERA-3) that were consistent with structure analysis, plus two other groups obtained by the differentiation of the lines in group 3 (INERA-2) from structure analysis. PCA identified a subgroup that consisted of ESPOIR derived lines from INERA-2 group comprised of FBC6 derived and ESPOIR derived lines. PCA of the temperate, CIMMYT and IITA lines (Figure 4.7.b) indicated a separation of the lines into three groups, representing temperate, IITA and a mixture of CIMMYT and IITA lines.

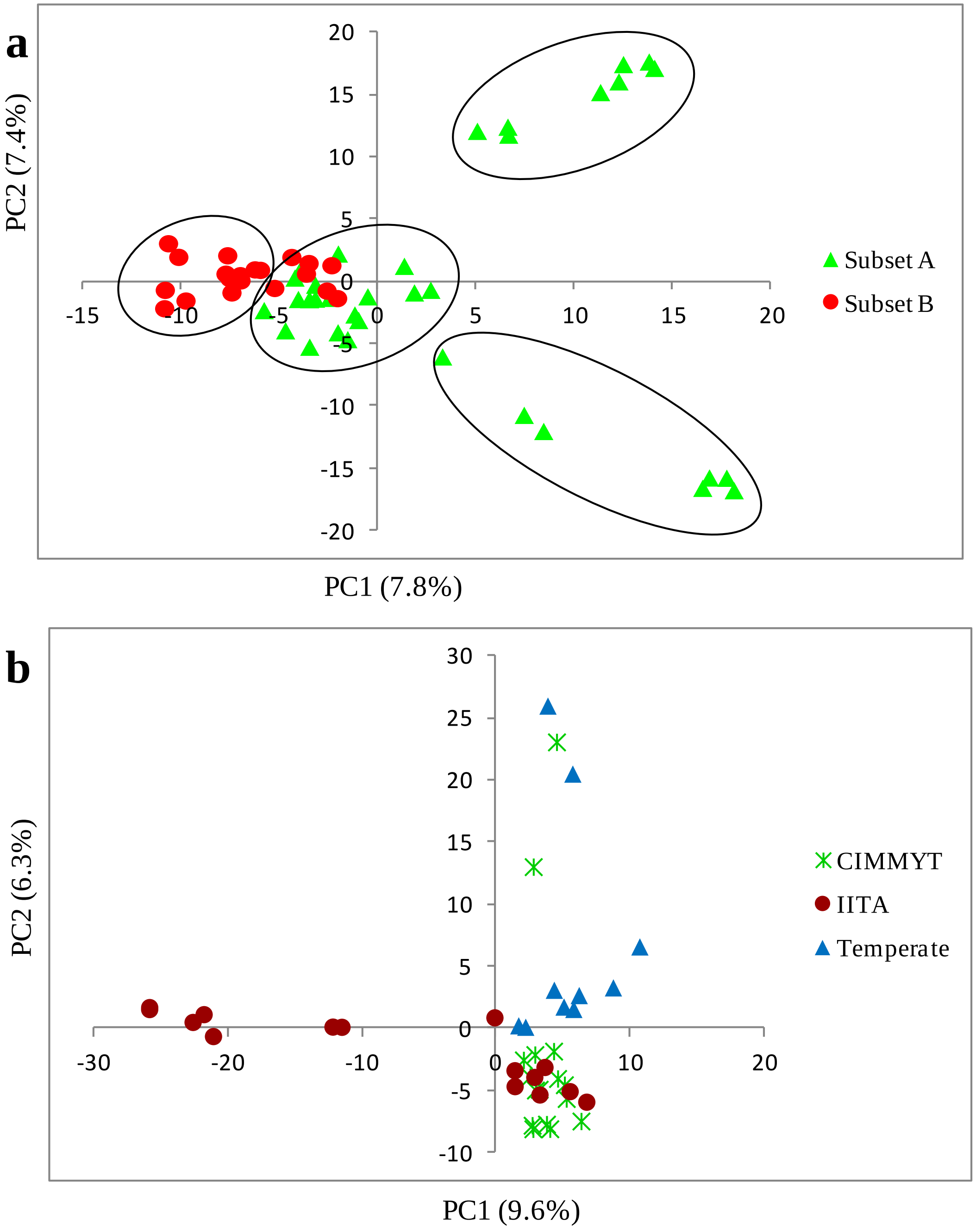


Figure 4. 7. Principal component analysis of INERA inbred lines (a) and a set of temperate, CIMMYT and IITA inbred lines (b)

In order to gain further insight into genetic differentiation between INERA germplasm and CIMMYT, IITA and Temperate germplasms, a principal component analysis was performed on the following sets of inbred lines: set 1 consisted of INERA and Temperate lines, set 2 INERA and CIMMYT lines and set 3 INERA and IITA lines. PCA on set 1 showed a clear separation of INERA and Temperate germplasm (Figure 4.8.a). However, the PCA graph of set 2 did not separate the inbred lines on the basis of the breeding program. The first PC divided the lines into two groups, a group consisted of a subset of FBC6 derived lines while the other group contained a mixture of CIMMYT and INERA (FBC6 derived and ESPOIR derived) lines (Figure 4.8.b). Inbred lines of set 3 were separated into three groups with the first PC separated IITA and INERA (FBC6 derived) lines while the second PC separated the two groups from a mixed group (Figure 4. 8.c).

Formation of new genetically distinct groups

Based on the structure and principal component analysis, four groups which were distinct from each other were constituted. The Temperate group consisted of 10 temperate and 2 CIMMYT lines (VL056516 and VL057903) (Figure 4.7.b). The second group, designated Tropic-IT, contained 7 IITA lines well separated from other germplasms (Figure 4.7.b and 8.c). The third group, designated FBMLs, contained 18 INERA lines (FBC6 derived). These lines were were clearly separated in PCA of set 2 and 3 (Figure 4.8.b and c). The fourth group was the mixed group, designated as the Tropic-M, 15 CIMMYT, 36 INERA and 8 IITA lines which were not separated in the PCA.

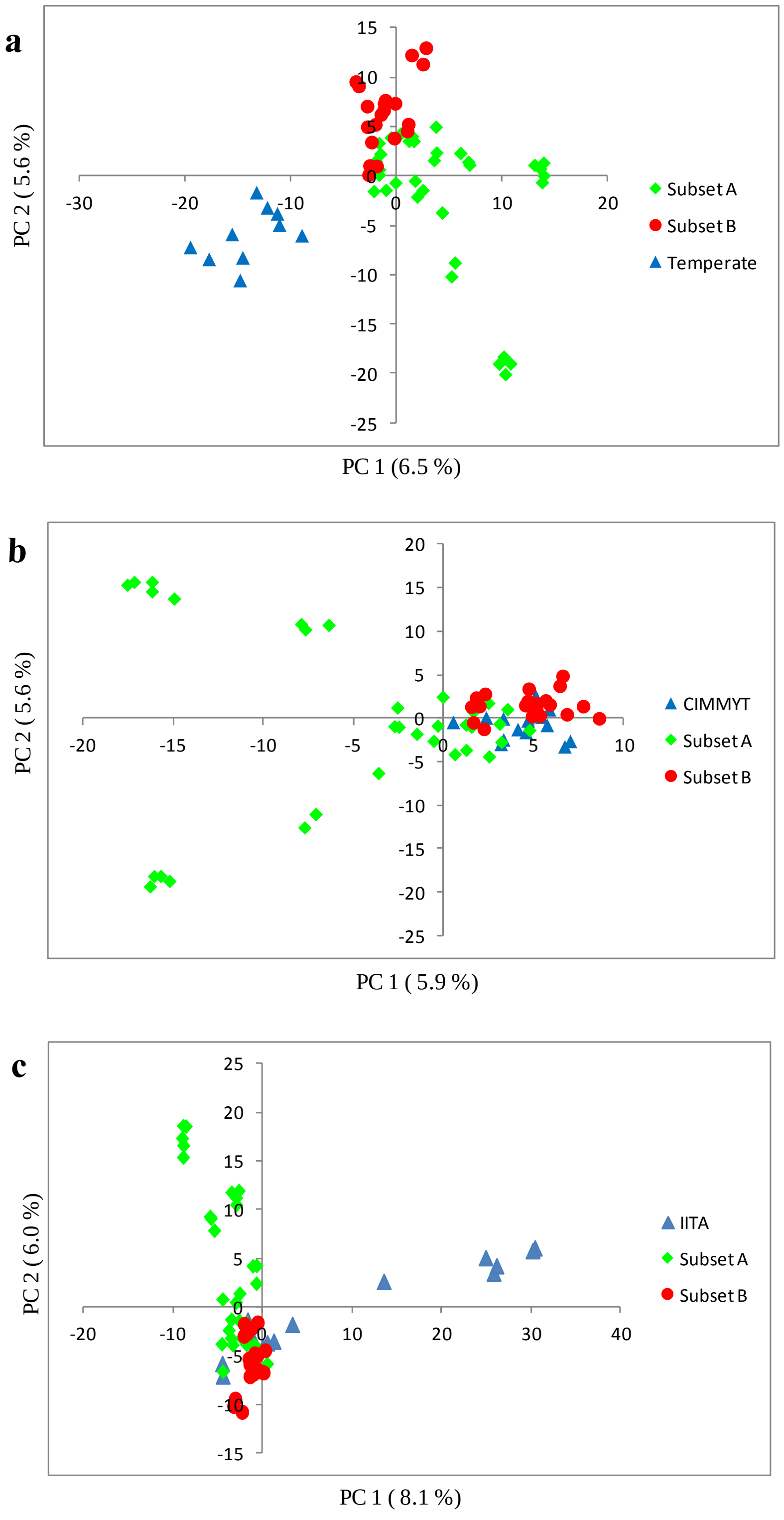


Figure 4. 8. Principal component analysis of set 1(INERA-Temperate lines) (a), set 2 (INERA-CIMMYT lines)(b) and set 3 (INERA-IITA lines)(c) with Subset A and Subset B representing INERA FBC6 and ESPOIR derived lines respectively.

4.3.5 Germplasm differentiation

4.3.5.1 Genetic Distance

Roger's genetic distance of the 96 lines ranged from 0.0205 to 0.448, with the overall average distance of 0.314. However, the majority (64.2%) of the inbred lines fell between 0.300 and 0.400 (Figure 4. 9). Genetic distance based on pairwise comparisons of the 54 INERA lines ranged from 0.029 to 0.348, with the overall average distance of 0.283 while 64.4% of the inbred lines fell between 0.200 and 0.300 (Figure 4.9).

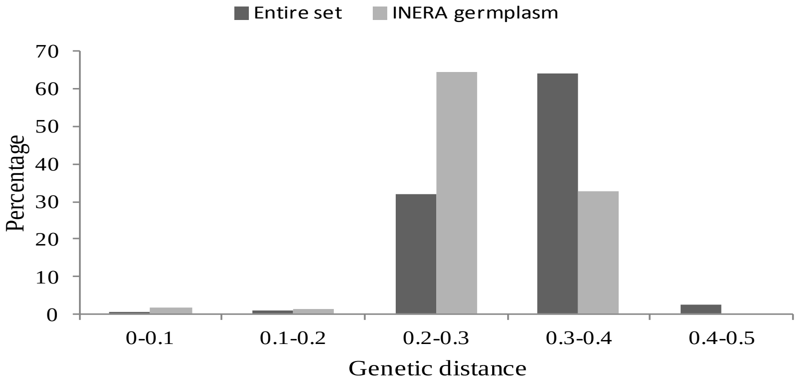


Figure 4. 9. Distribution of pairwise Roger'genetic distance for 96 (entire set) and 54 (INERA) maize inbred lines.

Genetic distances (GD) were also calculated among the four groups according to germplasm origin (Temperate, CIMMYT, IITA and INERA) and among the four new groups (Temperate, Tropic-IT, Tropic-M and FBMLs) formed based on comprehensive structure and principal component analysis. Based on the origin of germplasm, the largest genetic distance was observed between Temperate and IITA lines while the smallest was between CIMMYT and INERA lines (Table 4.6-a). The genetic distance calculated among the new groups revealed that the largest distance was between Temperate and Tropic-IT, and the smallest between FBMLs and Tropic-M (Table 4.6-b).

Table 4. 6. Genetic distance between germplam collection(a) and groups formed based on population structure and PC analysis (b).

a)					b)				
	CIMMYT	IITA	INERA	Temp		FBMLs	Temp	Tropic-IT	Tropic-M
CIMMYT	0.000	0.167	0.134	0.182	FBMLs	0.000	0.217	0.258	0.144
IITA		0.000	0.147	0.206	Temp		0.000	0.289	0.167
INERA			0.000	0.190	Tropic-IT			0.000	0.247
Temp				0.000	Tropic-M				0.000

Temp =Temperate

A broad range of GD values was found for pairs of lines in FBMLs group (Table 4.7). The average GD values for pairs of lines within each group were different indicating that the lines in one group were more or less closely related. The smallest minimum GD value was recorded between pairs of lines from Tropic-IT group.

Table 4. 7. Average, standard deviation (SD), minimum and maximum values of genetic distance estimates among inbred lines in Tropic-IT, Temperate, FBMLs and Tropic-M groups

	Number of pairs of lines	MEAN	SD	MIN	MAX
TROPIC-IT	42	0.154	0.062	0.021	0.247
TEMPERATE	132	0.363	0.055	0.138	0.447
FBMLs	306	0.243	0.068	0.051	0.323
Tropic-M	3422	0.302	0.032	0.029	0.374
All Combinations	9120	0.302	0.033	0.029	0.374

4.3.5.2 Differences in Allele frequencies

To reveal genetic differences among local and exotic maize inbred lines, comparative analysis of allele frequencies was performed for three pairwise comparisons: INERA versus CIMMYT lines, INERA versus IITA, and INERA versus Temperate. Of the 1057 SNPs, a significant difference in allele frequency ($P<0.01$) was observed for 331 (31.3%) SNPs in INERA versus Temperate, 263 (24.6%) SNPs in INERA versus CIMMYT lines, and 252 (23.8%) SNPs in INERA versus IITA. A distribution of allele frequency difference observed in the three pairwise comparisons, ranging from 0.1 % to 71% is shown in Figure 4.10..

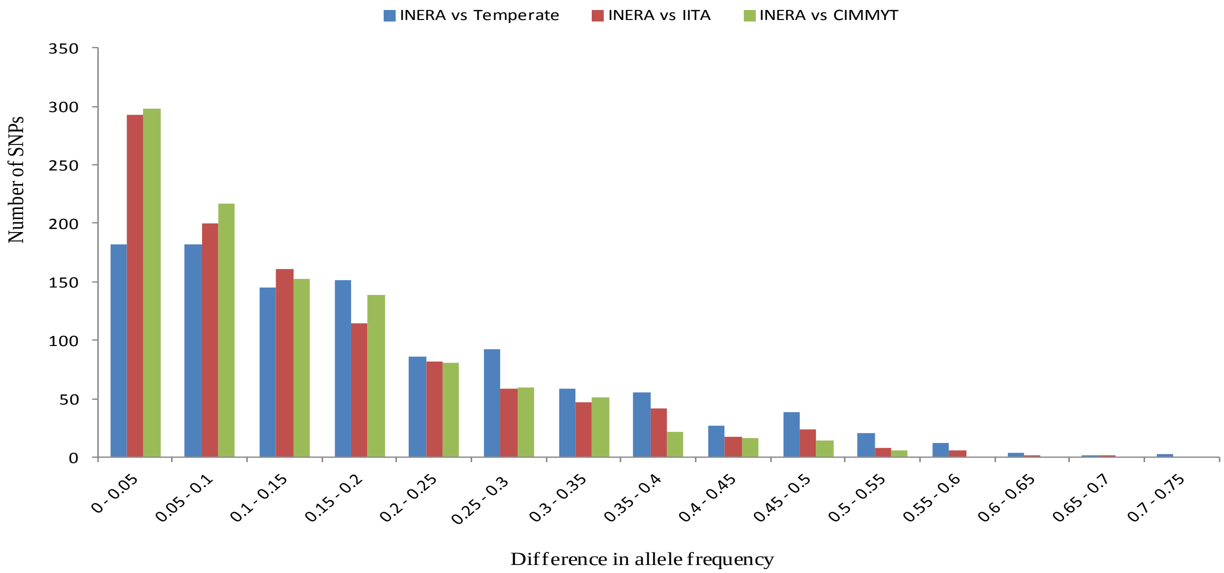


Figure 4. 10. Differentiation of allele frequencies between maize germplasm.

The minimum difference was found in INERA versus IITA comparison whereas the maximum was between INERA versus Temperate. In INERA versus Temperate comparison, the highest difference was 71%. for PZA01352.5 and PZA00643.13 for alleles A/G and G/T, the highest difference in INERA versus IITA was 67%. for PZA02398.2 for alleles A/G and the least highest difference among the three pairwise comparison was 57 % for PZA01073.1 for alleles A/G in INERA versus CMMYT comparison (Table 4.8).

Table 4. 8. Top ten SNPs with significant different allele frequencies among different germplasm origins

SNP Number	SNP Name	chr	Position	Allele	Allele frequency		Allele frequency difference
					I	II	
INERA (I) versus Temperate (II)							
53	PZB01062.3	1	56846728	A	0.25	0.80	0.55
				G	0.75	0.20	0.55
69	PHM12633.15	1	102664092	A	0.99	0.40	0.59
				C	0.01	0.60	0.59
186	PZA00172.12	2	4177515	A	0.14	0.80	0.66
				C	0.86	0.20	0.66
244	PZA00637.6	2	169524491	A	0.35	0.95	0.60
				G	0.65	0.05	0.60
281	PZA01352.5	2	226450168	A	0.86	0.15	0.71
				G	0.14	0.85	0.71
309	PZA00297.2	3	39992968	C	0.24	0.78	0.54
				G	0.76	0.22	0.54
593	PZA00643.13	5	91096945	G	0.91	0.20	0.71
				T	0.09	0.80	0.71
778	PZA02854.13	7	137834376	A	0.32	0.90	0.58
				G	0.68	0.10	0.58
900	PZA01799.1	9	9885916	A	0.76	0.11	0.65
				G	0.24	0.89	0.65
901	PHM11946.19	9	9886093	A	0.26	0.90	0.64
				G	0.74	0.10	0.64
INERA (I) versus CIMMYT (II)							
233	PZA01537.2	2	150984061	A	0.78	0.27	0.51
				G	0.22	0.73	0.51
253	PZA00824.2	2	193996904	A	0.92	0.43	0.50
				G	0.08	0.57	0.50
497	PZA02479.1	4	218367682	A	0.57	0.09	0.48
				G	0.43	0.91	0.48
627	PHM532.23	5	193208321	A	0.98	0.47	0.51
				C	0.02	0.53	0.51
670	PHM2551.31	6	85125455	A	0.68	0.20	0.48
				G	0.32	0.80	0.48
688	PZA01591.1	6	125113941	A	0.08	0.57	0.49
				G	0.92	0.43	0.49
870	PHM12749.13	8	155071379	C	0.12	0.59	0.48
				G	0.88	0.41	0.48
875	PZA00838.2	8	158949206	A	0.15	0.67	0.51
				G	0.85	0.33	0.51
1033	PZA01073.1	10	144549995	A	0.36	0.93	0.57
				G	0.64	0.07	0.57
1045	PZA00311.5	-	-	A	0.08	0.59	0.51
				G	0.92	0.41	0.51

Chr=chromosome

continued

SNP Number	SNP Name	chr	Position	Allele	Allele frequency I	Allele frequency II	Allele frequency difference
INERA (I) versus IITA (II)							
343	PZB02179.1	3	157640380	A	0.8	0.27	0.53
				G	0.2	0.73	0.53
403	PHM2423.33	3	227682146	A	0.25	0.85	0.6
				C	0.75	0.15	0.6
468	PZA03116.1	4	166002318	A	0.71	0.17	0.54
				G	0.29	0.83	0.54
521	PZA01887.1	5	656148	A	0.05	0.6	0.55
				G	0.95	0.4	0.55
864	PZB01454.1	8	145713853	A	0.19	0.75	0.56
				G	0.81	0.25	0.56
997	PZB00409.1	10	84002430	A	0.13	0.69	0.56
				G	0.87	0.31	0.56
1001	PZA02398.2	10	99471436	A	0.06	0.73	0.67
				G	0.94	0.27	0.67
1032	PHM5435.25	10	144239494	A	0.25	0.82	0.57
				G	0.75	0.18	0.57
1035	PZA01001.2	10	146538889	A	0.33	0.86	0.53
				G	0.67	0.14	0.53
1054	PZA02474.1	-	-	A	0.69	0.07	0.62
				G	0.31	0.93	0.62

4.3.5.3 Missing and unique alleles in different germplasm collections

The Temperate, CIMMYT and IITA inbred lines were included in this study for comparison purpose. They are not representative of all Temperate, CIMMYT, and IITA germplasms. Thus, we only identified missing alleles present in INERA lines. In total, there were one hundred missing alleles identified in INERA germplasm which were present in other germplasm. The counterpart allele frequency in Temperate, CIMMYT and IITA germplasm ranged from 3 to 70%. There are 28, 7 and 3 of such alleles that were frequent only in Temperate, CIMMYT and IITA germplasm, respectively, but completely lacking in INERA germplasm (Table 4.9). In addition, 26, 11 and 3 of the missing alleles were present in both CIMMYT-Temperate, Temperate-IITA and CIMMYT-IITA germplasms respectively and 22 were frequent in the three germplasms (Temperate, CIMMYT and IITA) (see Appendices Table S3). For instance, the the allele C at PHM537.22 (chromosome 10) was missing in INERA germplasm but present in 70, 50 and 13.3% of IITA, Temperate and CIMMYT germplasm, respectively.

Table 4. 9. Markers with missing alleles identified in INERA collection compared with CIMMYT, IITA and Temperate germplasm collections

SNP Number	**SNP Name**	**chr**	**Position**	**Missing allele in INERA**	**Allele frequency in counterpart**
INERA versus CIMMYT					
305	PZA03054.5	3	31695534	G	0.13
311	PHM13473.12	3	42534072	G	0.06
344	PZA00828.2	3	157646430	T	0.13
390	PHM1600.31	3	214887907	G	0.09
506	PHM5780.15	4	237583793	G	0.22
763	PZA01210.2	7	75098939	A	0.13
913	PHM7584.7	9	18683799	A	0.06
INERA versus IITA					
265	PHM2094.34	2	208384148	G	0.07
551	PZA00517.7	5	18240488	G	0.07
619	PZA01575.1	5	182337304	A	0.07
INERA versus Temperate					
28	PHM3951.25	1	31928634	A	0.50
60	PZA01135.1	1	82563962	G	0.20
61	PZB01235.4	1	88380865	A	0.11
64	PZA03465.1	1	91279351	C	0.20
108	PZA02655.9	1	217504762	C	0.50
181	PZA00902.1	2	3069068	G	0.30
321	PHM12153.9	3	57612577	A	0.29
325	PHM2439.25	3	69892464	G	0.20
335	PZD00016.4	3	133480598	G	0.11
336	PZD00015.5	3	133481473	G	0.10
367	PHM3075.15	3	189861169	G	0.10
369	PHM824.17	3	190451804	T	0.20
394	PZA02514.1	3	216915474	A	0.20
396	PZA02668.2	3	218119861	A	0.40
463	PZA03317.1	4	149531093	A	0.43
507	PZA00513.1	4	238553534	A	0.10
519	PZA00682.17	4	244992721	A	0.10
625	PZA02426.1	5	191642443	A	0.22
635	PZA02667.1	5	202777978	G	0.20
674	PZA03488.1	6	95789142	A	0.56
681	PZA01055.1	6	120059553	A	0.20
694	PHM3438.2	6	133843693	A	0.20
776	PZA03728.1	7	137207838	G	0.43
856	PHM1668.12	8	133149270	A	0.20
909	PZA02702.1	9	16228585	G	0.10
922	PHM4495.14	9	26765204	A	0.20
993	PZA01677.1	10	70797556	A	0.30
1012	PHM2270.15	10	122667719	A	0.10

By comparing allele frequencies of a specific germplasm collection with those in the entire germplasm set (96 lines), 55 unique alleles were identified that only existed in that specific germplasm collection but not in others (Table 4. 10;11;12). The highest number of unique alleles was found in the temperate lines (Table 4.11). All the temperate lines had at least one unique allele and 9 of the 10 lines had more than 2 unique alleles. Allele frequency of unique allele in temperate, CIMMYT and IITA collection varied from 0.01 to 0.05 while it varied from 0.01 to 0.1 in INERA germplasm.

Table 4. 10. Unique alleles identified in IITA, and CIMMY germplasm

SNP Number	**SNP Name**	**Chr**	**Position**	**UA**	**Allele frequency**	**Inbred lines with unique alleles**
IITA						
265	PHM2094.34	2	208384148	G	0.02	TZI35
551	PZA00517.7	5	18240488	G	0.01	TZEI10
619	PZA01575.1	5	182337304	A	0.01	TZI18
CIMMYT						
305	PZA03054.5	3	31695534	G	0.02	T020.58, VL058014
311	PHM13473.12	3	42534072	G	0.01	VL0511298
344	PZA00828.2	3	157646430	T	0.02	VL057967, VL058589
390	PHM1600.31	3	214887907	G	0.02	VL054794, VL054881
506	PHM5780.15	4	237583793	G	0.04	VL054794,VL054881,VL057903, VL081466
763	PZA01210.2	7	75098939	A	0.02	VL05615, VL0511247
913	PHM7584.7	9	18683799	A	0.01	VL057967

Table 4. 11. Unique alleles identified in Temperate germplasm

SNP Number	**SNP Name**	**Chr**	**Position**	**UA**	**Allele frequency**	**Inbred with unique alleles**
28	PHM3951.25	1	31928634	A	0.05	B73, EP1, F2, F252, W182E
60	PZA01135.1	1	82563962	G	0.02	C103, F7
61	PZB01235.4	1	88380865	A	0.01	DE811
64	PZA03465.1	1	91279351	C	0.02	F2, OH43
108	PZA02655.9	1	217504762	C	0.05	EP1, F2, F7, OH43, W182E
181	PZA00902.1	2	3069068	G	0.03	DE811, F2, F7
321	PHM12153.9	3	57612577	A	0.02	B73, F2
325	PHM2439.25	3	69892464	G	0.02	F2, F7
335	PZD00016.4	3	133480598	G	0.01	F252
336	PZD00015.5	3	133481473	G	0.01	F252
367	PHM3075.15	3	189861169	G	0.01	F7
369	PHM824.17	3	190451804	T	0.02	DE811, F7
394	PZA02514.1	3	216915474	A	0.02	B73, DE811
396	PZA02668.2	3	218119861	A	0.04	B73, DE811, F7, W182E
463	PZA03317.1	4	149531093	A	0.03	DE811, F2, F7
507	PZA00513.1	4	238553534	A	0.01	F252
519	PZA00682.17	4	244992721	A	0.01	F7

continued

SNP Number	SNP Name	Chr	Position	UA	Allele frequency	Inbred with unique alleles
625	PZA02426.1	5	191642443	A	0.02	C103, Mo17
635	PZA02667.1	5	202777978	G	0.02	B73, DE811
674	PZA03488.1	6	95789142	A	0.05	B73, C103, DE811, F252, OH43
681	PZA01055.1	6	120059553	A	0.02	B73, DE811
694	PHM3438.2	6	133843693	A	0.02	EP1, F7,
776	PZA03728.1	7	137207838	G	0.03	EP1, F2, F7
856	PHM1668.12	8	133149270	A	0.02	B73, OH43
909	PZA02702.1	9	16228585	G	0.02	W182E
922	PHM4495.14	9	26765204	A	0.02	C103, DE811
993	PZA01677.1	10	70797556	A	0.03	EP1, F7, W182E,
1012	PHM2270.15	10	122667719	A	0.01	F7

Chr Chromosome, UA unique allele

Table 4. 12. Unique alleles identified in INERA germplasm

SNP Nb	SNP Name	Chr	Position	UA	Allele frequency	Inbred lines with unique alleles
26	PZB01957.1	1	26283293	A	0.02	ERL242153, ERL242154
31	PZA02271.1	1	38607726	A	0.02	ELN41271, ELN41273
43	PHM4313.17	1	45788920	A	0.03	ELN41271, ELN41273, ERL21811
56	PHM5098.25	1	65825628	A	0.09	ELN41111, ELN41113, ELN41114, ELN424103 ELN443101, ERL227213, ERL23025, ERL230212 ERL24212, ERL263222, ERL264411
59	PZA00752.1	1	80803012	A	0.08	ELN402213, ELN40823, ELN41112, ELN41113 ELN41114, ELN41115, ELN41273, ELN45111 ERL221712, ERL24132
121	PHM5293.11	1	243339423	A	0.04	ELN39382, ELN40441, ELN40791, ELN443101
160	PZA00235.9	1	292728976	C	0.02	ERL221712, ERL24211, ERL24212
165	PZA01238.1	1	294334019	A	0.09	ELN401172, ELN41111, ELN41112, ELN45111 ERL242152, ERL242153, ERL242154, ERL24132
287	PHM343.7	3	1233789	A	0.02	ELN401172, ELN43574
377	PZA02122.9	3	197797720	A	0.10	ELN402213, ELN431251, ELN43453, ELN43574 ELN443101, ERL242152, ERL242153, ERL242154 ERL251323, ERL26411
378	PHM5716.60	3	198309265	A	0.10	ELN424103, ELN42441, ELN42442, ELN42444 ELN42445, ELN443101, ERL242152, ERL242153 ERL242154
641	PHM4349.3	5	206329214	A	0.02	ELN40823, ELN47132
723	PHM2108.61	6	161663814	C	0.03	ERL21811, ERL242153, ERL242154
876	PZA00706.16	8	159897914	G	0.10	ELN39111, ELN39382, ELN40791, ERL21811 ERL230212, ERL242152, ERL242153, ERL242154 ERL257112
891	PZA00189.23	8	170014475	C	0.07	ELN41111, ELN41112, ERL221712, ERL222722 ERL225132, ERL24212
987	PZA00079.1	10	18903460	A	0.01	ERL23025
989	PZA02443.8	10	29194886	A	0.10	ELN39381, ELN39382, ELN39427, ELN40823 ELN48392, ERL21811, ERL242152, ERL242153 ERL242154, ERL24211, ERL24212, ERL264411

Chr Chromosome, UA unique allele.

4.3.6 LD and LD decay across the maize genome and germplasm sets

LD across 935 SNPs with minor allele frequency (MAF) >0.05 was investigated among the entire set (96 lines), Tropical (CIMMYT, IITA and INERA lines) and INERA sets. In the 96 inbred lines, 43.32% of the pairwise SNPs had the r^2 more than 0.1, in which LD was significant at the 0.01 level between 6.04% of the pairwise SNPs (Table 4.13). In the INERA set, about 58% of pairwise SNPs across 10 chromosomes showed LD with r^2 value more than 0.1, and 4% of the pairwise SNPs with r^2 >0.1 was significant. In the tropical set, 46.73% of pairwise SNPs showed LD with r^2 >0.1, which was significant (5.6%). The extent of LD across 10 chromosomes had distinct differences. The percentage of pairwise SNPs in significant LD with r^2 >0.1 on chromosome 10 in the entire set (~10%) was higher than others (Table 4.13).

Table 4. 13. Percent pairwise SNP markers in linkage disequilibrium (LD) at P=0.01 level in different (entire, tropical and INERA) sets of maize germplasm.

Chromosome	Pairwise SNPs in LD with r^2 > 0.1 (%)			Pairwise SNPs in LD with r^2 > 0.1 (%) significant (P <0.01)		
	Entire	Tropical	INERA	Entire	Tropical	INERA
1	44.13	47.44	59.11	5.56	5.03	3.76
2	40.53	43.19	54.13	4.64	4.58	2.86
3	40.31	43.53	52.58	4.89	4.67	2.85
4	44.19	48.44	60.37	7.54	7.60	5.78
5	43.62	48.26	59.93	5.72	4.99	3.27
6	42.13	46.04	56.65	6.16	5.45	3.52
7	43.67	46.88	57.48	5.56	5.55	5.47
8	43.69	46.67	57.48	7.16	6.31	4.09
9	42.76	46.23	59.72	5.44	4.83	4.07
10	50.60	51.84	61.43	9.94	8.91	7.85
All	43.32	46.73	57.88	6.04	5.60	3.99

The percentage of pairwise SNPs in significant LD with r^2 >0.1 on chromosome 2 was the lowest (4.6%) for the entire set. The proportion of pairwise SNPs with significant LD and r^2 >0.1 on the remaining chromosomes ranged from 5% to 7%.

The mean r^2 values pooled over all 10 chromosomes in different categories of map distance for the entire, Tropical and INERA maize germplasm sets are summarized in Table 4.14. In the three maize germplasm sets, mean r^2 decayed rapidly with increasing physical distance but it did not decrease with the increase of physical distance between 0.2 and 2 kbp (Figure 4.11). The LD decay distance for r^2 to decrease to 0.1 in INERA set (10– 100 kb) was much larger than in entire and tropical sets (5–10 kb).

Table 4. 14. Mean LD among all SNPs with a minimum allelic frequency greater than 0.05, over different physical distances and across 10 chromosomes for entire, tropical and INERA germplasm sets

Distance	**Entire set**				**Tropical set**			
	N	Mean r^2	Range r^2	SD	N	Mean r^2	Range r^2	SD
0-0.2kb	53	0.407	0-1	0.291	50	0.415	0-1	0.284
0.2-0.5kb	37	0.212	0-0.68	0.192	36	0.214	0-0.68	0.19
0.5-1kb	6	0.232	0-0.54	0.218	6	0.238	0-0.5	0.219
1-2kb	8	0.406	0-0.92	0.289	8	0.387	0-0.91	0.284
2-5kb	15	0.15	0-0.68	0.209	12	0.173	0-0.65	0.219
5-10kb	10	0.06	0-0.18	0.063	7	0.087	0-0.19	0.078
10-100kb	53	0.053	0-0.5	0.098	49	0.044	0-0.51	0.083
100-500kb	267	0.038	0-0.57	0.077	236	0.043	0-0.53	0.081
0.5-1Mb	388	0.027	0-0.54	0.05	346	0.031	0-0.5	0.056
1-10Mb	5136	0.021	0-0.53	0.034	4599	0.023	0-0.55	0.036
10-100Mb	21612	0.016	0-0.42	0.025	19241	0.019	0-0.42	0.028
>100Mb	19476	0.015	0-0.42	0.023	17345	0.017	0-0.41	0.025

continued

Distance	**INERA set**			
	N	Mean r^2	Range r^2	SD
0-0.2kb	51	0.439	0-1	0.335
0.2-0.5kb	32	0.241	0-1	0.258
0.5-1kb	6	0.391	0-0.79	0.382
1-2kb	8	0.463	0.03-1	0.38
2-5kb	10	0.195	0-0.78	0.258
5-10kb	4	0.104	0.01-0.33	0.151
10-100kb	574	0.049	0-0.75	0.079
100-500kb	2163	0.034	0-0.84	0.053
0.5-1Mb	17018	0.029	0-0.78	0.044
1-10Mb	49	0.019	0-0.08	0.021
10-100Mb	2015	0.04	0-0.59	0.061
>100Mb	15709	0.027	0-0.65	0.04

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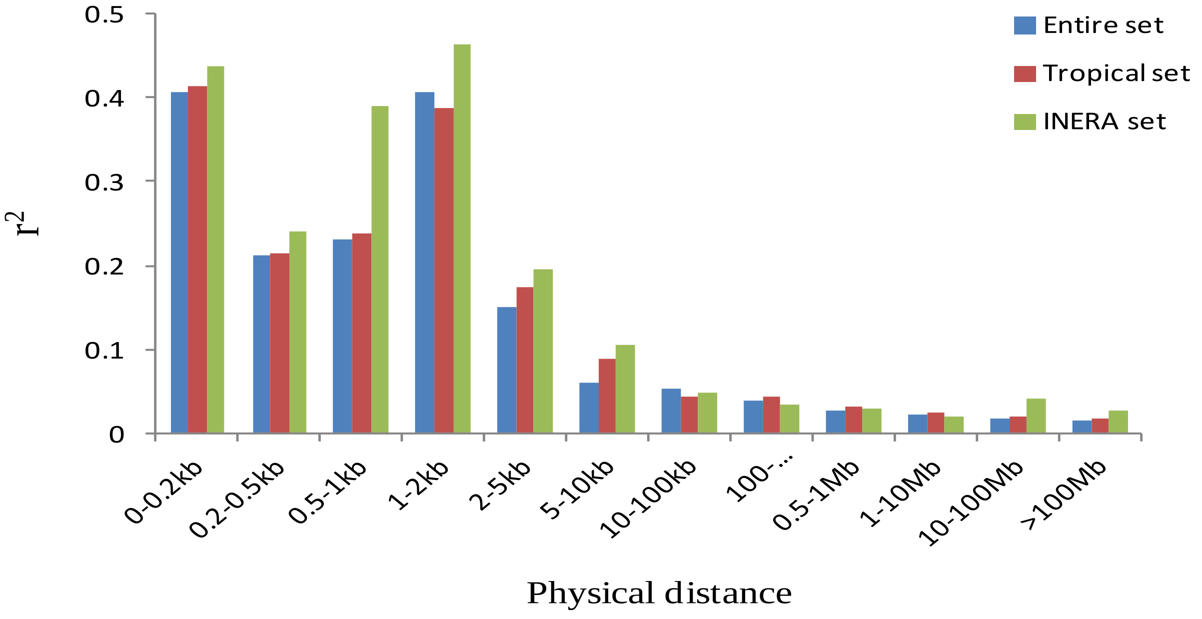


Figure 4. 11. Mean r^2 over different physical distances for INERA, tropical and entire germplasm sets.

The extent of LD also varied greatly from chromosome to chromosome and from region to region within chromosomes. Linkage disequilibrium decay varied from an average 2–5 kb for chromosome 2 and 9 to 100–500 kb for chromosomes 6 and 10 (Table 4.15). LD patterns on chromosomes 4 and 5 had a significant small LD blocks (Figure 4.12). On chromosome 4, the distinct LD block was significant at P<0.0001 with $r^2 > 0.8$ and $r^2 = 1$ in entire and INERA sets, respectively, which spanned around 60 bp and contained 3 SNPs . On chromosome 5, the LD block was significant at P<0.0001 with $r^2 > 0.7$ and $r^2 > 0.9$ in entire and INERA sets, respectively, which spanned around 120 bp and contained 3 SNPs.

Table 4. 15. Average LD decay distance of the 10 chromosomes for $r^2 < 0.1$

Chromosome	LD decay (kbp)
1	5 - 10
2	2 - 5
3	10 - 100
4	5 - 10
5	5 - 10
6	100 - 500
7	10 - 100
8	10 - 100
9	2 - 5
10	100 - 500

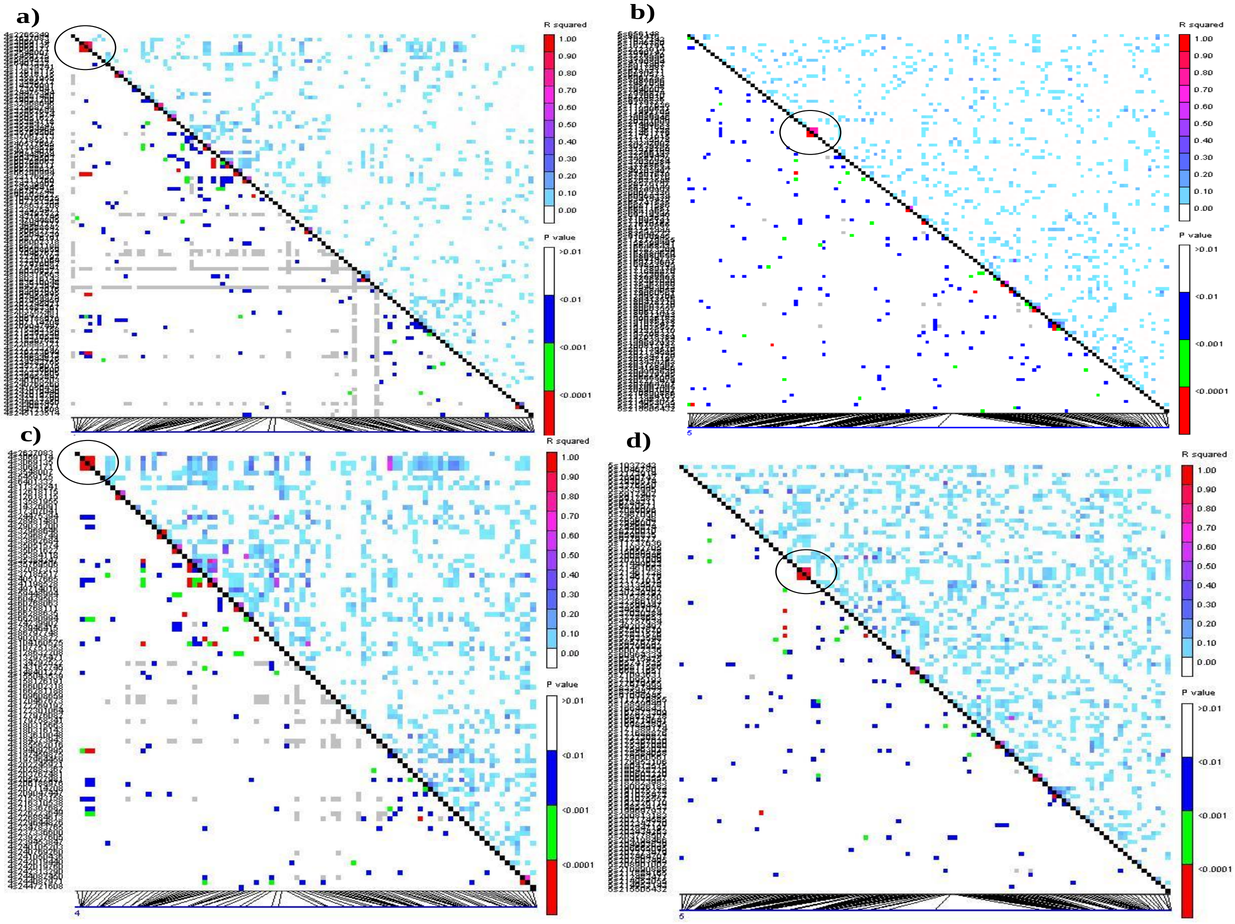


Figure 4. 12. Linkage disequilibrium (LD) patterns on chromosomes 4 and 5 in Entire maize germplasm set (a and b) and INERA maize germplasm set (c and d) genotyped with 935 and 835 SNPs, respectively. The squared correlation coefficients (r^2) for each pair of markers are presented in the upper triangle and their corresponding tests in the lower triangle: white $p>0.01$, blue $0.01>p>0.001$, green $0.001>p>0.0001$ and red $p<0.0001$. The SNPs on each chromosome were aligned up from the left to right long the x-axis.

4.4. Discussion

4.4.1 Performance and quality of SNP genotyping with KASPar technology

A total of 1237 SNPs were scored on 100 diverse maize genotypes with the KASPar technology (Cuppen, 2007). Missing data and heterozygosity levels were low and 93% of the SNPs were scorable. In a cost comparison of KASPar technology, Andrés *et al*. (2011) reported that the failure rate per genotype price and delivery time were lower and competitive in comparison with other SNP genotyping technologies. They also found close correspondence between Sanger sequencing and KASPar genotyping. In the present study, the monomorphic ratio (6.52%) was lower compared to 19.5% in the study of Hao *et al*. (2011b) using a collection of 95 Chinese lines but the ratio was close to 6.3% in the study of Yan *et al*. (2009), which was based on a global collection of 632 lines from tropical and temperate regions. The PIC value (0.256) was consistent with the highest reported value of 0.259 using 1034 informative SNPs and 770 maize inbred lines (Lu *et al*., 2009) and was higher than the value of 0.239 found by Hao *et al*. (2011b) using 1006 informative SNPs and 80 maize inbred lines. Our results showed that, using all the 1057 informative SNPs and a subset of 580 high quality markers, the order of genetic diversity in different germplasm changed contrary to what has been reported in the study of Lu *et al*. (2009), which showed that different subsets of SNPs did not change the order of genetic diversity in different germplasm collections. The sample size could explain this difference. However, there was a significant increase in the estimates of PIC and gene diversity for all germplasm collections, which were consistent in the two studies. Lu *et al*. (2009) recommended 449 out of 1034 SNPs that were found to be the best for the detection of genetic diversity in temperate, subtropical and tropical maize germplasm while Semagn *et al*. (2012) recommended 644 out of 1065 SNPs for routine genetic diversity and mapping studies in tropical and subtropical

CIMMYT maize germplasm. In the present study, we identified 580 high quality SNP markers which can give good result with lost for genetic characterization of tropical maize lines. Out of 580 SNPs 327 (56.4%) and 278 (47.9%) were common between the present study and, Lu *et al*. (2009) and Semagn *et al*. (2012) studies, respectively.

4.4.2 Linkage disequilibrium

Our results showed a total of 6.04, 5.6 and 4% of the SNP pairs across the genome exhibited significant LD with $r^2 > 0.1$ in the entire, tropical and INERA germplasm sets, respectively. The percentage of the entire set was low compared to 34 % found by Lu *et al*. (2011) who tested 280 tropical and 167 temperate maize inbred lines, but we found the percentage of 5.6 % in the subset defined as tropical in the two studies. A rapid LD decline was observed with increasing physical distance in the present study. The average LD decay (5-10 kb) is similar to that observed in 632 (Yan *et al*., 2009) and 447 (Lu *et al.*, 2011) maize inbred lines, but a little greater than the previous estimates reported by Remington *et al*. (2001). The relative rapid LD decay in the tropical maize inbred lines was explained by the fact that tropical maize has experienced much more intensive recombination and contains more rare alleles than temperate lines (Lu *et al*. 2009, Yan *et al*. 2009). LD observed in a population is the result of interplay of many factors including linkage, population structure, relatedness, selection and genetic drift (Flint-Garcia *et al*. 2003). LD generated by linkage is considerably useful for Genome-Wide Association study, but LD generated by population structure and genetic drift would result in spurious marker-trait associations. The effects of selection, mutation and relatedness on LD depend on the population under consideration (Wang *et al.* 2008). A rapid breakdown of linkage-related LD would be favorable for association testing of candidate genes that are located nearby the mapped quantitative trait loci (QTL) and have functional relevance to trait variation (Zhu *et al.,* 2008).

The higher average r^2 for INERA lines was consistent with the lower diversity observed for this material compared to low r^2 for entire set with a high diversity. This is consistent with the results of the previous studies that LD level detected by SNPs or SSRs was higher in narrow germplasm than in diverse germplasm (Ching *et al.*, 2002; Liu *et al.,* 2003). Linkage disequilibrium is the basis of association mapping. The decay of LD over physical distance in a population determines the density of marker coverage needed to perform association mapping. Rapid LD decay requires more markers to capture functional polymorphic markers located close enough to functional sites (Yu and Buckler, 2006).

4.4.3 Genetic diversity in maize germplasm in Burkina Faso

There are few studies on molecular characterization of maize germplasm from Burkina Faso. Sanou (1996) characterized a collection 104 materials (100 local landraces, 3 improved OPVs (SR21, SR22 and IRAT 171) and an ecotype, Maka) using 10 enzymatic markers (ACP, ADH, CAT, DIA, IDH, MDH, GOT, βGLU, PGI, PGD) associated with 18 loci distributed on 9 of the 10 maize chromosomes. The one hundred local landraces comprised 33 grown in farmers' fields and 67 backyard landraces. The results showed that the two alleles Idh1-2 and Idh3-4-210, present in SR22 with a frequency of 0.402 and 0.134, respectively, were also present in 10 landraces at low frequency indicating the introgression of these alleles. In addition, F_{st} estimator differentiated the non introgressed landraces into two groups by farm localizations with significant genetic difference. The genetic structure observed was associated with the specific farming conditions and the long-term selection by farmers for diverse purposes. A recent study by Morais (2012) using 19 SSR markers that showed a large number of Burkina landraces had genes introgressed from SR22, indicating a wide adoption of this improved OPV. These results should be taken into consideration in Burkina maize hybrid breeding program. The present study is the first molecular characterization of maize inbred lines developed in maize breeding program of Burkina Faso. Model-based population structure and PCA of the 54 INERA maize lines, extracted from two different sources, included in this study showed a separation of the lines into three distinct subgroups and a mixed group, which is believed to include recombinant lines since the two parental sources had a common parent. The extraction of inbred lines from FBC6, which was developed by mixing many different varieties, might have resulted in the separation of the lines into genetically distinct subgroups. In contrast, the lines extracted from ESPOIR did not show any major differentiation suggesting that the separation of lines from FBC6 could be explained by the presence in its genetic background of genes from divers geographic origins. Lines extracted from FBC6 and tested in this study consisted of three groups, two are closely related in terms of the pedigree. In other studies of tropical maize that were considered extremely diverse, SSR markers variation did not provide any evidence of population structure other than among individuals closely related by pedigree (Warburton *et al*. 2002, 2005). It has been suggested that relatedness among highly diverse maize lines is difficult to measure accurately regardless of the marker system (Hamblin *et al*. 2007).

4.4.4 Population structure and genetic relationship

The extent of genetic differentiation, population structure, and patterns of relationship among a set of 96 maize inbred lines was investigated using 1057 SNP markers. The model-based population structure analysis, NJ-cluster analysis, and principal component analysis were used to explore whether the population of the 96 maize lines from diverse origins (Temperate, CIMMYT, IITA and INERA) was homogeneous or contained genetically distinct subgroups. All the different multivariate methods supported the presence of genetically distinct groups. The model-based population structure and principal component analysis showed, to some extent, a separation by origin of the lines with related lines tending to cluster together. It has been reported that the clustering observed in the tropical populations is largely consistent with the pedigree information (Warburton *et al.*, 2005, Semagn *et al.*, 2012). Comparisons of the different multivariate analysis revealed high concordance among the PCA, and model-based population methods in terms of the number of groups and members of each group. However, cluster analysis showed low concordance with the other methods in terms of assigning genotypes into their respective groups. Similar results were reported by Semagn *et al*. (2012). This could be explained by the fact that, in cluster analysis, different combinations of genetic distance/similarity matrix and clustering algorithms can give rise to different groups. Even a single distance matrix and a single clustering algorithm may produce several alternative clusters that often create ambiguity in selecting the best one. PCA produces 2 or 3-dimensional scatter plot of the samples in which geometrical distances among samples in the plot reflect the genetic distances among them with a minimum of distortion and ambiguity compared to cluster analysis (Karp *et al*., 1997). Therefore, our population differentiation was based on PCA and population structure analysis which is more reliable than the NJ-clustering. Population structure grouping and different pairwise PCAs between different sets of inbred lines led to the identfication of 4 distinct groups. All the temperate lines, 19% of INERA and 13% of IITA lines were well differentiated, CIMMYT lines could not be divided into groups with significant genetic differences. This corroborates the results of previous studies that showed no clear grouping in CIMMYT germplasm. Although 394 maize lines from CIMMYT's global maize breeding programs were tested in the study of Lu *et al*. (2009), the lines were not differentiated into groups supporting the previous reports that no clear clustering or heterotic patterns could be identified in either CIMMYT lowland tropical maize (Xia *et al.*, 2004) or subtropical, tropical midaltitude and highland maize populations (Xia *et al*., 2005). A set of INERA, CIMMYT and IITA lines, representing 61% of the 96 lines included in this study, were not separated by origin. All the INERA maize lines derived from ESPOIR were grouped with 88% and 53% of CIMMYT and IITA lines respectively, in PCA. These maize lines might share a common genetic background. The source of ESPOIR was population 66 developed by CIMMYT in collaboration with IITA. In addition, two varieties from IITA were included in FBC6. Although the three breeding programs share common germplam, results of this study identified subgroups between INERA and IITA germplam with a large genetic differentiation which was not observed in the INERA and CIMMYT germplasm. A genetic distance observed between maize inbred lines from IITA and a national breeding program program (the Cameroon Institute of Agronomic Research) has been reported (Menkir *et al*., 2004). The same authors suggested that maize breeding programs isolated in space can play a significant role in generating divergent inbred lines. A clear separation between temperate and INERA lines was observed in this study thus confirming the results of previous study (Lu *et al.* 2009) on genetic difference between temperate and tropical germplasm. However, the largest distance of 0.2888 observed in this study between Tropic-IT and Temperate groups was less than the 0.3494 reported by Lu *et al*..(2009) between CIMMYT lines and Chinese BSSS group in a diversity study with 770 inbred lines covering diverse temperate and tropical/subtropical maize germplasm. Genetic distance and kinship analysis showed that the lines tested in this study are distantly related, with 0.61% of the pairwise comparison the 96 lines falling within a genetic distance less than 0.1. In addition, 61.3% of the pairwise kinship estimates had a value of zero. The results on kinship coefficient estimation are a little lower than that of Hao *et al.* (2011b) who reported pairwise kinship values close to zero for about 66.6% of 80 inbred maize lines. The lack of redundant lines among the germplasm suggests that each line is probably contributing new alleles to the breeding program (Semagn *et al.,* 2012). This is supported by this study with identification of missing alleles or unique alleles and significant allelic frequency differences among germplasm collections observed. The higher rate of alleles in temperate germplasm but missing in a tropical germplasm in the present study has been previously reported (Lu *et al.,* 2009). As heterotic group assignment is made based on combining ability from diallel or line by tester experiments, several authors have suggested the use of molecular markers in heterotic grouping (Reif *et al.*, 2003a,b; Flint-Garcia *et al.*, 2009). In this study, cluster analysis separated the testers of well-known heterotic groups into different clusters. In addition, population structure analysis separated some testers into the groups. However, assign lines into heterotic groups based on this result might not be consistent with field experiments. Semagn *et al.,* (2012) used three multivariate analysis (population structure, NJ-clustering and PCA) to separate 220 CIMMYT lines into heterotic groups A and B, but the SNP markers did not reveal clear population structure and genetic differentiation of most of the inbred lines in heterotic groups A and B, as defined by CIMMYT breeders.

4.4.5 Breeding implications and recommendations

Several hybrid maize programs were initiated at INERA but little progress has been made. The recent hybrid breeding program uses improved OPVs, adapted to local environments and adopted by famers, as source populations for extraction of inbred lines. FBC6 and ESPOIR are the main sources from which the available advanced inbred lines were extracted. On the basis of theoretical and experimental results, Melchinger (1999) demonstrated that organization of germplasm into genetically divergent heterotic groups is beneficial for a systematic and optimum exploitation of heterosis. The present study identified genetic variation and subgroups among INERA inbred lines but there was not a clear separation for the majority of the lines. Nor was the separation indicative of heterotic groups. Since INERA inbred lines from OPVs, with the mixed genetic constitution of CIMMYT populations and pools, the task of assigning the lines to genetically diverse and complementary heterotic groups zould be very difficult. The lack of clustering or heterotic grouping of CIMMYT germsplam is well documented. One option for the hybrid breeding program in Burkina Faso would be to identify among local lines or CIMMYT/IITA lines appropriate testers representing heterotic groups A and B. The Burkina inbred lines could then be grouped into the heterotic groups A and B, or A/B based on the hybrid performance with testers. Another possibility is to choose elite inbred parents of local or/and CIMMYT/IITA released hybrids as testers. Inbred line, FBLM10, in combination with B73, DE811 and ELN43453 were identified as high performing single cross hybrids. These elite parental lines could be use as testers. The present study showed that INERA inbred lines are fixed (lower level of heterotozygosity) and exhibited an amount of genetic diversity between different lines. This makes them a valuable source for association mapping studies. A panel of CIMMYT, IITA and INERA lines had a linkage disequilibrium decay of 2 to 10 time less than the panel comprised of INERA lines alone. Allelic frequency differences observed between INERA and; temperate, CIMMYT, and IITA lines, together with unique alleles identified within each germplasm set, suggests that a mutual improvement between INERA and each of temperate, CIMMYT and IITA sets of germplasm is possible. Specific recommendations can be made for exploiting maize inbred lines tested to produce hybrids with good yield potential. Within the same grain color, CIMMYT lines can be crossed with the two distinct subgroups of

INERA lines identified and the subgroup of IITA lines can be crossed with FBC6 derived lines in tropic-M group as well as with ESPOIR derived lines.

4.5. Conclusions

The present study investigated the genetic diversity among maize inbred lines developed at INERA and the relationship between these lines with temperate elite lines and CIMMYT and IITA lines. The 1057 informative SNP markers revealed genetic variation among the whole set of inbred lines as well as in the INERA germplasm set. The two subsets of INERA germplasm included in this study were divergent but also present a mixed group indicating they share a common genetic background. There appeared to be substantial progress in national program inbred line development as revealed by the low level of heterozygosity and the uniqueness of the majority of the lines. Principal component analysis showed genetic differentiation between INERA and, temperate and IITA germplasm but not with CIMMYT germplasm. However, the unique alleles identified within each germplasm suggest that all the exotic germplasms can provide new desirable alleles for local lines. This study has confirmed a set of SNPs previously reported by Lu *et al*. (2009) and Semagn *et al*. (2012) which can provide good results at low cost in genetic characterization of tropical maize germplasm.

CHAPTER FIVE

5.0 Performance *per se* of maize inbred lines and identification of genetic variation associated with drought traits using association mapping

5.1 Introduction

The response of plants to drought stress is very complex and involves expression of a lot of genes and pathways for diverse mechanisms and interactions with environments. In maize, QTL mapping for grain yield (GY) and other associated traits under drought stress, such as anthesis-silking interval (ASI), has been an active area of research, especially in the past two decades (Almieda *et al*., 2013). Using meta-analysis approach, Hao *et al*. (2010) combined QTL mapping results from many studies and found constitutive and adaptive QTLs. In a similar study, Almieda *et al*. (2013) identified, across three tropical bi-parental populations and multiple environments, seven genomic regions for GY and one for ASI, of which six mQTL on chromosome 1, 4, 5 and 10 for GY were constitutively expressed across stressed and well watered environments. The first extensive report on meta-analysis of data from over 3100 individuals developed from 18 bi-parental maize populations, genotyped using the same SNP platform and evaluated in the same conditions across a wide range of managed water-stressed and well-watered environments, identified 9 QTLs specific to ASI, 15 specific to GY, and 44 for both GY and ASI (Semagn *et al*., 2013). There has also been progress in identifying QTLs for carbohydrate and ABA accumulation during stress (Pelleschi *et al.,* 2006; Tuberosa and Salvi 2007; Capelle *et al*., 2010). However, QTL studies with biparental populations only sample a small proportion of the total allelic diversity expected to be present in diverse germplasm. Furthermore, locating the causal gene underpinning a significant QTL requires a lengthy and involved process of fine structure mapping (Setter *et al.*, 2011). Recently, QTL identification without the need for a mapping population is possible using the development of analytical approaches that explore the residual linkage disequilibrium between markers and closely linked QTLs present in pools of unrelated germplasm (Meuwissen and Goddard, 2000 cited by Tuberosa *et al.*, 2002). The underlying principles of association mapping derives from genetic association studies, in human genetic, for a correlation between disease status and genetic variation to identify candidate genes or genome regions that contribute to a specific disease. (Jorde, 2000; Cardon and Bell, 2001 cited by Tuberosa *et al*., 2002). Association mapping in a population of diverse maize lines allows sampling of greater genetic diversity and also provides higher mapping resolution because more recombination events have occurred during historical diversification than during production of a biparental mapping population (Setter *et al*., 2011). Some studies have shown the potential of tropical maize for association mapping investigation (Warburton *et al*., 2008; Wen *et al*., 2011). Genetic variation to identify candidate gene associated to maize grain yield and secondary traits using association mapping under drought stress has been little studied. Hao *et al.* (2011b) used this powerful tool to study the functional genetic variations underlying drought tolerance using 95 maize inbred lines that were parents of popular hybrids in China. Setter *et al.* (2011) evaluated a panel of 350 tropical and subtropical maize inbred lines with association mapping to identify loci involved in accumulation of carbohydrates and ABA metabolites during stress. A global collection of 305 lines representing temperate, tropical and subtropical maize germplasm was also used to identify genomic loci or genes that are significantly associated with plant height and biomass as measured by the Normalized Difference Vegetation Index (NDVI) under well-watered and water-stressed conditions using association mapping (Lu *et al*., 2012).

The objectives of this study were to evaluate the performance *per se* of a diverse group of maize inbred lines and to use association mapping to identify the genetic variations underlying drought tolerance.

5.2 Materials and Methods

5.2.1 Plant Materials

In this study, 54 maize inbred lines were selected from the panel used in the previous chapter (4). All were tropical and subtropical lines representing INERA, IITA and CIMMYT/Zimbabwe lines. The panel was composed of 28 white (set 1) and 26 yellow lines (set 2). The list of lines with their characteristics including origin, grain color, maturity and reaction to drought stress (where available) are provided in Table 5.1.

5.2.2 Molecular Data

The SNP genotyping procedure and data analysis including genetic diversity, population structure and relative kinship have been extensively described in Chapter 4. However, in the present study, the burn- in length and iterations were set to 100,000 with 10 runs for each K in population structure analysis. Of the 1151 SNPs scored on the 54 inbred lines, we kept only the SNPs with the lower percentage of missing data (<20%). Monomorphic markers (101 markers) were also discarded yielding a total of 994 markers.

5.2.3 Phenotypic Evaluation

A prior screening of the inbred lines showed that there are grouped in two maturities (Table 5.1), intermediate (40 lines) and early (14 lines) in maturity. In addition, one INERA line, ELN39382, which failed to produce enough seed for field evaluation was not included in the trials. In total, 53 inbred lines were evaluated in two experiments, A and B of 39 and 14 inbred lines, respectively. The two trials were evaluated side by side during the off-season under well-watered and water stressed conditions over two years (2011/2012 and 2012/2013).

Table 5. 1. Characteristics of inbred lines used in the study

No	Line	Ori	Col	Maturity	DT	No	Line	Ori	Col	Maturity	DT
1	T02058	CIM	W	Inter	T	28	ELN41272	IN	W	Inter	-
2	VL0511247	CIM	W	Inter	T	29	ELN41273	IN	W	Inter	-
3	VL0511298	CIM	W	Inter	T	30	ELN424101	IN	W	Inter	-
4	VL0512593*	CIM	W	Early	T	31	ELN42441	IN	W	Inter	-
5	VL054794	CIM	W	Inter	T	32	ELN42442	IN	W	Inter	-
6	VL054881	CIM	W	Inter	T	33	ELN42444	IN	W	Inter	-
7	VL05615	CIM	W	Inter	T	34	ELN42445	IN	W	Inter	-
8	VL05616	CIM	W	Inter	S	35	ELN431251	IN	Y	Inter	-
9	VL057903*	CIM	W	Early	T	36	ELN43453	IN	Y	Inter	-
10	VL057967	CIM	W	Inter	T	37	ELN43574	IN	Y	Inter	-
11	VL058014*	CIM	W	Early	S	38	ELN45111	IN	Y	Inter	-
12	VL058025*	CIM	W	Early	T	39	ELN47132	IN	Y	Inter	-
13	VL058589*	CIM	W	Early	T	40	ELN48392	IN	W	Inter	-
14	VL081464	CIM	W	Inter	T	41	FBML10	IN	Y	Inter	-
15	VL081466*	CIM	W	Early	T	42	TZEI10*	IT	Y	Early	-
16	ELN39382	IN	Y	Inter	-	43	TZEI124*	IT	Y	Early	S
17	ELN39427	IN	Y	Inter	-	44	TZEI146*	IT	Y	Early	T
18	ELN402213	IN	Y	Inter	-	45	TZEI148*	IT	Y	Early	T
19	ELN40791	IN	Y	Inter	-	46	TZEI149*	IT	Y	Early	T
20	ELN40823	IN	Y	Inter	-	47	TZEI158	IT	Y	Inter	T
21	ELN40941	IN	Y	Inter	-	48	TZEI16	IT	Y	Inter	T
22	ELN41111	IN	W	Inter	-	49	TZEI161	IT	Y	Inter	T
23	ELN41112	IN	W	Inter	-	50	TZEI17*	IT	Y	Early	-
24	ELN41113	IN	W	Inter	-	51	TZEI177	IT	Y	Inter	T
25	ELN41114	IN	W	Inter	-	52	TZEI23*	IT	Y	Early	T
26	ELN41115	IN	W	Inter	-	53	TZEI8*	IT	Y	Early	T
27	ELN41271	IN	W	Inter	-	54	TZI18	IT	Y	Inter	T

Ori=Origine; CIM=CIMMYT/Zimbabwe; IN= INERA; IT= IITA, Col=Grain color, W=White, Yellow; DT=Drought tolerance; T=tolerant, S=Susceptible; Inter=intermediate, - = unknown; lines with * were included in experiment B. The maturity of CIMMYT and IITA lines indicated in the table was determined based on a screening in local environment using national maize breeding program guideline: extra-early (70-84 days), early (85-94 days), intermediate (95-110 days) and late (more than 110 days) (Sanou, 2006).

Managed drought trials

In each trial and in both water regimes, lines were planted in single-row plots of 5-m length and 25 cm between plants. Plots were overplanted, with two seeds per hill, and then thinned to one after seedling establishment, giving an average density of 50 000 plants/ha. All the trials were planted using randomized complete block design (RCBD) with three replications in two adjacent blocks that received different irrigation regimes. The first block, well watered (WW), received irrigation water throughout the life cycle of the crop whereas the second block, water-stressed (WS), received irrigation water up one to two weeks to anthesis so that water stress could coincide with the time of flowering. Drought stress was imposed for 21 in experiment A and 14 days of stress were applied in experiment B (early inbreds) because the plants could not stand beyond the two weeks.

5.2.4 Phenotypic data analysis

Six phenotypic traits were measured in all the environments using the average value of at least five plants per line: Grain yield (GY), total number of ears per plant (EPP), plant (PHT) and ear height(EHT), Days to 50% anthesis (AD), Days to 50% silking (SD) and anthesis-silking interval (ASI). In addition, Relative Decrease in Yield (RDY) and Selection Tolerance Index (STI) were calculated. Relative decrease in yield (RDY) was defined as:

$\mathrm{RDY} = 100 - (\mathrm{Y_s}/\mathrm{Y_p}\ \mathrm{x}\ 100)$ (Farshadfar *et al.*, 2013) with Yp and Ys as yield potential under well-watered and drought stress respectively. The STI is a modification of the formula of Banzinger *et al*. (2000) proposed by Hao *et al.* (2011a). The algorithm is as follows for STI:

$$\mathrm{S_{ij}} = (\chi_{ij} - \mu_j)/\sigma_j \qquad (1)$$

$$\mathrm{MP_{ij}} = (\mathrm{S_{ijd}} + \mathrm{S_{ijw}})/2 \qquad (2)$$

$$\mathrm{STI} = \mathrm{b_1 MP_{i1}} + \mathrm{b_2 MP_{i2}} + \cdots + \mathrm{b_j MP_{ij}} \qquad (3)$$

In the above three equations, *j* range was 1–4, representing traits GY, EPP, ASI, and AD, respectively. S_{ij} in the equation 1, indicates the standardized phenotypic value of trait *j* to genotype *i* under the WW regime (w) and under the WS regime (d), χij indicates the real phenotypic value of genotype *i* on trait *j*, μj indicates the mean value of trait *j* for all materials, and σj indicates the standard deviation of trait *j*. $MPij$ in equation 2, indicates the mean productivity of trait *j* on genotype *i*; b_j in equation 3, indicates the given weight value of trait *j*. Individual analysis of variance of each tested trait in each year and water regime were conducted using the PROC GLM procedure in SAS (SAS, 2002). For the combined analysis across years for well-watered and water-stressed conditions, the genotypes were considered as fixed and years as random. Consequently the genotype x year interaction mean squares were used to test the mean square of genotype. Student-Newman-Keuls test ($p < 0.05$) was used to compare the means of grain yield among the different groups of lines (CIMMYT, IITA and INERA) and between the set 1 (white inbred lines) and set 2 (yellow inbred lines).

5.2.5 Association Analysis

The phenotypic data of the line ELN39382, which was not included in the field evaluations but genotyped was considered as missing data in the association analysis. The association test was run as described by Setter *et al*. (2011). Q matrix from STRUCTURE was used to correct population stratification and to account for relatedness among individuals. All the SNP data were used to generate a relative kinship matrix of similarity between each pair of lines in the association panel (the K matrix) using the program SPAGeDi vs 1.02 and the Loiselle algorithm. The Q matrix was set as a fixed effect, and K matrices were incorporated as a covariate structure of random effects representing the total of polygene effects in a mixed linear model (MLM). Each SNP was set as a fixed effect, one at a time, to test the association between the SNP and phenotype. The analysis was performed using the TASSEL software package (Version 2.1). Variance components of polygene effects and the residuals were estimated using the likelihood equations employing the EMMA algorithm (Kang *et al*., 2008), implemented in TASSEL.

5.3 Results

5.3.1 Molecular diversity, Population structure and Relatedness

The average unbiased gene diversity of the 994 polymorphic SNPs was 0.308 with a range of 0.021 - 0.492 and heterozygosity range of 0 - 1.241 and an average of 0.046 . The average PIC value was 0.253, with a range of 0.021 - 0.428. Of the 994 SNPs, 140 had a minor allele frequency (MAF) less than 0.05 and they were discarded. Finally, 854 informative SNPs with a MAF > 0.05 were used to estimate associations with phenotypic traits related to drought tolerance in the set of 54 lines of this study. Population structure and kinship relationship of 54 inbred lines were respectively analyzed with 994 polymorphic SNPs. Based on the log likelihood results and previous results (Chapter 4), we selected k = 3 as the highest population structure (Figure 5.1). Relative kinship coefficients between pairs of lines varied from 0 to 1.20, with an overall average of 0.06, and 66.1% of the pairwise kinship estimates had a value of zero, indicating that the lines were unrelated.

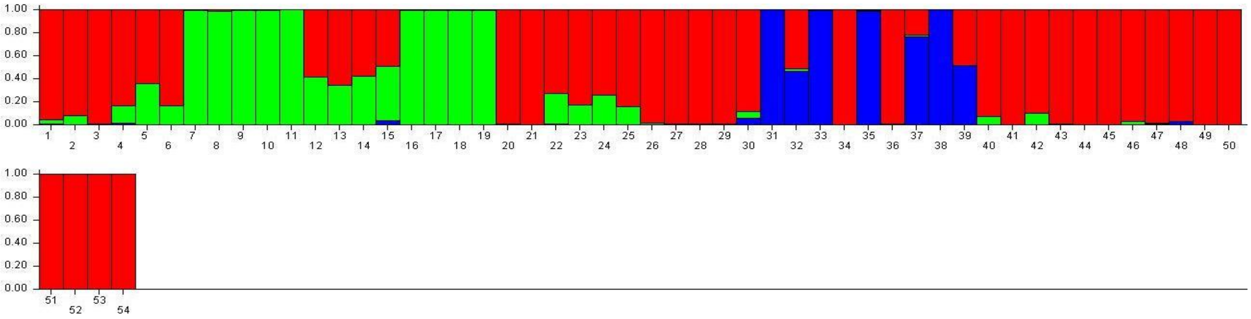


Figure 5. 1. Population structure of the 54 maize lines shown as membership coefficients (Q values). Each horizontal bar represents one maize line, which is partitioned into up to k colored segments.

5.3.2 Phenotypic variation

The genetic effect was significant in general for all the traits across water regimes at 0.05 to 0.001 level (Table 5.2, 3). The experiment B (with 14 inbred lines) showed less variability for most of the traits in water-stressed condition (Table 5.3). Mean square of genotype by year interactions were significant for grain yield across water regimes and experiments (Table 5.2,3).

Table 5. 2. Mean squares from combined analysis for grain yield and 6 traits of 39 and 14 maize inbred lines tested under well-watered environment over 2 years.

Source	**DF**	**AD**	**SD**	**ASI**	**PHT**	**EHT**	**EPP**	**GY**
Experiment A								
Year	1	1583.58***	798.29***	98.81***	115489.49***	6786.21***	0.14	285605.61
Rep(year)	4	69.23***	56.06***	2.14	1309.16***	744.03***	0.16*	186746.77
Geno	38	85.13***	94.31***	9.86***	1911.26***	752.07***	0.23**	1102519.2
Geno x year	33	12.79***	10.8**	2.64	320.07***	170.08***	0.07	675770.93***
Error	142	3.87	5.4	2.18	115.44	63.86	0.05	141822.59
R2		0.91	0.87	0.68	0.93	0.84	0.66	0.78
Experiment B								
Year	1	1898.09***	747.82***	210.55***	19968.59***	1146.3*	1.19***	46012.88
Rep(year)	4	5.59	10.53	5.28	512.78	269.16	0.21***	582029.66**
Geno	13	131.26***	135.17***	9.36	742.36	354.79**	0.31**	1706518.14**
Geno x year	13	17.28***	17.5***	5.71	308.97	84.41	0.06	387168.83**
Error	50	2.41	4.35	3.31	373.45	204.35	0.04	119944.94
R2		0.97	0.93	0.73	0.67	0.45	0.78	0.84

***, ** and * significance at *P* <0.001, 0.01 and 005 respectively.

Table 5. 3. Mean squares from combined analysis for grain yield and 6 traits of 39 and 14 maize inbred lines tested under water-stressed environment over 2 years.

Source	**DF**	**AD**	**SD**	**ASI**	**PHT**	**EHT**	**EPP**	**GY**
					Experiment A			
Year	1	88.21**	28.98	164.72***	131873.9***	6365.96***	8.66***	2381682.36***
Rep(year)	4	93.16***	59.01***	21.97*	222.89	324.49**	0.66***	211104.47**
Geno	38	82.12***	107.97***	20.08	1162.06**	487.48**	0.24*	324839.72**
YearxGeno	33	20.58**	16.93	13.23*	344.01**	163.91**	0.11*	135316.35***
Error	142	11.18	11.71	8.13	189.01	72.91	0.07	51384.17
R^2		0.73	0.78	0.6	0.89	0.76	0.71	0.75
					Experiment B			
Year	1	1341.34***	143.16***	549.74***	9096.96***	923.19***	2.73***	2360929.83***
Rep(year)	4	16.52*	7.23	5.05	261.56	14.99	0.05	60765.12
Geno	13	183**	191.6***	28.37*	548.04	543.55	0.18	285113.43
YearxGeno	13	31.35***	27.81**	10.03	323.03*	154.84**	0.1	117566.42***
Error	50	5.57	8.6	5.1	136.99	57.81	0.06	44773.53
R^2		0.93	0.9	0.83	0.78	0.79	0.73	0.8

***, ** and * significance at *P* <0.001, 0.01 and 005 respectively.

The grain yield across trials over two years in well-watered condition ranged from 32 kg/ha for VL058589 to 2212 kg/ha for TZEI149 with an average of 941 kg/ha. Under drought stress the average yield was 335 kg/ha ranging from 0 kg/ha for ELN41113 and VL058589 to 1125 kg/ha for ENL43453. The grain yield reduction was 64% while the increased ASI was 36% under drought stress.

Table 5. 4. Range and average mean of traits for 53 maize inbred lines across trials evaluated over 2 years

Trait	**Well-watered condition**					**Water-stressed condition**				
	Min	Max	Mean	SE (±)	CV	Min	Max	Mean	SE (±)	CV
GY	31.89	2212.45	941.41	218.81	40.26	0.00	1124.93	334.60	130.31	67.46
EPP	0.11	1.19	0.83	0.12	25.24	0.00	0.96	0.55	0.15	48.37
AD	58.60	89.83	77.37	1.12	2.51	57.83	89.40	76.53	1.86	4.22
SD	63.00	94.00	80.00	1.37	2.98	63.50	96.50	80.85	1.92	4.11
ASI	0.67	6.40	2.92	0.92	54.36	1.00	13.67	4.56	1.59	60.18
PHT	98.17	204.67	133.54	7.90	10.25	89.33	179.00	123.24	7.77	10.92
EHT	28.58	84.50	53.23	5.98	19.45	26.40	77.50	51.16	4.85	16.41

Min=minimum, Max=maximum, SE= standard error

In general, the yellow lines in this study performed better than white lines (Figure 5.2) in both water regimes. No significant difference was found between groups of lines under drought stress for average grain yield whereas IITA lines (yellow) yielded significantly higher than INERA white inbred lines under well-watered condition. The IITA and CIMMYT inbred lines were generally earlier in maturity than the INERA lines (Figure 5.2)

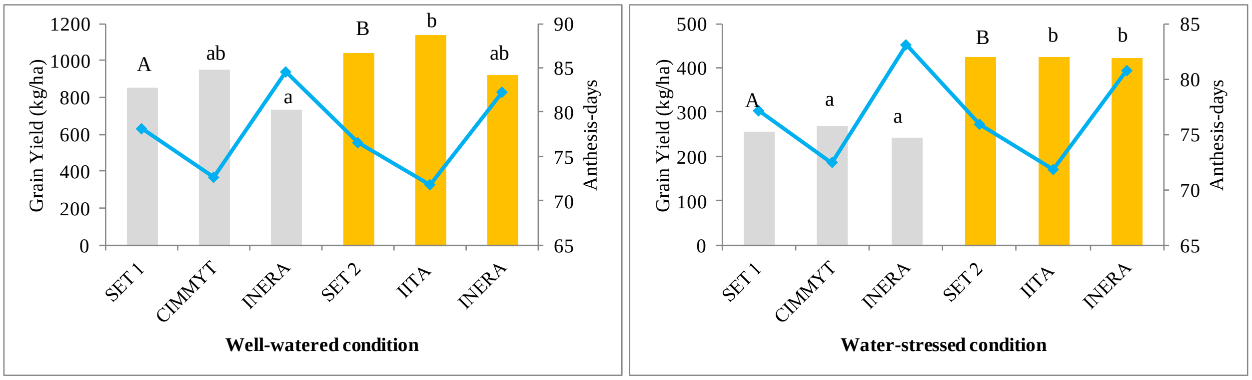


Figure 5. 2. Distribution of average grain yield and anthesis-days for white (set 1) and yellow (set 2) lines and, for CIMMYT, IITA and INERA inbred lines under well-watered and water-stressed conditions. Means with the same letter are not significantly different (small letter for comparison between group of CIMMYT, IITA and INERA lines and capital letter for comparison between set 1 and 2).

Drought tolerance

The tolerance of the 53 inbred lines to drought was ranked according to STI and RDY (Table 5.5). If two lines had similar values of STI, the line that showed the lower RDY was ranked first. Results showed that 25% of the lines had a yield reduction more than 75% due to drought stress. The selection index (STI) varied from -8 to 10. Inbred lines ELN43453, TZEI149, ELN40791, VL054794 and TZEI10 were the top 5 drought tolerant lines based on the selection index (STI) and relative decrease in yield (RDY) indices.

Table 5. 5. Grain yield (kg/ha) of the line in different environment and the selection criteria for drought tolerance (RDY and STI).

Line	**Yield (kg/ha)**		**RDY (%)**	**STI**	**Rank**
	WW	WS			
ELN39427	944.1	248.9	74	0	28
ELN402213	563.7	209.0	63	0	32
ELN40791	1583.0	919.0	42	5	3
ELN40823	509.2	243.4	52	-7	48
ELN40941	1275.1	589.2	54	2	18
ELN41111	277.9	170.1	39	1	26
ELN41112	645.9	51.7	92	-5	45
ELN41113	298.6	0.0	100	-3	41
ELN41114	41.6	16.9	60	-4	43
ELN41115	152.1	57.6	62	-1	34
ELN41271	750.8	272.9	64	2	19
ELN41272	1006.7	556.6	45	5	7
ELN41273	524.8	21.3	96	1	24
ELN424101	1358.4	720.0	47	3	16
ELN42441	906.5	159.7	82	-2	38
ELN42442	1567.1	377.1	76	-8	52
ELN42444	1006.4	488.3	51	1	23
ELN42445	869.0	370.5	57	0	31
ELN431251	784.5	348.5	56	3	15
ELN43453	1661.7	1124.9	32	10	1
ELN43574	179.3	81.7	54	-5	46
ELN45111	451.4	196.0	57	-8	51
ELN47132	536.9	183.8	66	2	22
ELN48392	749.4	177.8	76	-8	53
FBML10	1382.0	368.4	73	-2	37
T02058	563.6	31.8	94	-3	40
TZEI10	1294.8	576.6	55	5	5
TZEI124	444.6	190.5	57	-6	47
TZEI146	814.6	217.7	73	-7	49
TZEI148	1050.3	435.0	59	0	27
TZEI149	2212.5	1062.8	52	7	2
TZEI158	1339.0	792.6	41	4	9
TZEI16	357.9	175.4	51	-5	44
TZEI161	965.4	308.5	68	4	12
TZEI17	1238.0	210.0	83	2	20
TZEI177	1205.3	461.0	62	5	8
TZEI23	1220.5	304.4	75	3	14
TZEI8	1328.3	471.1	65	5	6
TZI18	1097.2	452.6	59	3	13
VL0511247	1317.2	191.7	85	0	29
VL0511298	1261.7	275.5	78	0	30
VL0512593	631.3	309.1	51	-3	39
VL054794	1637.7	418.3	74	5	4
VL054881	420.0	118.7	72	0	33
VL05615	1119.5	422.7	62	1	25
VL05616	873.0	217.3	75	-2	35
VL057903	810.6	264.0	67	3	17
VL057967	1027.6	373.2	64	4	11
VL058014	1283.1	387.4	70	-2	36
VL058025	1010.1	330.6	67	2	21
VL058589	31.9	0.0	100	-4	42
VL081464	1745.5	442.3	75	4	10
VL081466	197.4	84.7	57	-8	50

The higest STI value is considered as the most drought tolerant

5.3.3 Association mapping with phenotypic traits related to drought tolerance

A total of 277 associations involving 83 SNPs which showed strong associations with at least two drought tolerance-related traits ($P < 0.01$) across the 10 maize chromosomes were identified. Among the significant associations, 22 SNPs were associated with at least one phenotypic trait in 2011, 2012 and across the two years are presented in Table 5.6. For a given water regime (WW or WS), there were 5 significant SNPs identified in both 2011 and 2012 to have significant association with at least one trait including ASI (2 SNPs - WS), GY (2 SNPs - WW) and RDY (1 SNP). In addition, 3 SNPs were associated with ASI in WS condition in 2011, 2012 and across the two years. The trait, selection tolerance index (STI), showed highly signficant associations across the 10 chromosomes except chromosome 6. The largest associations with the lowest P-value were identifed on chromosomes 1, 2, 5, 8 and 9 [(−LOG10(P) > 10.00], associated with trait STI of 2011.

Table 5. 6. Information on 22 SNPs associated with at least two phenotypic traits investigated under water-stressed (WS) and well-watered (WW) regimes in 2011 and 2012 at $P < 0.01$.
Chr=chromosome; Env= environment. Gene information obtained from MaizeGDB, Panzea, and NCBI websites

Bin	SNP	Allele	Chr	Position	-LOG(P)	Year	ENV	Trait	Gene
1.03	PZA02686.1	G/A	1	34724094	2.20	2011	WS	GY	AY110856
					11.71	2011		STI	
					2.04	2012	WS	EPP	
					2.70	2012	WW	ASI	
					2.82	Across	WW	GY	
1.04	PZB00872.3	G/A	1	46255059	2.21	2011	WS	GY	MAGI_81726
					11.46	2011		STI	
					2.00	2012	WS	EPP	
					2.64	2012	WW	ASI	
					2.77	Across	WW	GY	
5.00	PHM9807.9	G/C	1	294311411	2.36	2011	WS	EPP	AY105715
					2.09	2012	WS	ASI	
					2.30	Across	WW	GY	
2.03	PHM4425.25	A/G	2	19837545	2.25	2011	WS	GY	AY107316
					11.45	2011		STI	
					2.05	2012	WW	ASI	
					2.44	Across	WW	GY	

continued

Bin	SNP	Allele	Chr	Position	-LOG(P)	Year	ENV	Trait	Gene
2.04	PZA01336.1	G/A	2	31394426	3.54	2011	WW	EPP	AY103944
					2.82	2011	WS	ASI	
					2.41	2011		STI	
					2.08	2012	WS	EPP	
					2.32	2012	WS	ASI	
					2.43	Across	WW	EPP	
					2.21	Across		RDY	
2.05	PHM3626.3	A/G	2	125642617	4.46	2011		RDY	AY108532
					2.41	2012	WW	GY	
					2.29	Across	WS	EPP	
					2.03	Across		RDY	
3.04	PZA00279.2	G/A	3	52804070	3.74	2011		STI	AY105471
					3.04	2011		RDY	
					2.06	2012	WS	EPP	
					2.36	2012	WW	ASI	
					3.38	2012	WS	ASI	
					2.4	2012		RDY	
					3.06	Across	WW	EPP	
					2.92	Across	WW	GY	
3.05	PZB02002.1	A/G	3	133483395	2.41	2011	WW	EPP	NCBI_X80206
					2.48	2011	WS	ASI	
					3.87	2012	WS	GY	
					2.28	2012	WS	EPP	
					2.48	2012	WS	ASI	
					3.13	Across	WW	ASI	
					2.16	Across	WS	ASI	
4.05	bt2.7	G/A	4	66290994	2.44	2011	WW	GY	bt2
					2.19	2011		STI	
					2.09	2012	WW	GY	
					3.09	Across	WS	EPP	
5.01	PZA02653.12	A/G	5	5917587	2.39	2011	WW	EPP	AY111251
					2.55	2011	WS	ASI	
					3.13	2012	WS	GY	
					2.77	2012	WW	ASI	
					2.46	2012	WS	ASI	
					3	Across	WW	ASI	
					4.65	Across	WW	GY	
					3.61	Across	WS	ASI	
					2.46	Across	WS	GY	
5.05	PHM5337.18	G/A	5	180611413	2.24	2011	WS	GY	AY106267
					11.46	2011		STI	
					2.04	2012	WS	EPP	
					2.64	2012	WW	ASI	
					2.82	Across	WW	GY	
5.09	PZA02769.1	T/A	5	215505432	2.54	2011	WW	GY	AY112190
					2.08	2012	WW	GY	
					2.77	Across	WS	EPP	

continued

Bin	SNP	Allele	Chr	Position	-LOG(P)	Year	ENV	Trait	Gene
6.04	PZA00571.1	G/A	6	120679943	2.17	2011	WS	GY	AY112197
					2.34	2011	WS	ASI	
					3.42	2012	WS	EPP	
					3.21	2012		RDY	
					2.06	Across	WW	ASI	
7.01	PZA02872.1	A/G	7	13058813	2.06	2011	WS	ASI	AY104876
					3.54	2012	WS	GY	
					2.15	2012	WS	ASI	
					2.8	Across	WW	ASI	
					2.22	Across	WW	EPP	
7.02	PZA01230.1	G/A	7	46238189	2.32	2011		STI	AY109061
					4.14	2012	WS	EPP	
					3.41	2012	WW	ASI	
					3.12	2012	WS	ASI	
					3.63	2012		RDY	
					2.12	Across	WW	GY	
8.05	PHM448.23	A/G	8	133775120	2.68	2011		RDY	AY108725
					4.89	2012	WW	GY	
					2.28	2012	WS	ASI	
					2.96	Across	WS	EPP	
8.05	PZA00766.1	A/G	8	133775220	2.15	2011		RDY	AY108725
					3.13	2012	WW	GY	
					2.17	Across	WS	EPP	
8.05	PZA02011.1	G/A	8	140212456	2.6	2011	WW	GY	AY109767
					6.38	2011		RDY	
					4.81	2012	WW	GY	
					2.89	2012	WS	ASI	
					2.12	Across	WW	EPP	
					4.74	Across	WS	EPP	
8.08	PHM5468.25	G/A	8	129163361	2.04	2011	WS	GY	AY106091
					10.32	2011		STI	
					2.3	2012	WS	EPP	
					2.92	2012	WW	ASI	
					2.96	Across	WW	GY	
9.03	PZB00014.1	A/G	9	46590628	10.53	2011		STI	BE186703
					2.01	2012	WW	ASI	
					2.28	Across	WW	GY	
10	PZA02554.1	G/A	10	1939921	2.18	2011	WW	EPP	AY111722
					2.51	2011	WS	ASI	
					2.3	2012	WS	GY	
					2.96	2012	WS	EPP	
					2.68	2012	WW	ASI	
					2.36	2012	WS	ASI	
					2.14	Across	WW	GY	
10	PHM5740.9	G/A	10	8773358	4.42	2011		STI	AY109514
					3.49	2012	WW	ASI	
					3.78	Across	WW	GY	

5.4 Discussion

This study showed that the exotic maize inbred lines performed as well or even better than local lines in Burkina Faso environments. Local lines were more sensitive to cold weather which prolonged the flowering time suggesting that breeders should screen local inbred lines for adaptation to cold since off-season production in dry and cold seasons is an important period for maize hybrid production under irrigation. Based on STI and RDY, the 10 most drought tolerant maize inbred lines included 5 IITA, 3 INERA and 2 CIMMYT lines. The IITA and CIMMYT lines confirmed their tolerance since they were previously selected for drought tolerance. STI is a selection index based on yield and secondary traits developed by CIMMYT (Bänzinger *et al.*, 2000) to identify drought tolerant genotypes. The modified version used in the present study showed a highly significant association of all the traits used with SNP markers. A similar result was reported by Hao *et al.* (2011b) confirming the value of the selection index in selecting in drought tolerant genotypes.

In this study, SNP variations detected by SNP–trait associations related to drought tolerance were distributed on all 10 maize chromosomes indicating that drought tolerance might be controlled by a large number of genes with small effects which is consistent with the results of Hao *et al.*, (2011b). A similar result was also reported for flowering time studied with nested association mapping (NAM) (Buckler *et al.*, 2009). When a gene/QTL is detected under well-watered and mositure-stressed conditions, it is 'constitutive' while an ‘adaptive’ gene/QTL is detected only in moisture-stress condition (Hao *et al.*, 2011b; Collins *et al.*, 2008 cited by Almeida *et al.*, 2013). Based on this assumption, the 22 SNPs identified are believed to be associated with the genotypic variation underlying drought tolerance, since they were detected under both water regimes. For example, PZA02653.12 in chromosome 5 was associated with 9 different phenotypic traits in 2011, 2012 and across the two years: ASI-WW in 2012 and cross years; ASI-WS in 2011, 2012 and cross years; EPP-WW in 2011; GY-WS in 2012 and cross years; and GY-WW cross years.

The SNPs identified in this study were located in some of the gene positions which were common for detecting consensus QTLs and candidate genes by meta-analysis and association mapping. The SNP, PZB00872.3 located in bin 1.04 on chromosome 1 associated with GY-WS, GY-WW, ASI-WW, EPP-WS and STI was also reported to be associated with a QTL related to ASI-WS (Lu *et al.*, 2010). SNP PZA02653.12 seated in bin 5.01 of chromosome 5 was reported to be associated with the biomass measured by the Normalized Difference Vegetation Index (NDVI) in a WS regime (Lu *et al.*, 2012). It was associated, in the present study, with GY-WW, GY-WS, ASI-WW, ASI-WS and EPP-WW. In addition, 4 SNPs including PHM5740.9, PZA00279.2, PZA01336.1 and PZA02769.1 identified in the present study as drought tolerance traits were detected as flanking markers for many QTLs associated with grain yield and ASI in WW and WS conditions (Almieda *et al.*, 2013). For example PZA01336.1 was one of flanking markers of 4 QTLs for GY and ASI.

Gene sequences related to common SNP variations identified across studies could be important genes underlying drought-tolerance mechanisms, and can be converted into functional markers and then used for maize improvement by marker-assisted selection.

5.5 Conclusions

There was extensive phenotypic variation among inbred lines evaluated for grain yield and secondary traits, indicating the potential of the lines for selection of good parents for white and yellow endosperm hybrids development in Burkina Faso. This study is the first report on performance of CIMMYT inbred lines with subtropical adaptation in Burkina Faso environments, and the average performance of these lines was favorable, suggesting that CIMMYT subtropical inbred lines can contribute to INERA's maize breeding program.

The panel of diverse inbred lines tested exhibited genetic variation associated with drought tolerance traits. Some of these lines carried important drought genes, and would be good sources for drought tolerant hybrid development or improvement of farmers preferred varieties by backcrossing or marker assisted selection.

To the best of our knowledge, this study is the first report on association mapping for drought tolerance with CIMMYT, IITA and a NARS (National Agricultural Research System) maize inbred lines in Sub-Sahara Africa.

CHAPTER SIX

6.0 Heritability and path analysis of secondary traits on yield of tropical maize hybrids under drought

6.1 Introduction

Selection for improved genotypes under drought based on grain yield alone has often been inefficient (Ludlow and Muchow, 1990). The use of secondary traits in addition to yield has been suggested (Bolaños and Edmeades, 1996). The value of a secondary trait for improving genotype tolerance under drought is determined by many parameters including genetic variability, high heritability under drought and genetic correlation with gain yield (Bänziger *et al*., 2000; Araus *et al*., 2008). In general, heritability for grain yield is reduced under drought whereas secondary traits have relatively high heritabilities (Bolaños and Edmeades, 1996; Almeida *et al*., 2013). High genetic correlation between grain yield and a secondary trait shows that high yielding genotypes could be selected through selection for that trait. However the simple correlation does not give information on relative contribution of direct and indirect effects of the traits on grain yield. Studies have shown that the correlation coefficient between grain yield and secondary traits was explained by the direct contribution of the trait on grain yield as well as the indirect contribution through other traits, indicating these traits should also be considered during selection (Arias *et al*., 1999; Khalily *et al*., 2010).

The objective of this study was to determine the most appropriate secondary traits to be included in the selection index for selecting drought tolerant genotype. Thus, broad-sense heritability of the traits and phenotypic correlations were computed. The correlation was further portioned in to direct and indirect effects using path coefficient analysis.

6.2 Materials and Methods

6.2.1 Plant Materials

A total of one hundred maize testcross hybrids were used in this study. The hybrids were generated using line by tester mating design from the inbred lines used in chapter 5. The 100 hybrids were divided into two sets according to kernel color, genetic background, and geographical origin. Set 1 included 48 white maize hybrids derived from mid-altitude (CIMMYT) and lowland (INERA) tropical maize inbred lines while set 2 consisted of 52 yellow hybrids which included only lowland tropical (IITA and INERA) maize inbred lines. In addition, 7 checks were included in each set, 4 commercial varieties (Bondofa, Sanem, Barka and Wari) were common among the two sets. Bondofa and Sanem are varietal and single cross hybrids, respectively, while Barka and Wari are drought tolerant OPVs. The hybrid between the two testers (VL0511298 x VL054881 for white hybrid set and TZEI7 x TZE10 for yellow hybrid set) and two drought tolerant hybrids (VH052527 and VH053024 in set1 trial and, TZEI17 x TZEI24 and TZI18 x FBML10 in set2 trials) were also used as checks.The list of the inbred parents is presented in Tables 6.1.

6.2.2 Field evaluation and Experimental design

The trials were conducted during the off-season in 2011/2012 and 2012/2013 at the experimental station of INERA in Valley du kou located at 11°22′ N Latitude, 4°22′ W Longitude; and at 300 m above sea level, characterized by ferruginous and acid soils with silty texture. Hybrids of each set were randomized separately and laid out in different trials but planted at the same time, and received the same treatment. The trials were established and managed according to procedures developed by CIMMYT (Bänziger *et al*., 2000). Adequate irrigation was applied in both water regimes from planting and throughout the vegetative phase, using furow irrigation system.

Based on the prior evaluation of a sample of hybrids in 2011 during the rainy season, the average pollination date for the trial was predicted. In addition, the crop was monitored on a daily basis. Drought stress was achieved in water stressed regime by withholding water two weeks before the expected flowering time for 21 days.

Table 6. 1. Parent inbred lines used in line by tester mating scheme

No	Inbred lines	Origin	Grain color	Pedigree
1	VL0512593	CIMMYT	white	Syn01E2-64-2-B-2-BB
2	VL058589	CIMMYT	white	INTA-F2-192-2-1-1-1-B*7-2-B-3
3	T02058	CIMMYT	white	[CML389/CML176]-B-29-2-2-B*6-B
4	VL0511247	CIMMYT	white	[INTA-2-1-3/INTA-60-1-2]-X-11-6-3-BBB
5	VL054794	CIMMYT	white	[[[K64R/G16SR]-39-1/[K64R/G16SR]-20-2]-5-1-2-B*4/CML390]-B-38-1-B-2-#-1-B-2
6	VL05616	CIMMYT	white	[SC/CML204//FR812]-X-30-2-3-2-1-B*6
7	VL057903	CIMMYT	white	ZEWAc1F2-151-6-1-B-1-BBB-2-2-BB
8	VL057967	CIMMYT	white	ZEWAc1F2-219-4-3-B-1-B*4-1-3-BB
9	VL058014	CIMMYT	white	ZEWAc1F2-254-2-1-B-1-BB-1-3
10	VL058025	CIMMYT	white	ZEWAc1F2-164-3-2-B-1-BBB-2-2-BB
11	VL05615	CIMMYT	white	ZEWBc1F2-216-2-2-B-2-B*4-4-2-8-B
12	VL081464	CIMMYT	white	ZEWBc2F2-101-2-BB
13	VL081466	CIMMYT	white	ZEWBc2F2-110-1-BBB
14	TZEI16	IITA	Yellow	TZE Comp5-YS C6 S6 Inbred 31
15	TZEI177	IITA	Yellow	TZE Comp5-Y C6 S6 Inbred 62-1-2
16	TZEI124	IITA	Yellow	TZE-Y Pop DT STR Co S6 Inbred 3-1-3
17	TZEI146	IITA	Yellow	TZE-Y Pop DT STR Co S7 Inbred 49-3-3
18	TZEI148	IITA	Yellow	TZE-Y Pop DT STR Co S6 Inbred 62-1-3
19	TZEI149	IITA	Yellow	TZE-Y Pop DT STR Co S6 Inbred 66-2-2
20	TZEI151	IITA	Yellow	
21	TZEI158	IITA	Yellow	TZE-Y Pop DT STR Co S6 Inbred 102-2-2
22	TZEI161	IITA	Yellow	TZE-Y Pop DT STR Co S6 Inbred 103-2-3
23	TZEI23	IITA	Yellow	TZE-Y Pop DT STR Co S6 Inbred 62-2-3
24	TZEI8	IITA	Yellow	TZE-Y Pop DT STR Co S6 Inbred 62-3-3
25	TZI18	IITA	Yellow	Sete Lag. 7728 x TZSR
26	ELN41111	INERA	white	FBC6 x FBMS1
27	ELN41112	INERA	white	FBC6 x FBMS1
28	ELN41114	INERA	white	FBC6 x FBMS1
29	ELN41115	INERA	white	FBC6 x FBMS1
30	ELN41271	INERA	white	FBC6 x FBMS1
31	ELN41272	INERA	white	FBC6 x FBMS1
32	ELN42441	INERA	white	FBC6 x FBMS1
33	ELN42442	INERA	white	FBC6 x FBMS1
34	ELN42444	INERA	white	FBC6 x FBMS1
35	ELN42445	INERA	white	FBC6 x FBMS1
36	ELN48392	INERA	white	FBC6 x FBMS1
37	ELN39382	INERA	Yellow	FBC6 x FBMS1
38	ELN39427	INERA	Yellow	FBC6 x FBMS1
39	ELN402213	INERA	Yellow	FBC6 x FBMS1
40	ELN40791	INERA	Yellow	FBC6 x FBMS1

continued

No	Inbred lines	Origin	Grain color	Pedigree
41	ELN40823	INERA	Yellow	FBC6 x FBMS1
42	ELN40941	INERA	Yellow	FBC6 x FBMS1
43	ELN431251	INERA	Yellow	FBC6 x FBMS1
44	ELN43453	INERA	Yellow	FBC6 x FBMS1
45	ELN43574	INERA	Yellow	FBC6 x FBMS1
46	ELN45111	INERA	Yellow	FBC6 x FBMS1
47	ELN462121	INERA	Yellow	FBC6 x FBMS1
48	ELN464171	INERA	Yellow	FBC6 x FBMS1
49	ELN47132	INERA	Yellow	FBC6 x FBMS1
50	FBML10	INERA	Yellow	Derived from Ku1414
	Testers			
51	VL0511298	CIMMYT	white	MAS[MSR/312]-117-2-2-1-B*4-2-14-BB
52	VL054881	CIMMYT	white	[Ent2:92SEW1-EarlySel-22/[DMRESR-W]EarlySel-#1-3-2-B/CML390]-B-26-1B-1-#-1-BB-3-1
53	TZEI 17	IITA	Yellow	TZE Comp5-Y C6 S6 Inbred 35
54	TZEI 10	IITA	Yellow	TZE-Y Pop DT STR Co S6 Inbred 152

Testers No 51 and 53 belong to heterotic group A while testers No 52 and 54 belong to heterotic group B

Soil moisture level was determined weekly by collecting soil sample from two depth (0-30 and 30-45 cm) in the field. Weights of the wet samples were recorded, oven dried, and the dry weights recorded. This provides the values for total soil moisture content.

The 48 white hybrids plus 7 checks were randomized in incomplete block, 11 x 5 alpha lattice design with three replications. The 52 yellow hybrids plus 7 checks were randomized in 6 incomplete blocks with 10 entries per block but the last block which had 9 entries, all in 3 replications. The experimental unit was a single row of 5 m spaced 80 cm. Two seeds were planted per hill spaced 25 cm and thinned to one plant per hill to give a final population density of 50, 000 plants/ha.

6.2.3 Field Measurements

Grain yield and 12 secondary traits were measured or calculated. Grain yield was calculated according to the procedure described by (Menkir *et al.*, 2003). In Well-watered environments, all ears harvested from each plot were weighed and representative samples were shelled to determine percent moisture. Therefore, grain yield adjusted to 15% moisture was computed from ear weight and grain moisture assuming a shelling percentage of 80%, based on the following formula: Grain yield (kg/ha) = Ear weight (kg) x 0.8 x (100-moisture)/85) x (10/area m^2) x 1000. In water-stressed environments, all ears harvested from each plot were shelled to determine percent moisture. Grain yield adjusted to 15% moisture was computed from the shelled grain based on the following formula : Grain yield (kg/ha) = Grain weight (kg) x (100-moisture)/85) x (10/area m^2) x 1000. The secondary traits measured in the experiment are listed and defined in Table 6.2

Table 6. 2. Description of the phenotypic traits measured or calculated

No	**Trait**	**Description**
1	Anthesis Date (AD)	Number of days from planting to when 50% of plants had shed pollen.
2	Silking Date (SD)	Number of days from planting to when 50% of plants had silks extruded.
3	Anthesis to Silking Interval (ASI)	SD minus AD.
4	Ears per Plant (EPP)	Number of cobs with at least one grain, divided by the total number of plants.
4	Plant height (PHT)	The distance from the base of the plant to the first tassel branch measured in cm, obtained from five competitive plants
6	Ear Height (EHT)	The distance from the base of the plant to the node bearing upper ear measured in cm, obtained from five competitive plants
7	Ear Aspect (EASP)	Visual score: 1 (clean, uniform, large and well-filled ears) to 5 (rotten, variable, small and partially filled ears)
8	Leaf Rolling (LR)*	Visual score: 1 (unrolled leaves) to 5 (rolled leaves). LR scores were recorded three times at weekly interval commencing a week after stress application.
9	Leaf Erectness (LE)*	Visual score: 1 (erect leaves) to 5 (lax leaves). LE scores were recorded twice weekly intervals commencing three weeks after stress application.
10	Leaf senescence (SEN)*	Visual score: 1 (0% senesced leaves) to 10 (100% leaves dead). SEN scores were recorded twice at weekly intervasl commencing two weeks after stress application.
11	Tassel size (TS)*	Visual score: 1 (small tassel size) to 5 (large tassel size). TS scores were recorded twice at weekly intervals commencing three weeks after stress application.
12	Plant Recovery (PR)*	Visual score: 1 (all plants recovered from stress) to 5 (all plants dead). PR scores were recorded twice at weekly interval commencing one week after irrigation was stopped

* traits measured only in water-stressed environment.

6.2.4 Statistical Analysis

Analysis of variance

Individual analysis of variance of each measured trait in each year and water regime were conducted with the PROC MIXED procedure from SAS with genotypes considered as fixed effects, and replication and blocks within replication as random effects. Because the alpha lattice did not provide significant efficiency over randomized complete block design (RCBD), data were analyzed as from RCBD. Combined analysis of variance were conducted with PROC GLM in SAS (SAS, 2002) using RANDOM statement with Test option. For combined analysis in well-watered (WW) and water-stressed (WS) conditions, mean square for genotypes, years and genotypes x years (G x Y) were tested against the mean squares for G x Y, replications within years, and the pooled error, respectively.

Heritability estimates

Variance components were estimated from plot raw data using VARCOMP in SAS with years, replications, genotypes and the genotype x year interactions as random factors.

Broad-sense heritability of traits (h_b^2) was also calculated on an plot basis as the ratio between the genetic variance (σ_G^2) and the sum of the genetic variance, the variance of the genotype x year interaction (σ_{GY}^2) divided by the number of years (y) and the variance of the residuals (σ_e^2) divided by the number of replications (r) and years : $\mathrm{h_b^2} = \frac{\sigma_G^2}{\sigma_G^2 + \sigma_{GY}^2/y + \sigma_e^2/ry}$

Path coefficient analysis

Path coefficient analysis were performed to evaluate the relative importance of direct and indirect effects of secondary traits on yield. Pearson correlation (phenotypic correlation) was first calculated and then the correlation coefficients were partitioned in to direct and indirect effects through path coefficient analysis using PATHSAS program developed by Cramer *et al.* (1999).

6.3 Results

6.3.1 Phenotypic variation

Table 6.3 shows the average phenotypic data per water regime and the mean squares of genotype and genotype by year interactions of the 13 measured traits. Results showed significant variability within white hybrids (set1) for many traits while the yellow hybrids (set2) showed significant variability for few traits. Significant genotype x year interaction was also observed for different traits including grain yield (GY) and anthesis-siking days interval (ASI) in both water regimes. In general, drought stress significantly reduced the GY and increased the ASI in both sets. GY reduction was 46% in set 2 compared to 34% in set1. However, the increase in ASI under drought was around 37% in both sets. The average maturity, plant and ear height of the hybrids in both sets was fairly equal.

Table 6. 3. Phenotypic variation for two maize hybrid sets under Well-watered (WW) and Water stress (WS) conditions across years

Well-watered condition				
Trait	Range	Mean ± SE	MS_G	MS_{GY}
		SET 1		
GY	847.31 - 3243.55	2286.53±409.49	1663882.25**	801898.25*
EPP	0.56 - 1.02	0.88±0.08	0.03	0.02
EASP	2.00 - 3.58	2.78±0.36	0.98*	0.6*
AD	70.00 - 83.67	76.14±0.96	58.13***	6.96***
SD	73.67 - 89.00	79.47±1.08	59.70***	11.11***
ASI	1.50 - 7.33	3.43±0.82	8.02**	3.3*
PHT	147.50 - 196.17	175.12±7.94	641.31***	159.42
EHT	41.33 - 89.00	67.85±6.26	496.5***	104.7
		SET 2		
GY	1512.27 - 2494.53	2531.46±352.27	1072950.66*	573375.8*
EPP	0.70 - 1.10	0.92±0.07	0.03	0.024*
EASP	1.00 - 3.42	2.49±0.34	0.84	0.53*
AD	72.17 - 82.33	76.34±0.99	20.42***	5.89***
SD	75.17 - 86.33	79.27±1.02	23.73***	7.3***
ASI	1.67 - 5.33	2.95±0.77	2.83	2.35
PHT	151.33 - 209.67	173.45±7.89	555.3**	239.97
EHT	62.50 - 106.00	79.91±6.3	330.6**	151.32

continued

		Well-stressed condition		
Trait	Range	Mean ± SE	MS_G	MS_{GY}
		SET 1		
GY	642.93 - 2494.53	1514.16±299.39	1325157.49***	481411.35**
EPP	0.51 - 1.04	0.83±0.11	0.074*	0.04
EASP	2.5 - 3.83	3.08±0.28	0.33	0.25
AD	70.50 - 86.40	77.77±1.35	62.84***	10.86**
SD	77.67 - 90.17	83.47±1.24	37.44***	7.1*
ASI	1.33 - 9.00	5.53±0.88	12.64**	5.47***
PHT	119.33 - 147.50	135.29±7.56	325.17	261.09*
EHT	45.60 - 70.50	59.79±5.19	230.78***	77.49
SEN	2.92 - 5.25	3.98±0.59	1.9	1.38
LR	1.56 - 2.56	1.99±0.26	0.34	0.32*
LE	1.67 - 3.75	2.62±0.3	1.15**	0.50**
TS	2.00 - 3.50	2.75±0.24	0.66***	0.27*
PR	1.92 - 4.50	2.89±0.51	1.88***	0.64
		SET 2		
GY	602.76 - 2408.87	1360.23±357.07	836422.9	605965.05*
EPP	0.54 - 1.16	0.85±0.11	0.06	0.04
EASP	2.00 - 3.5	2.86±0.31	0.45	0.56**
AD	74.67 - 86.33	78.96±1.63	27.52***	9.61
SD	78.67 - 89.00	83.84±1.59	25.76	21.22***
ASI	2.00 - 8.17	4.62±1.07	7.73	5.70*
PHT	106.39 - 161.83	134.06±10.17	529.27	415.23
EHT	46.22 - 86.83	66.92±6.98	276.57	184.24
SEN	2.33 - 7.50	4.82±0.75	1.96	2.54*
LR	1.44 - 3.33	2.48±0.4	0.43	0.55
LE	1.42 - 3.58	2.58±0.28	1.08***	0.37*
TS	2.33 - 3.42	2.87±0.24	0.34	0.34**
PR	1.33 - 4.17	2.91±0.53	1.67	1.23*

SE= standard error *, ** and *** significant at P=0.05, 0.01 and 0.001 respectively

6.3.2 Genetic and environmental variances and broad sense heritability.

The magnitude of genotypic (σ^2_G), genotype by year interaction (σ^2_{GY}) and error (σ^2_e) variances, as well as the estimated broad-sense heritability (h^2_b) of specific traits for each water regime and hybrid set are presented in Table 6.4. Under both water regimes, values of σ^2_G were greater for white hybrids (set1) developed froù mid-altitude and lowland tropical inbred lines than for yellow hybrids (set2) which included only lowland tropical lines, but genotype by year interaction and error variances were greater in set 2 than in set 1.

Genotypic variance (σ^2_G) and to some extent broad sense heritability for grain yield decreased under drought for hybrids in both set 1 and 2. Among the two hybrid sets, heritability for GY ranged from 0.36 to 0.64 under water-stressed condition and had a value around 0.50 under well-watered conditions. In contrast, the σ^2_G and h^2_b for ASI and number of ears per plant (EPP) either increased or remained fairly constant under drought. The heritability of ear aspect (EASP) was around 0.40 under well-watered conditions, but also decreased under drought. Leaf erectness (LE), Tassel size (TS) and plant recovery (PR) score had a value around 0.60 of heritability for hybrids in set1 but only LE showed a higher heritability (0.69) in set 2. Leaf rolling (LR) and leaf senescence (SEN) showed low heritability ranging from 0 to 0.3 for the two hybrid sets.

Table 6. 4. Estimates of genetic variance components and broad sense heritabilities of grain yield and other traitsof both hybrid sets evaluated under two water regimes over 2 years

Trait	**SET 1**				**SET 2**			
				Well - watered condition				
	σ^2_G	σ^2_{GY}	σ^2_e	h^2_b	σ^2_G	σ^2_{GY}	σ^2_e	h^2_b
GY	148379.40	101085.90	503163.00	0.53	92866.90	65867.80	373652.80	0.49
EPP	0.00	0.00	0.02	0.34	0.00	0.00	0.02	0.30
EASP	0.07	0.07	0.40	0.40	0.06	0.06	0.34	0.39
AD	8.70	1.41	2.75	0.88	2.67	1.00	2.92	0.73
SD	9.04	2.62	3.51	0.83	3.05	1.49	3.14	0.71
ASI	0.84	0.42	2.04	0.60	0.06	0.23	1.77	0.12
PHT	77.89	0.00	183.54	0.72	58.70	15.22	186.54	0.60
EHT	66.23	0.00	115.40	0.78	33.65	9.05	119.11	0.58
				Water-stressed condition				
GY	141711.90	71506.60	268908.70	0.64	52507.10	61989.40	386307.20	0.36
EPP	0.01	0.00	0.04	0.48	0.00	0.00	0.03	0.27
EASP	0.01	0.01	0.23	0.25	0.00	0.07	0.30	0.00
AD	9.05	1.79	5.50	0.83	3.47	0.71	7.99	0.67
SD	5.25	0.79	4.65	0.82	1.15	4.23	7.68	0.25
ASI	1.46	1.30	2.32	0.59	0.47	0.72	3.62	0.33
PHT	11.50	27.65	173.14	0.21	25.18	30.50	310.10	0.27
EHT	25.77	0.00	80.24	0.66	18.43	11.04	146.02	0.38
LR	0.01	0.04	0.20	0.08	0.00	0.00	0.48	0.00
LE	0.10	0.08	0.28	0.55	0.13	0.04	0.23	0.69
TS	0.06	0.03	0.18	0.58	0.01	0.05	0.18	0.09
SEN	0.09	0.11	1.06	0.27	0.00	0.18	1.68	0.00
PR	0.19	0.00	0.75	0.60	0.09	0.12	0.85	0.31

σ^2_G = Genetic variance, σ^2_{GY} =Variance of Genotype by year interaction, σ^2_e = Error variance, h^2_b = Broad-sense heritability.

6.3.3 Path coefficient analysis

The phenotypic correlation between secondary traits and grain yield along with their direct and indirect effects are presented in tables 6.5 and 6.6

The phenotypic correlation values of the traits with grain yield computed for hybrids in set 1 versus those computed for hybrids in set 2 were variable. However, the coefficient of correlations between grain yield and ears per plant was consistently high and positive under both water regimes, and hybrid sets, while that with ear aspect was negative but high across both trials. In both hybrid sets, ASI was significant and negatively associated with grain yield under well-watered conditions but the coefficient of correlation was lower and non-significant under drought stress.

The correlation of LR and SEN with grain yield was negative and larger for hybrids in set 2 (r=-0.52*** and r= - 0.62*** respectively) than set 1 (r= 0.08 to -0.13 respectively). The coefficients of correlation of PR with grain yield was the same value (r= -0.33**) in the both hybrid sets but that of tassel size with grain yield was significant and negative in set1 while it was positive and non-significant in set 2.

The phenotypic correlation between the measured traits and grain yield was partitioned into direct and indirect effects using path coefficient analysis. Similar patterns were observed for EASP and EHT in both hybrid sets and water regimes while the patterns were variable for the other traits between set 1 and 2. EASP showed the highest and negative direct effect on grain yield across all the trials ranging from - 0.4 to - 0.6 with several traits having important indirect effects on grain yield via this trait. In general, EPP, PHT and EHT had positive indirect effect on grain yield through EASP while AD, ASI, LR and SEN, and PR had negative indirect effect on grain yield via EASP.

Under drought stress, EHT had positive and high direct effect, and negative indirect effect via plant height on grain yield indicating that the ratio between ear and plant heights (R_EPH) was associated to grain yield which was calculated and; significant and positive phenotypic correlation was found between R_EPH and grain yield (r=0.44** and r=0.36** in set 1 and 2 respectively). EPP showed high positive direct effect on grain yield for hybrids in set 1 while its direct effect was low for hybrids in set 2. Under drought stress, EPP in set 2 had important indirect effect on grain yield via LR (-0.16) and SEN (0.2). AD had, under drought stress, negative and positive effect for hybrids in set 1 and 2, respectively. High (0.27) and low (0.01) positive direct effects were observed for ASI for hybrids in set 2 and 1 respectively under water-stressed conditions whereas its indirect effect on grain yield via EASP was the same in magnitude (-0.11) in both hybrid sets.

Among the drought adaptive traits represented by LR, SEN, LE, TS and PR, four of them including SEN (-0.55), LR (0.39), TS (0.19) and LE (-0.10) showed important direct effect on grain yield for hybrids in set 2 while three, PR (- 0.15), TS (- 0.14) and LR (0.11) showed high direct effects for hybrids in set 1.

Table 6. 5. Partition of correlation coefficients into direct and indirect effects on grain yield under well-watered conditions

	Direct effect	**Indirect effect**						**Phenotypic correlation with grain yield**
		EPP	EASP	EHT	AD	PHT	ASI	
				SET 1				
EPP	0.36		0.24	0.05	0.01	-0.03	0.00	0.63***
EASP	-0.56	-0.15		-0.10	-0.01	0.05	0.01	-0.78***
EHT	-0.36	-0.05	-0.16		-0.03	0.36	0.00	-0.23
AD	-0.03	-0.10	-0.23	-0.30		0.27	0.00	-0.40**
PHT	0.45	-0.02	-0.06	-0.29	-0.02		0.00	0.06
ASI	0.01	-0.07	-0.27	0.02	0.00	-0.08		-0.38**
				SET 2				
EPP	0.15		0.12	-0.01	0.02	0.00	0.11	0.39**
EASP	-0.36	-0.05		-0.07	-0.02	-0.03	-0.08	-0.61***
EHT	0.23	-0.01	0.11		-0.02	0.05	-0.02	0.35**
AD	-0.05	-0.05	-0.13	0.07		0.01	-0.03	-0.18
PHT	0.06	0.00	0.18	0.19	0.00		-0.03	0.40**
ASI	-0.25	-0.07	-0.12	0.01	-0.01	0.01		-0.42*

SET 1: R2=0.78 N=55; SET 2 : R2= 0.50 N=59

SET 1= White hybrids; SET2= Yellow hybrids

EPP= Number of ear per plant; EASP=Ear aspect; EHT=Ear height; AD=Anthesis-days; PHT=Plant height; ASI= Anthesis-silking interval.

Table 6. 6. Partition of correlation coefficients into direct and indirect effects on grain yield under drought stress

	Direct effect	**Indirect effect**											**Phenotypic correlation with grain yield**
		EPP	EASP	EHT	AD	PHT	LR	SEN	LE	TS	PR	ASI	
							SET 1						
EPP	0.27		0.23	0.17	-0.05	0.01	-0.02	0.00	0.00	0.02	0.03	0.00	0.62***
EASP	-0.48	-0.13		-0.08	0.03	0.05	0.01	0.00	-0.01	-0.05	-0.06	0.00	-0.70***
EHT	0.51	0.09	0.08		-0.16	-0.17	0.00	0.00	0.02	0.00	0.05	0.00	0.42**
AD	-0.28	0.05	0.04	0.30		-0.04	0.00	0.00	0.03	0.00	0.07	0.00	0.16
PHT	-0.28	-0.01	0.08	0.32	-0.04		-0.02	0.00	0.01	0.00	0.05	0.00	0.13
LR	0.11	-0.06	-0.05	0.00	-0.01	0.06		-0.01	0.02	0.04	-0.04	0.00	-0.08
SEN	-0.01	-0.04	-0.07	-0.15	0.08	0.10	0.07		-0.02	0.01	-0.11	0.00	-0.13
LE	-0.06	0.00	-0.10	-0.16	0.15	0.07	-0.03	0.00		-0.06	-0.07	0.00	-0.29*
TS	-0.16	-0.03	-0.16	-0.01	-0.01	0.00	-0.03	0.00	-0.02		0.00	0.00	-0.42**
PR	-0.15	-0.05	-0.18	-0.16	0.12	0.09	0.03	-0.01	-0.03	0.00		0.00	-0.33**
ASI	0.01	-0.05	-0.11	-0.10	0.16	0.02	0.02	0.00	-0.03	-0.02	-0.04		-0.13
							SET 2						
EPP	0.13		0.26	0.10	-0.02	-0.03	-0.16	0.25	0.00	0.00	0.01	-0.01	0.50***
EASP	-0.62	-0.05		-0.14	0.05	0.06	0.22	-0.25	0.03	-0.01	-0.01	0.05	-0.66***
EHT	0.42	0.03	0.20		-0.02	-0.15	-0.16	0.18	-0.03	-0.03	0.00	-0.02	0.44***
AD	0.17	-0.02	-0.17	-0.05		0.00	0.15	-0.19	0.02	0.04	0.01	-0.11	-0.17
PHT	-0.20	0.02	0.18	0.31	0.00		-0.12	0.09	-0.04	-0.01	0.01	0.02	0.26
LR	0.39	-0.05	-0.34	-0.17	0.07	0.06		-0.43	0.02	-0.01	0.00	-0.01	-0.52***
SEN	-0.55	-0.06	-0.28	-0.13	0.06	0.03	0.31		0.00	-0.02	-0.01	0.01	-0.62***
LE	-0.10	-0.01	0.15	0.12	-0.03	-0.07	-0.08	0.00		-0.03	0.00	0.02	-0.04
TS	0.19	0.00	0.02	-0.07	0.04	0.01	-0.02	0.04	0.02		0.00	0.00	0.22
PR	-0.03	-0.03	-0.25	-0.04	-0.05	0.09	0.05	-0.10	-0.01	-0.02		0.06	-0.33**
ASI	0.27	-0.01	-0.11	-0.03	-0.07	-0.01	-0.01	-0.02	-0.01	0.00	-0.01		-0.00007

SET 1: R2=0.74 N=51; SET 2 : R2= 0.72 N=59

LR=leaf rolling; SEN=Leaf senescence; LE=Leaf erectness; TS=Tassel size; PR=Plant recovery.

6.4 Discussion

Grain yield. The genetic variance for grain yield estimated for the two hybrid sets was lower under drought conditions compared to well-watered conditions. Reduced genetic variance for grain yield under moisture stress conditions was also reported by Messmer *et al*. (2009) and Almeida *et al*. (2013). In hybrid set1, the heritability estimates for grain yield unders well-watered condition was less than under moisture stressed (WS) conditions, indicating high yield stability of white hybrids (set1). According to Almeida *et al*. (2013), high heritability estimates of grain yield under WS environments implies stability of drought tolerant genotypes across diverse environments.

Ear per plant (EPP) and grain yield. The phenotypic correlation coefficient between EPP and grain yield was significant and positive with the direct effect of EPP positive and high for hybrids in set1. Similarly, for hybrids in set 2, correlation between the two traits was significant with the direct effect of EPP positive but low. On the other hand, positive indirect effect of EPP on grain yield via EASP and SEN was found which seemed to be the cause of the strong correlation between EPP and grain yield for hybrids in set2. Therefore, these traits must be considered if selection is made through EPP. The heritability value (0.48) of EPP for hybrids in set1 was similar to the 0.54 reported by Bolaños and Edmeades (1996) but it was lower (0.27) for hybrids in set 2.

Ear aspect (EASP) and grain yield. EASP had a high negative direct effect on grain yield. The phenotypic correlation between the traits was also negative and significant. Therefore, the correlation reprented the true relationship between the two traits. Ear aspect scores captured the different yield components such as ear diameter, ear length, kernels rows and kernel per row. High positive direct effect of ear diameter and number of kernels per row on maize ear weight was reported (Arias *et al*., 1999). Ahmad and Saleem (2003) showed that the direct effect of kernel rows on grain yield per plant was also positive and greater in magnitude than that of genotypic correlation. Heritability of EASP in the present study was low under drought. Because of the small variance, selection for this trait may not be effective even though the correlation of EASP with grain yield under drought was high.

Anthesis days (AD) and grain yield. The phenotypic correlation between AD and grain yield was low and significant for both hybrid sets but the signs were different. Similarly the direct effects were high with different direction. For hybrids in set 1, the direct effect of AD was negative but the positive and high indirect effect through EHT and PR may have caused the positive correlation observed. For hybrids in set 2, the negative and high indirect effect of EASP, SEN and ASI seemed to be the cause of the negative correlation between AD and grain yield while the direct effect was positive and high. Heritability of AD was high which is consistent with earlier reports (Magorokosho *et al*., 2003; Bolaños and Edmeades, 1996). Grain yield potential of hybrids tested was less affected by their maturity since the correlation between AD and grain yield was low, however earliness and high yield was associated, to some extent for hybrids in set 2 under drought stress because of negative but low correlation between the two traits.

Anthesis-silking interval (ASI) and grain yield. The phenotypic correlation coefficient was negative and low between the two traits. The magnitude of direct effect of ASI on grain yield was variable depending on hybrid set, the same trend was also found for the heritability of this trait. This result contrasts with results from several drought tolerance studies in maize (Edmeades *et al*., 1995; Bolaños and Edmeades, 1996; Messmer *et al*., 2009; Almeida *et al*., 2013). This unexpected result was explained by the relative high number of missing values recorded for silking days, since some plots under drought stress failed to reach 50% silking approximately 20 to 30 days after 50% anthesis, and were declared missing values. Usefulness of ASI as an indirect selection trait for grain yield under drought stress was suggested, but not conclusively established in this study though high heritability was found for this trait under drought.

Plant height (PHT) and grain yield. The phenotypic correlation coefficient was positive and low between the two traits, but the direct effect of plant height was negative and high which was also found under severe drought conditions by Khalili *et al*. (2013), indicating that hybrids with high plant height were more sensitive to drought stress than medium height plant. The indirect positive and high effect of ear height and ear aspect are the possible cause of positive and low correlation between plant height and grain yield. Therefore, these traits must be considered if selection is made through plant height. Similar result was reported by Ahmad and Saleem (2003) in non-drought condition.

Ear height (EHT) and grain yield. The phenotypic correlation between EHT and grain yield and direct effect of ear height were both positive and almost equal in magnitude. Guan-zheng *et al*. (2008) reported also positive direct effect of EHT on single ear yield of maize inbred lines under medium drought conditions. Moreover high heritability was found for EHT in the present study under drought as well as under well-watered conditions, indicating that characteristic of ear position should be considered in selection of high yielding drought tolerant genotypes.

Leaf rolling (LR) and senescence (SEN) and grain yield. The phenotypic correlation coefficient was significant and negative between LR and SEN and grain yield of yellow lowland tropical hybrids in set2. The direct effect on grain yield was also greater and negative for SEN but positive for LR. The positive indirect effect of SEN on grain yield through LR and inversely the negative indirect effect of LR on grain yield via SEN, indicated that the two traits should definitely be selected simultaneously. However the genetic variance of the two traits was zero indicating the low variability within the hybrids tested for these traits. In contrast, Bolaños and Edmeades (1996) found that the heritability of LR and SEN was moderate while the genotypic correlation between these traits and grain yield was low using 3509 inbred progenies (S_l to S_3 level) in their study. The type of genetic material included in the two studies could explain the contradictory results. The phenotypic correlation coefficient and the heritability of LR and SEN of the white hybrids in set1 were all low. This result showed that any progress can be made by including these traits in selection index for selecting high yielding drought tolerant hybrids in set1 .

Leaf erectness (LE) and grain yield. The direct effect of leaf erectness was negative and variable in magnitude. The phenotypic correlation between LE and grain yield with grain yield was non-significant and negative, however the heritability under drought was higher. Similar result was reported by Bolaños and Edmeades (1996) for the heritability.

Tassel size (TS) and grain yield. Hybrids in set1 showed a negative direct effect of TS on grain yield as well as negative and significant phenotypic correlation between TS and grain yield. Moreover this trait showed a high heritability, indicating that TS can be included in the selection index as an indirect selection trait for grain yield under drought conditions. However the high negative contribution to grain yield was through ear aspect, therefore this trait should also be included in the selection index. Conversely the use of TS in selection for hybrids in set 2 would not be effective since opposite results was found for the hybrids.

Plant recovery (PR) and grain yield. Results showed a significant and negative phenotypic correlation between PR score and grain yield. The magnitude of the direct effect on grain yield was negatively high and low in sets 1 and 2, respectively. The same trend was observed for the heritability. The use of plant recovery as secondary trait would be more effective for selection of drought tolerant genotypes among the white hybrids (set1) than the yellow (set2) included in the present study.

6.5 Conclusions and Recommendations

Results of the present study showed that important genetic variability for grain yield and secondary traits existed among the white hybrids derived by crossing two CIMMYT testers with 24 inbred lines from CIMMYT and INERA breeding programs. Similary, genetic variability was found but was less important in the yellow hybrids generated by line x tester mating with 2 IITA testers and, 26 IITA and INERA lines. This variability will facilitate selection for drought tolerant hybrids with high yield potential, either by drought escape mechanisms or by using secondary traits in a selection index. The relative value of secondary traits for indirect selection for a primary trait is determined by the magnitudes of their genetic variances, heritabilities and genetic correlations with the primary trait (Falconer, 1981). This study identified EPP and to some extent leaf rolling and senescence, tassel size and plant recovery as indirect selection criteria for grain yield under drought conditions. The usefulness of ASI and LE as an indirect selection trait for grain yield under drought stress were not conclusively established in this study. The path coefficient analysis showed that the traits identified as useful secondary traits were significantly correlated to grain yield but, their direct effect on grain yield were low and/or had important contribution to grain yield through other traits which should also be considered in selection for drought tolerance.

In the light of these findings, GY, EPP, TS and PR were suggested for use in the selection for white hybrids in set1. In addition EASP and R_EPH should also be added. They are not drought tolerant traits by themselves but EPP, TS and PR had important indirect effect on grain yield via

EASP. Moreover its correlation coefficient with grain yield and direct effect on grain yield was higher. The ratio, R_EPH would avoid selection of genotypes with undesirable ear position and plant height characteristics. As for yellow hybrids in set 2, GY, LR, SEN and PR were recommended, plus EASP and R_EPH. Like in set 1, EPP, LR, SEN and PR had high indirect effects on grain yield via EASP which had significant correlation coefficient with grain yield and high direct effects on grain yield. The raison to include R_EPH in the selection index is the same as stated in set 1.

The function of the selection index in each of the two hybrid sets would be,

SET 1: Selection index = 5 f (GY) + 3 f (EPP) - 2 f (TS) - 2 f (PR) - 2 f (EASP) + 1 f (R_EPH)

SET 2 : Selection index = 4 f (GY) + 3 f (EPP) - 2 f (LR) - 2 f (SEN) - 2 f (PR) - 2 f (EASP) +1 f (R_EPH)

In set 1 the weight of GY is higher than that in set 2 because the heritability for grain in the first set was higher in WS than the corresponding in WW conditions, indicating stability of grain yield (Almeida *et al*., 2013) while the opposite result was found in set 2.

CHAPTER SEVEN

7.0 Genetic analysis and heterotic relationships among lowland and mid-altitude tropical maize inbred lines under drought and non-drought conditions

7.1 Introduction

The successful adoption of the hybrid "Bondofa" in Burkina Faso has emphasized the need for development of more maize hybrids in the INERA maize breeding program. In developing countries, judicious application of available crop improvement methods and use of both local and exotic germplasm to improve yields and yield stability are required to meet the increasing demand of improved maize hybrids (Dhliwayo *et al*., 2009). A wide range of inbred lines adapted to the mid-altitude regions of southern and eastern Africa (CIMMYT, 1998) and western and central Africa (Everett *et al*., 1994a, 1994b) developed by CIMMYT and IITA have been made available to national agricultural research system (NARS). In addition, INERA has extracted inbred lines from adapted and adopted OPVs, but little is known about the heterotic relationships between CIMMYT and INERA and between IITA and INERA maize inbred lines. To make effective use of the available local and exotic germplams, information on the heterotic relationships and combining abilities are desirable.

General combining ability (GCA) and specific combining ability (SCA) effects represent additive and non-additive gene effects which control the variation of quantitative traits. The contribution of GCA and SCA can differ among environments (Betrán *et al*., 2003b,c; Worku *et al*., 2008). Drought stress is among the major abiotic stresses causing yield reduction in maize grown in the tropics (Edmeades *et al*., 1995). Understanding of the genetic basis of hybrid performance under this stress is crucial to the design of appropriate breeding strategies (Hallauer and Miranda, 1988; Betrán *et al*., 2003a,b). Moreover, the evaluation of hybrids under drought and non-drought stress conditions will facilitate the identification of hybrids with consistent performance across environments (Menkir *et al*., 2003). An integrated approach is essential to identify high yielding and stable drought tolerant hybrids.

The objectives of this study were to: (i) determine the relative importance of GCA and SCA in white and yellow mid-altitude and lowland maize inbred lines of CIMMYT, IITA and INERA; (ii) examine the heterotic relationships among 24 CIMMYT and INERA white lines, 26 IITA and INERA yellow inbred lines for grain yield and, classify them into complementary heterotic groups; (iii) identify high yielding and stable drought tolerant hybrids.

7.2 Materials and Methods

7.2.1 Plant Materials

The same plant materials presented in Chapter 6 was used for the current study. Testers, VL0511298 and VL054881 from CIMMYT, belonging to heterotic group A and B, respectively, were developed from two different populations. VL0511298 had CML312SR as a parent and VL054881 had CML390 in its genetic background. Four lines (VL057903, VL058025, VL057967 and VL058014) were developed from an extra-early population developed through reciprocal recurrent selection from a wide pool of Southern and Eastern Africa adapted inbred lines of N3, Kitale, A and Tuxpeño population backgrounds. VL05615, VL081464, VL081466 were developed from an extra-early population developed through reciprocal recurrent selection from a wide pool of Southern and Eastern Africa adapted inbred lines from SC, Ecuador, B, and ETO population backgrounds. VL05616 traces 50% of its pedigree to a temperate inbred line

(FR812). No known relationship exists between the testers and the lines except that the tester VL054881 and line VL054794 have CML390 in common in their genetic background. Tester, TZEI 17, from heterotic group A and two lines (TZEI 177 and TZEI 16) were derived from the broad-based *Striga hermonthica* resistant early yellow population, TZE COMP5-Y. Tester, TZEI 10 which belongs to heterotic group B and 8 inbred lines including TZEI 158, TZEI 161, TZEI 124, TZEI 148, TZEI 23, TZEI 8, TZEI 149 and TZEI 146, were derived from the broad-based *Striga hermonthica* resistant and drought tolerant early yellow population, TZE-Y Pop DT STR. The eleven white and thirteen yellow lines from INERA used in the present study (Chapter 6, Table 6.1) were all extracted from the early yellow drought tolerant Open-pollinated variety, FBC6, which was created by the mixture of 8 different yellow and white varieties (see materials and methods of chapter 4 for more detail).

*Generation of testcross hybrids and constitution of hybrid groups***.**

A total of one hundred testcross hybrids were generated using line by tester mating design. Based on grain color of the inbred lines, two hybrid sets were constituted. Two CIMMYT testers, VL0511298 and VL054881, were crossed onto 13 CIMMYT and 11 INERA white inbreds giving 48 white hybrids (set 1). Two IITA testers, TZEI17 and TZEI10, were crossed onto 12 IITA and 14 INERA yellow inbred lines giving 52 yellow hybrids (set 2).

7.2.2 Field evaluation and experimental design.

The two hybrid sets were evaluated under managed drought trials over 2 years as described in Chapter 6. To assess the yield potential of hybrids under optimum conditions and examine the heterotic patterns of the inbred lines in contrasting environments, two additional trials were planted, one during rainy season of 2012 and another during the dry and hot season of 2013 under well-watered and high plant density . However the rainy season trial was affected by the floods at seedling stage and was discarded.

Evaluation under well-watered and high plant density (WWHPD)

Hybrids of the inbred line TZEI151 with the two testers were not included in this trial because of the small amount of seed available. Consequently, 50 of the 52 yellow hybrids plus 4 checks (TZEI17 x TZEI10, TZEI17 x TZEI24, Bondofa, Sanem) and all the 48 white hybrids plus 3 checks (VL0511298 x VL054881, Bondofa, Sanem) were seperatly evaluated. Hybrids of each set were randomized separately in different trials. The trials were conducted at Valley du kou. The experimental unit was a single row of 5 m spaced at 80 cm. Three seeds were planted per hill spaced 40 cm and later thinned to two plants to give a final planting density of 62, 500 plants/ha. The 51 white hybrids and 54 yellow hybrids were laid out in 17 x 3 and 18 x 3 alpha lattices with 3 replications.

7.2.3 Statistical Analysis

Details on the observations recorded for grain yield and other agronomic traits as well as the statistical analysis were presented in Chapter 6.

Analysis of variance for combining ability

Individual analysis of variance was performed for all characters prior to combined analysis. Line x tester analysis was performed for each growing condition including well-watered (WW) and water-stressed (WS) conditions over 2 years, and well-watered high plant density (WWHPD) in one year and across all environments. The hybrid source of variation was partitioned into variations due to lines, testers and line x testers. For WW and WS trials combined over two years, the significance of line GCA , tester GCA and line x tester SCA mean squares were determined using the corresponding interaction with years as error terms. The significance of

GCA_{lines} × year, GCA_{tester} x year and SCA × year interactions was determined using the pooled error. In WWHPD trial, the significance of line, tester and line x tester mean squares were determined using the error mean square. For trials combined over environments (combinaison year by water-regime was considered as an individual environment), the significance of GCA and SCA sources of variation were determined using the corresponding interaction with environments as error terms. The significance of GCA × E and SCA × E interactions was determined using the pooled error.

Furthermore, the relative contribution of lines (GCA_{Line}), testers (GCA_{Tester}) and line x tester ($SCA_{linextester}$) were calculated as the ratio sum of squares of each component and entry sum of squares among the crosses (Singh and Chaudhary, 1985). Estimate of GCA of a tester was obtained in terms of its performance in F1 hybrid combinations with all possible lines. Similary, GCA of a line was determined in terms of its performance in F1 hybrid combinations with the two testers. The lines and testers were considered as fixed effects. Years was considered as random effects. GCA and SCA effects were determined for grain yield as follows:

GCA_{line}= Line mean ($X.j$) – Overall mean (X..);

GCA_{tester}= Tester mean ($Xi.$) – Overall mean (X..);

$SCA_{line\ x\ tester}$ = Cross mean (Xij) – Line mean ($X.j$) – Tester mean ($Xi.$) + Overall mean (X..), where Xij is the mean yield of the cross between i^{th} tester and j^{th} line, $Xj.$ is the mean yield of the i^{th} tester and $X.j$ is the mean yield of jth line.

Standard errors (SE) for general and specific combining ability were calculated following Mohammed (2009) as follows:

$SE_{Lines} = (MS_{LxY}/rty)^{½}$, $SE_{Testers} = (MS_{TxY}/rly)^{½}$, and

$SE_{Line\ x\ Tester} = (MS_{LxTxY}/ry)^{½}$, Where MS_{LxY} and MS_{TxY} are the respective mean squares of line x year and tester x year divided by number of observations (replicates (r), years (y), testers (t) or lines (l)). MS_{LxTxY} is the mean square for line x tester year divided by number of observations (replicates and years). The critical difference (C.D) was calculated as follows: C.D. = SE x t (tabulated). When the absolute effect of GCA or SCA was greater than the C.D., it was considered significantly different from zero. Genetic analysis were computed using SAS program (SAS, 2002). The checks were excluded in the analysis of variance.

Classification of inbred lines into heterotic groups

The specific combining ability (SCA) effects and mean grain yields of testcrosses of the lines with the two testers were used to classify the inbred lines into heterotic groups for each of the three growing conditions and across environments using the procedure described by Menkir *et al*. (2003). Lines that showed positive SCA effects with tester A with testcross mean yields equal to or greater than 1SE of the mean yield of hybrid between the two testers but had negative SCA effects with tester B were placed into anti-A or opposite heterotic group of tester A. When lines exhibited positive SCA with tester B with testcross mean yield equal to or greater than 1 SE of the mean yield of the cross between the two testers but had negative SCA with tester A were assigned into opposite heterotic group of tester B (anti-B). Lines that showed positive SCA effects with both testers A and B with testcross mean yields equal to or greater than 1SE of the mean yield of hybrid between the two testers were placed into anti-A and anti-B groups (or opposite heterotic groups of A and B) whereas lines that exhibited negative SCA effects with both testers A and B or lines that had testcross mean yields less than 1 SE of the mean of the cross between the two testers were not assigned into either heterotic group.

Heterosis estimates

Mid-parent heterosis (MPH) and high-parent heterosis (HPH) for grain yield under well-watered and water-stressed conditions and across water regimes were estimated. Mid-parent heterosis was calculated as: $MPH = \frac{(F1-MP)}{MP}\ x\ 100$, where F1 is the mean performance of the F1 hybrid and MP is the mean of the two inbred parents. Similarly, high-parent heterosis (HPH) was calculated as : $HPH = \frac{(F1-HP)}{HP}\ x\ 100$, where F1 is the mean performance of the F1 hybrid and HP is the mean of the best parent. The grain yield means of parental inbreds were presented in Chapter 5.

Drought tolerance index

Two selection criteria were used, selection tolerance index (STI) and relative decrease in yield (RDY). The formulae for the two indices were presented and discussed in Chapter 5.

AMMI stability value (ASV). The AMMI stability value (ASV) as described by Purchase (2000) was calculated as follows: $\mathrm{ASV} = \sqrt{\left[\frac{\mathrm{SS_{IPCA1}}}{\mathrm{SS_{IPCA2}}}\ \mathrm{x}\ (\mathrm{IPCA1_{Score}})\right]^2 + [\mathrm{IPCA2_{Score}}]^2}$

Where $\frac{\mathrm{SS_{IPCA1}}}{\mathrm{SS_{IPCA2}}}$ is the weight given to the IPCA1-value by dividing the IPCA1 sum of squares by the IPCA2 sum of squares. ASV takes into account both IPCA1 and IPCA2 that justify most of the variation in GE interaction. Smaller ASV scores indicated a more stable genotype across environments. AMMI analysis was performed using GenStat (12th edition) (Payne *et al*., 2009).

Yield stability index (YSI)

YSI was calculated as follows: YSI = RASV + RY, where RASV is the rank of AMMI stability value and RY is the rank of mean grain yield of hybrids (RY) across environments. YSI incorporates both mean yield and stability in a single entity. Low value of this parameter shows desirable hybrids with high mean yield and stability.

Principal component analysis (PCA)

PCA was performed for each hybrid set using 4 variables : high parent heterosis (HPH), selection tolerance index (STI), relative decrease in yield (RDY) and yield stability index (YSI). The first two axes, PCA1 and PCA2, were selected. For each axe, variables and individuals (hybrids) which had high contribution to the formation of the axe were determined. XLSTAT software (Addinsoft, 2007) was used to compute principal component analysis.

7.3 Results

7.3.1 Mean grain yield in managed drought trials

Mean grain yield under well-watered conditions varied from 3243.55 kg/ha to 847.31 kg/ha for white hybrids (set 1), and from 3595.29 kg/ha to 1512.27 kg/ha for yellow hybrids (set 2). Under drought stress, mean grain yields of the trials ranged from 2494.53 kg/ha to 642.93kg/ha for hybrids in set 1, and from 2408.87 kg/ha to 602.76 kg/ha for hybrids in set 2. The average grain yield across water regimes varied from 2832.52 kg/ha for VL0511298 x VL057967 to 777.69 kg/ha for VH052527 among the white hybrids (Table 7.1) while it varied from 2781.34 kg/ha for TZEI17 x ELN43574 to 1243.41 kg/ha for Barka among yellow hybrids (Table 7.2). Ten white and three yellow hybrid out yielded all the 7 checks included in the trials (Table 7.1,2). Sanem was the best check and was ranked 11th (of the 55 entries) with 2311.22kg/ha and 4th (of the 59 entries) with 2504.50kg/ha in hybrid set 1 and 2 trials, respectively. The relatively high performance of this hybrid (Sanem), a cross between FBML10, drought tolerant tropical inbred line, and B73 in managed drought trials suggested that FBML10 contributed favorable alleles for drought tolerance and exhibited high heterosis with B73.

Table 7. 1. Mean grain yield and days to anthesis of 48 white hybrids and 7 checks.

No	**Entry**	**Grain yield(kg/ha)**						**Anthesis-days**		
		WW	Rank	WS	Rank	Across	Rank	WW	WS	Across
1	VLA x ELN41111	2033.84	40	1483.22	25	1758.53	39	81	82	81
2	VLA x ELN41112	2196.87	32	1348.93	36	1772.9	35	80	82	81
3	VLA x ELN41114	1537.65	49	741.78	52	1139.71	53	82	82	82
4	VLA x ELN41115	2033.55	41	1917.43	10	1975.49	22	80	82	81
5	**VLA x ELN41271**	**2415.48**	**25**	**2277.24**	**3**	**2346.36**	**7**	78	80	79
6	**VLA x ELN41272**	**2593.37**	**16**	**2067.08**	**8**	**2330.23**	**9**	79	80	79
7	**VLA x ELN42441**	**2928.77**	**6**	**1716.19**	**14**	**2322.48**	**10**	77	79	78
8	VLA x ELN42442	2512.11	18	2082.21	7	2297.16	12	79	79	79
9	VLA x ELN42444	2156.88	33	1631.68	16	1894.28	26	80	82	81
10	VLA x ELN42445	2738.09	11	1830.18	11	2284.14	13	78	79	78
11	VLA x ELN48392	2815.73	10	1572.73	22	2194.23	16	78	81	79
12	**VLA x T02058**	**2731.95**	**12**	**2266.45**	**4**	**2499.2**	**3**	77	80	78
13	VLA x VL0511247	2235.87	29	1935.27	9	2102.27	18	79	79	79
14	VLA x VL0512593	2110.96	36	1407.86	33	1759.41	38	76	76	76
15	**VLA x VL054794**	**3000.5**	**5**	**2483.65**	**2**	**2742.08**	**2**	77	79	78
16	VLA x VL05615	2923.95	7	1495.65	24	2209.8	15	75	78	76
17	**VLA x VL05616**	**2471.16**	**22**	**2226.91**	**5**	**2349.04**	**6**	77	80	79
18	VLA x VL057903	2554.4	17	1368.46	35	1961.43	24	73	72	72
19	**VLA x VL057967**	**3170.51**	**3**	**2494.53**	**1**	**2832.52**	**1**	75	76	76
20	VLA x VL058014	3059.04	4	1477.88	26	2268.46	14	73	74	73
21	**VLA x VL058025**	**3243.55**	**1**	**1588.91**	**19**	**2416.23**	**4**	73	75	74
22	VLA x VL058589	2511.57	19	1427.32	32	1969.44	23	75	77	76
23	VLA x VL081464	2456.65	23	1745.87	13	2101.26	19	75	75	75
24	VLA x VL081466	1968.96	44	1287.58	38	1628.27	43	73	76	74
25	VLB x ELN41111	2131.85	35	1398.85	34	1765.35	36	78	80	79
26	VLB x ELN41112	1733.14	48	1187.21	40	1460.18	47	79	79	79
27	VLB x ELN41114	1297.15	53	1170.75	41	1228.2	51	80	82	81
28	VLB x ELN41115	1433.41	51	893.18	51	1163.3	52	79	81	80
29	VLB x ELN41271	2204.45	31	1442.34	31	1823.39	31	76	77	76
30	VLB x ELN41272	2147.55	34	1462.18	28	1804.86	32	77	77	77
31	VLB x ELN42441	2355.84	27	1302.07	37	1828.96	28	77	79	78
32	**VLB x ELN42442**	**3223.36**	**2**	**1455.72**	**29**	**2339.54**	**8**	77	78	77
33	**VLB x ELN42444**	**2909.41**	**8**	**1827.55**	**12**	**2368.48**	**5**	75	77	76
34	VLB x ELN42445	1980.22	43	1452.44	30	1716.33	41	76	77	77
35	VLB x ELN48392	990.84	54	723.45	53	857.15	54	76	77	76
36	VLB x T02058	2054.24	38	1601.46	17	1827.85	29	76	80	78
37	VLB x VL0511247	2905.48	9	1467.15	27	2186.32	17	77	80	79
38	VLB x VL0512593	1801.92	47	1113.8	46	1457.86	48	70	72	71
39	VLB x VL054794	1928.27	45	1583.11	20	1755.69	40	78	80	79
40	VLB x VL05615	2235.44	30	1091.65	48	1663.55	42	71	74	73
41	VLB x VL05616	2667.01	13	1252.69	39	1959.85	25	76	79	77
42	VLB x VL057903	2620.23	15	1110.2	47	1865.22	27	70	74	72
43	VLB x VL057967	2477.36	20	1591.45	18	2034.4	20	72	72	72
44	VLB x VL058014	2411.11	26	1119.21	45	1765.16	37	71	73	72
45	VLB x VL058025	2637.76	14	955.39	50	1796.58	33	70	73	72
46	VLB x VL058589	2004.12	42	973.8	49	1488.96	46	73	75	74
47	VLB x VL081464	2074.85	37	1575.1	21	1824.98	30	73	74	74
48	VLB x VL081466	2052.37	39	1126.33	44	1589.35	44	77	78	77

continued

No	Entry	Grain yield(kg/ha)						Anthesis-days		
		WW	Rank	WS	Rank	Across	Rank	WW	WS	Across
49	*VLA x VLB*	*2342.47*	*28*	*1549.49*	*23*	*1990.03*	*21*	*74*	*77*	*76*
50	*Bondofa*	*1388.09*	*52*	*1163.35*	*42*	*1275.72*	*50*	*82*	*83*	*82*
51	*Sanem*	*2453.97*	*24*	*2168.47*	*6*	*2311.22*	*11*	*78*	*79*	*78*
52	*VH052527*	*847.31*	*55*	*708.08*	*54*	*777.69*	*55*	*84*	*86*	*85*
53	*VH053024*	*2476.48*	*21*	*642.93*	*55*	*1559.7*	*45*	*70*	*71*	*70*
54	*Barka*	*1530.33*	*50*	*1159.94*	*43*	*1345.13*	*49*	*76*	*77*	*76*
55	*wari*	*1841.62*	*46*	*1709.68*	*15*	*1775.65*	*34*	*80*	*82*	*81*
	Mean	2282.89		1488.26		1886.57		76	78	77
	LSD$_{0.05}$	800.50		588.58		657.30		2	3	2

VLA x VLB = VL0511298 x VL054881 (cross between the two testers); Entries in bold were the best hybrids which out yielded all the checks; Entries in italic were the checks

Table 7. 2. Mean of grain yield and days to anthesis of 52 yellow hybrids and 7 checks.

No	Entry	Grain yield(kg/ha)						Anthesis-days		
		WW	Rank	WS	Rank	Across	Rank	WW	WS	Across
1	TZA x ELN39382	2797.42	15	1475.9	23	2136.66	18	77	81	79
2	TZA x ELN39427	1844.81	55	1677.36	13	1761.08	39	78	79	79
3	TZA x ELN402213	1780.96	57	1663.19	14	1722.08	43	78	80	79
4	TZA x ELN40791	2729.12	23	1898.91	5	2314.02	11	80	82	81
5	TZA x ELN40823	1926.47	54	1202.66	37	1564.57	53	79	82	80
6	TZA x ELN40941	3066.94	7	1653.41	15	2360.18	9	77	79	78
7	TZA x ELN431251	3364.08	4	1373.45	26	2368.77	8	76	79	78
8	TZA x ELN43453	2035.12	50	855.52	54	1498.94	54	79	83	81
9	**TZA x ELN43574**	**3196.44**	**6**	**2366.24**	**2**	**2781.34**	**1**	79	81	80
10	**TZA x ELN45111**	**3414.22**	**2**	**1839.04**	**7**	**2626.63**	**2**	79	83	81
11	TZA x ELN462121	2194.27	45	935.57	48	1564.92	52	78	84	81
12	TZA x ELN464171	2742.52	22	1787.52	10	2265.02	13	77	80	79
13	TZA x ELN47132	1963.62	53	1184.86	38	1574.24	51	79	82	81
14	TZA x FBML10	2578.13	29	1509.05	22	2043.59	24	78	82	80
15	TZA x TZE124	2511.31	32	1149.3	40	1892.21	34	76	80	78
16	TZA x TZE146	2868.47	13	1829.11	8	2348.79	10	75	77	76
17	TZA x TZE148	2636.8	28	1351.49	27	1994.15	27	75	77	76
18	TZA x TZE149	2223.39	41	1248.85	34	1736.12	41	75	76	75
19	**TZA x TZE151**	**2794.24**	**17**	**2408.87**	**1**	**2601.55**	**3**	78	78	78
20	TZA x TZE158	2214.79	43	1730.11	12	1972.45	31	75	78	76
21	TZA x TZE16	1968.75	52	602.76	59	1285.75	58	77	80	79
22	TZA x TZE161	2785.58	19	933.87	49	1859.72	35	73	76	74
23	TZA x TZE177	1970.96	51	1246.7	35	1608.83	48	76	79	78
24	TZA x TZE23	2216.54	42	1346.25	29	1781.4	38	74	79	76
25	TZA x TZE8	2668.48	26	1314.77	31	1991.63	28	73	76	75
26	TZA x TZI18	2995.33	9	838.06	56	1916.7	32	78	86	82
27	TZB x ELN39382	1802.37	56	872.92	53	1337.65	57	78	81	79
28	TZB x ELN39427	2835.36	14	2118.27	3	2476.82	5	77	80	79
29	TZB x ELN402213	2980.37	10	1336.96	30	2158.66	16	76	77	77
30	TZB x ELN40791	2787.68	18	1592.12	17	2189.9	15	76	78	77
31	TZB x ELN40823	2116.74	48	1841.27	6	1979	29	79	80	79
32	TZB x ELN40941	2681.97	25	1583.17	19	2132.57	19	76	77	76
33	TZB x ELN431251	2715.73	24	1279.18	32	1997.46	26	75	76	75
34	TZB x ELN43453	2157.18	46	1089.93	41	1623.55	47	77	80	78

Continued

No	Entry	Grain yield(kg/ha)						Anthesis-days		
		WW	Rank	WS	Rank	Across	Rank	WW	WS	Across
35	TZB x ELN43574	2753.37	20	1802.44	9	2277.9	12	77	80	78
36	TZB x ELN45111	2900.73	12	1374.81	25	2137.77	17	77	79	78
37	TZB x ELN462121	2342.18	37	953.2	46	1647.69	46	77	85	81
38	TZB x ELN464171	2999.4	8	951.71	47	1975.55	30	77	80	78
39	TZB x ELN47132	2795.1	16	1032.98	44	1914.04	33	78	81	79
40	TZB x FBML10	3595.29	1	1162.9	39	2379.1	7	76	81	79
41	TZB x TZE124	3364.76	3	1587.72	18	2476.24	6	74	75	74
42	TZB x TZE146	2463.33	33	898.17	52	1680.75	44	76	77	76
43	TZB x TZE148	2074.62	49	1390.08	24	1732.35	42	75	77	76
44	TZB x TZE149	2558.26	30	1540.42	21	2049.34	22	75	79	77
45	TZB x TZE151	2312.21	38	1735.57	11	2081.55	20	77	76	76
46	TZB x TZE158	2207.5	44	623.15	58	1415.33	55	75	77	76
47	TZB x TZE16	2370.45	36	1266.89	33	1818.67	37	77	78	77
48	TZB x TZE161	2280.43	40	1026.59	45	1653.51	45	74	75	74
49	TZB x TZE177	2453.49	35	1216.35	36	1834.92	36	72	77	75
50	TZB x TZE23	2661.38	27	1347.34	28	2004.36	25	76	77	76
51	TZB x TZE8	2554.01	31	930.23	50	1742.12	40	73	76	74
52	TZB x TZI18	3314.69	5	848.12	55	2081.41	21	75	82	79
53	*TZA x TZB*	*2123.21*	*47*	*1055.02*	*43*	*1589.12*	*50*	*77*	*78*	*78*
54	*TZI18 x FBML10*	*2752.66*	21	*1633.93*	16	2193.29	14	*78*	*78*	78
55	*TZEI17 x TZEI24*	*2460.1*	34	*1547.04*	20	2045.07	23	*76*	*77*	76
56	*Bondofa*	*1512.27*	59	*1083.21*	42	1340.65	56	*82*	*85*	83
57	*Sanem*	*2930.97*	11	*2078.03*	4	2504.5	4	*77*	*79*	78
58	*Barka*	*1748.81*	58	*738.01*	57	1243.41	59	*74*	*80*	77
59	*Wari*	*2284.82*	39	*915.90*	51	1600.36	49	*78*	*83*	81
	Mean	2531.46		1360.23		1950.47		76	79	78
	LSD	694.50		704.07		771.32		2	3	3

TZA x TZB = TZEI17 x TZEI10 (cross between the two testers); Entries in bold were the best hybrids which out yielded all the checks; Entries in italics were the checks

7.3.2 Analysis of variance

In the combined analysis of variance across the 5 environments, the mean squares for environmental effect was significant (P<0.001) for all traits in both hybrid sets (Table 7.3). The variation among INERA and CIMMYT white inbred lines in the testcrosses was significant for all the traits while the variation among the INERA and IITA yellow lines in the testcrosses was significant for all the traits except EPP and ASI. The variation between the two CIMMYT testers was not significant only for EPP and ASI whereas the variation between the two IITA testers differed significantly only for AD. The line x tester interaction (SCA) was significant for almost all traits, except for grain yield (GY) and ASI in both hybrid sets and for ear height (EHT) in set

1. The mean squares for line x environment, tester x environment and line x tester x environment interactions were significant for grain yield in both hybrid sets.

Table 7. 3. Mean squares from the combined ANOVA of grain yield and secondary traits

Source	**DF**	**GY**	**EPP**	**EASP**	**AD**
			SET 1		
Env	4	41745472.8***	0.60***	7.90***	7908.61***
GCA_{Line}	23	2819560.2***	0.07*	1.52***	229.14***
$\text{GCA}_{\text{Tester}}$	1	21909334.6*	0.20	6.50*	448.59**
$\text{SCA}_{\text{Line x Tester}}$	23	896161.60	0.04*	0.62*	19.14***
Env x GCA_{Line}	92	717088.3**	0.04**	0.50**	7.36***
Env x $\text{GCA}_{\text{Tester}}$	4	1107724.4*	0.08*	0.47	8.16
Env x $\text{SCA}_{\text{LinexTester}}$	92	619172.70*	0.02	0.33	6.10**
Error	478	455555.50	0.02	0.03	3.88
			SET 2		
Env	4	59953166.0***	0.48***	22.26***	6719.92***
GCA_{Line}	25	1815141.5**	0.03	1.23*	83.31***
$\text{GCA}_{\text{Tester}}$	1	64977.00	0.04	1.74	237.35**
$\text{SCA}_{\text{Line x Tester}}$	25	949418.40	0.05*	0.76**	11.35***
Env x GCA_{Line}	99	828317.1***	0.03**	0.63***	7.18*
Env x $\text{GCA}_{\text{Tester}}$	4	1275466.7*	0.09**	0.63	7.02
Env x $\text{SCA}_{\text{LinexTester}}$	99	593677.0*	0.03	0.31	3.61
Error	514	439264.00	0.02	0.33	5.40

continued

Source	**DF**	**ASI**	**PHT**	**EHT**
			SET 1	
Env	4	215.67***	121274.80***	6465.92***
GCA_{Line}	23	20.48***	1623.89***	1218.67***
$\text{GCA}_{\text{Tester}}$	1	34.46	1859.74**	4361.51**
$\text{SCA}_{\text{Line x Tester}}$	23	4.84	458.58**	143.98
Env x GCA_{Line}	92	7.53***	335.00***	99.46
Env x $\text{GCA}_{\text{Tester}}$	4	4.90	38.76	77.28
Env x $\text{SCA}_{\text{LinexTester}}$	92	3.51**	200.06	89.84
Error	478	2.21	187.58	93.20
			SET 2	
Env	4	96.86***	108963.71***	13226.45***
GCA_{Line}	25	5.39	1035.04***	507.36***
$\text{GCA}_{\text{Tester}}$	1	0.18	918.94	750.86
$\text{SCA}_{\text{Line x Tester}}$	25	3.55	417.87*	259.47**
Env x GCA_{Line}	99	5.41***	290.78	141.56
Env x $\text{GCA}_{\text{Tester}}$	4	6.09*	426.23	539.28**
Env x $\text{SCA}_{\text{LinexTester}}$	99	2.69	210.25	102.86
Error	514	1.99	284.17	130.44

GY=Grain yield (kg/ha); EPP = Number of ear per plant; EASP= Ear aspect; AD= anthesis-days; ASI= anthesis-silking interval; PHT=plant height; EHT=ear height, Env=Environment.

GCA mean square was significant for the CIMMYT and INERA lines for grain yield, anthesis-days (AD), anthesis-silking interval (ASI) and ear height (EHT) under both well-watered and water-stressed conditions, while GCA mean square was significant for the IITA and INERA lines only for anthesis-days under both water regimes (Table 7.4 and 5). The two testers within each set differed significantly for anthesis-days under drought stress. SCA effects were not significant for many traits in both hybrid sets under both water regimes. Among the drought adaptive traits measured under drought stress, GCA mean square were significant only for TS and PR in set 1 and for LE in both hybrid sets (Table 7.5). Mean square of tester GCA was significant only for plant recovery in set 2. No significant SCA effects were detected for the four adaptive drought traits in either hybrid set.

Table 7. 4. Mean squares for grain yield and 6 traits evaluated under <u>well-watered</u> conditions in 2 years

Source	DF	GY	EPP	EASP	AD	ASI	PHT	EHT
					SET 1			
GCA$_{\text{Line}}$	23	1609472.43	0.03	1.49**	84.61***	11.82**	1012.81***	704.69***
GCA$_{\text{Tester}}$	1	7903498.63	0.03	1.06	227.26*	9.72	418.68	1434.6
SCA$_{\text{Line x Tester}}$	23	1007732.78	0.02	0.47	9.55	2.28	302.93	97.02
Year x GCA$_{\text{line}}$	23	810380.17	0.03	0.53	8.70***	3.17	162.08*	92.81
Year x GCA$_{\text{Tester}}$	1	525546.42	0.04	0.73	0.46	5.88	25.76	259.24
Year x SCA$_{\text{line xTester}}$	23	864668.25*	0.01	0.68	5.48*	2.76	114.16	103.89
					SET 2			
GCA$_{\text{Line}}$	25	1244308.91*	0.030*	0.91	31.97***	3.47	501.66*	276.75
GCA$_{\text{Tester}}$	1	281516.00	0.03	1.66	55.13***	7.66	476.80	9.29
SCA$_{\text{Line x Tester}}$	25	832912.12	0.03	0.62	5.78	2.04	362.42**	236.77**
Year x GCA$_{\text{line}}$	25	521857.12	0.01	0.85**	5.79*	2.73*	221.08	198.19
Year x GCA$_{\text{Tester}}$	1	177151.92	0.14**	0.21	0.00	0.48	1423.81*	423.28
Year x SCA$_{\text{line xTester}}$	25	655583.69	0.01	0.37	5.12	1.80	120.61	71.99

GY=Grain yield (kg/ha); EPP = Number of ears per plant; EASP= Ear aspect; AD= anthesis-days; ASI= anthesis-silking interval; PHT=plant height; EHT=ear height; LR=leaf rolling; SEN=leaf senescence LE=leaf erectness; TS=tassel size PR=plant recovery

Table 7. 5. Mean squares for grain yield and 11 traits evaluated under water-stressed conditions in 2 years

Source	**DF**	**GY**	**EPP**	**EASP**	**AD**	**ASI**	**PHT**	**EHT**
					SET 1			
GCA$_{\text{Line}}$	23	1116980.33*	0.06	0.2	16.67***	16.672*	333.64	330.04***
GCA$_{\text{Tester}}$	1	14830470	0.29	2.92	16.64	16.64	869.47	1833.47
SCA$_{\text{Line x Tester}}$	23	404372.25	0.05	0.26	7.79	7.79	322.42	86.81
Year x GCA$_{\text{line}}$	23	480055.32	0.05	0.33	11.87**	7.039***	304.54	79.37
Year x GCA$_{\text{Tester}}$	1	1965615.53*	0.17*	0.35	26.75*	7.88	33.17	14.67
Year x SCA$_{\text{line xTester}}$	23	379870.25	0.03	0.19	9.43*	4.19	306.24	89.15
					SET 2			
GCA$_{\text{Line}}$	25	932409.8	0.04	0.42	35.35**	10.31	540.88	269.98
GCA$_{\text{Tester}}$	1	1953612.1	0.01	0.01	148.87	9.18	909.96	1424.87
SCA$_{\text{Line x Tester}}$	25	570438.11	0.06	0.33	8.09	6.15	308.9	140.53
Year x GCA$_{\text{line}}$	25	761359.86*	0.05	0.77**	12.17	8.29**	520.15	203.1
Year x GCA$_{\text{Tester}}$	1	371891.99	0.11	0.04	5.15	0.07	308.16	805.95*
Year x SCA$_{\text{line xTester}}$	25	501462.61	0.04	0.35	6.71	3.23	317.68	121.15

continued

Source	**DF**	**LR**	**SEN**	**LE**	**TS**	**PR**
				SET 1		
GCA$_{\text{Line}}$	23	0.21	2.44	1.58**	1.07***	2.40***
GCA$_{\text{Tester}}$	1	1.13	0.17	3.52	2.2	5.15
SCA$_{\text{Line x Tester}}$	23	0.38	1.53	0.48	0.31	1.18
Year x GCA$_{\text{line}}$	23	0.34	1.7	0.43	0.22	0.61
Year x GCA$_{\text{Tester}}$	1	1.04	1.25	0.98	1.28	0.73
Year x SCA$_{\text{line xTester}}$	23	0.3	1.14	0.41	0.24	0.72
				SET 2		
GCA$_{\text{Line}}$	25	0.45	2.52	1.45**	0.43	1.72
GCA$_{\text{Tester}}$	1	0.01	0	12.69	6.04	5.56*
SCA$_{\text{Line x Tester}}$	25	0.46	1.78	0.26	0.23	1.56
Year x GCA$_{\text{line}}$	25	0.52	2.99	0.46	0.37	1.38
Year x GCA$_{\text{Tester}}$	1	2.72	2.1	1.86	1.98	0.01
Year x SCA$_{\text{line xTester}}$	25	0.52	1.8	0.29	0.24	0.93

GY=Grain yield (kg/ha); EPP = Number of ears per plant; EASP= Ear aspect; AD= anthesis-days; ASI= anthesis-silking interval; PHT=plant height; EHT=ear height; LR=leaf rolling; SEN=leaf senescence LE=leaf erectness; TS=tassel size PR=plant recovery

In general, GCA mean squares for lines, GCA mean squares for testers and SCA effects were significant for most traits under well-watered high plant density (WWHPD) conditions in both hybrid sets (Table 7.6).

Table 7. 6. Mean squares for grain yield and 6 traits evaluated under <u>well-watered high plant density</u> condition in 1 year

	DF	**GY**	**EPP**	**EASP**	**AD**	**ASI**	**PHT**	**EHT**
					SET 1			
GCA$_{\text{Line}}$	23	1667471.9***	0.063***	0.96***	61.80***	11.66***	1156.32***	413.38***
GCA$_{\text{Tester}}$	1	1066236.21	0	3.36***	88.67***	3.36	663.06*	1125.04***
SCA$_{\text{Line x Tester}}$	23	725757.85	0.02	0.32	7.85***	1.72	216.70**	130.29**
Error	96	533664	0.01636	0.24826	1.72222	1.88889	99.0625	52.6476
					SET 2			
GCA$_{\text{Line}}$	24	1378324.21***	0.024**	0.77***	31.58***	3.86***	513.39***	199.11***
GCA$_{\text{Tester}}$	1	5611418.87***	0.26***	4.17***	72.11***	19.25***	440.61	157.08
SCA$_{\text{Line x Tester}}$	24	815470.73**	0.01	0.34	5.47***	1.44	148.49	78.29
Error	100	380220.1	0.01121	0.27833	1.98	1.21886	112.677	60.645

7.3.3 Relative importance of GCA and SCA

In both hybrid sets, the GCA (GCA $_{(\text{Line})}$ + GCA $_{(\text{Tester})}$) explained more than 50% of the total sum of squares among crosses (GCA $_{(\text{Line})}$ + GCA $_{(\text{Tester})}$ + SCA $_{(\text{Line x Tester})}$) for grain yield and almost all the secondary traits under the three growing conditions including WS, WW and WWHPD (Table 7.7). However, the proportion of SCA was higher than GCA for EPP in set 2 and for leaf rolling (LR) in both hybrid sets, under drought stress.

Table 7. 7 Proportion (%) of sum of squares for general combining ability (GCA) effects and specific combining ability (SCA) effects for grain yield and secondary traits under three growing environments

						Water-stressed (WS)						
	GY	EPP	EASP	AD	ASI	PHT	EHT	LR	SEN	LE	TS	PR
						SET 1						
GCA $_{(\text{Line})}$	51	49	35	84	69	48	66	33	61	72	72	63
GCA $_{(\text{Tester})}$	30	10	22	6	3	5	16	8	0	7	6	6
SCA $_{(\text{Line x Tester})}$	19	41	44	10	28	46	17	59	38	22	21	31
						SET 2						
GCA $_{(\text{Line})}$	59	44	56	72	71	61	58	49	59	65	48	49
GCA $_{(\text{Tester})}$	5	0	0	12	3	4	12	0	0	23	27	6
SCA $_{(\text{Line x Tester})}$	36	56	44	16	26	35	30	51	41	12	25	45

continued

	Well-watered (WW)							Well-watered high plant density (WWHPD)						
	GY	EPP	EASP	AD	ASI	PHT	EHT	GY	EPP	EASP	AD	ASI	PHT	EHT
				SET 1							SET 1			
GCA $_{(L)}$	53	55	74	81	81	76	82	68	75	67	84	86	82	70
GCA $_{(T)}$	11	3	2	9	3	1	7	2	0	10	5	1	2	8
SCA $_{(L \times T)}$	37	43	24	9	16	23	11	30	25	23	11	13	15	22
				SET 2							SET 2			
GCA $_{(L)}$	60	51	57	80	60	57	54	57	53	60	79	63	75	70
GCA $_{(T)}$	1	2	4	6	5	2	0	10	24	14	8	13	3	2
SCA $_{(L \times T)}$	40	47	39	14	35	41	46	34	23	27	14	24	22	28

7.3.4 General combining ability (GCA) effects

The four testers, two each from CIMMYT and IITA, showed opposite GCA effects for grain yield within each growing condition and across environments (Table 7.8; Figure 7.1). Tester VL0511298 exhibited a constant positive effect in all the evaluation conditions whereas the opposite tester, VL054881 showed a constant negative GCA effects (Figure 7.1). Tester, TZEI17 showed a good general combining ability effects with INERA and IITA yellow lines under drought stress but negative GCA effects in WW and WWPHD conditions and across environments. The opposite reaction was observed for the tester TZEI10 which showed negative GCA effects under drought but positive GCA effects in non-drought condition (Figure 7.1). This result implied that TZEI17 contributed many favorable alleles for drought tolerance in combination with the lines compared to TZEI10. Eight CIMMYT and five INERA white lines showed a positive GCA effects ranging from 0.1 to 0.4 t/ha, and 9 INERA and 5 IITA lines also had positive GCA effects varying from 0.02 to 0.5 t/ha. These inbred lines could be included in white and yellow synthetic varieties with other lines having a positive GCA effects.

Table 7. 8 Estimates of general combining ability (GCA) effects for grain yield of 24 white (set 1) and 26 yellow (set 2) inbred lines under three growing environments

LINES	SET 1					SET 2				
	WW	WS	WWHPD	Across		LINE	WW	WS	WWHPD	Across
ELN 41111	-266	-73	-475	-231		ELN 39382	-264	-190	-476	-293
ELN 41112	-384	-246	-620*	-377*		ELN 39427	-179	553*	149	172
ELN 41114	-921**	-557*	-1365**	-874**		ELN 402213	-129	121	134	18
ELN 41115	-616*	-108	-677*	-426*		ELN 40791	195	381	-233	167
ELN 41271	-39	346	-877**	-53		ELN 40823	-551*	128	652*	-33
ELN 41272	21	251	-140	80		ELN 40941	311	254	193	248
ELN 42441	293	-5	423	199		ELN 431251	476*	-38	361	231
ELN 42442	519	255	515	412*		ELN 43453	-468*	-381	-334	-398*
ELN 42444	184	216	476	254		ELN 43574	411	720**	-1225**	191
ELN 42445	10	128	66	68		ELN 45111	594**	242	726**	463*
ELN 48392	-446	-366	312	-263		ELN 462121	-296	-420	-269	-259
T02058	44	420*	1032**	391*		ELN 464171	307	5	309	170
VL0511247	252	141	57	193		ELN 47132	-184	-256	-100	-111
VL0512593	-393	-253	-5	-260		FBML 10	353	29	353	247
VL054794	115	520*	314	316		TZEI 124	374	24	378	251
VL05615	231	-220	318	67		TZEI 146	102	-1	-139	-4
VL05616	220	226	188	215		TZEI 148	-208	6	-456	-188
VL057903	238	-274	-191	-53		TZEI 149	-173	30	-28	-79
VL057967	475	529*	-14	398*		TZEI 151	-59	708**		204
VL058014	386	-215	573	182		TZEI 158	-353	-188	126	-207
VL058025	591*	-242	397	219		TZEI 16	-394	-430	-966**	-539*
VL058589	-91	-313	59	-151		TZEI 161	-31	-384	180	-146
VL081464	-83	147	-190	-13		TZEI 177	-352	-133	-88	-228
VL081466	-339	-307	-178	-294		TZEI 23	-125	-18	741**	75
$\text{SE}_{\text{GCA line}}$	260	200	298	155		TZEI 8	48	-242	-346	-163
TESTER						TZI 18	591**	-521*	358	212
VL0511298	169	228	86	177		$\text{SE}_{\text{GCA Lines}}$	209	252	252	166
VL054881	-165	-227	-86	-176		TESTER				
$\text{SE}_{\text{GCATester}}$	60	117	86	55		TZEI 17	-34	79	-193	-29
						TZEI 10	31	-72	193	18
						$\text{SE}_{\text{GCATester}}$	34	49	70	57

* and ** significant at $P < 0.05$ and 0.01 respectively

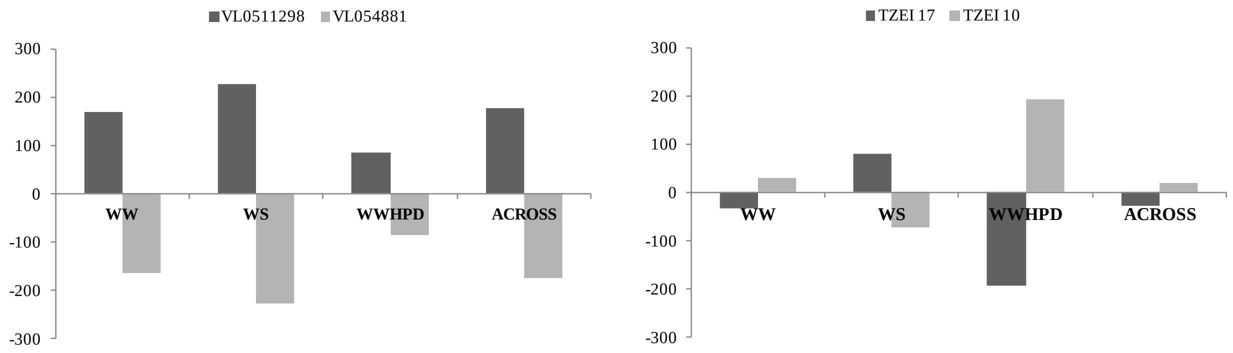


Figure 7. 1 GCA effects for grain yield of testers estimated under three growing conditions and across.

7.3.5 Specific combining ability (SCA) effects

SCA effects for grain yield estimated across environments showed that tester VL0511298 was a good specific combiner with 2 CIMMYT lines (T02058, VL054794) derived from biparental crosses and 3 (VL057967, VL058014 and VL058025) from source population with Tuxpeño background. It produced hybrids that yielded between 0.3 and 0.7t/ha greater than VL0511298 x VL054881 (Table 7.9). This suggested that the sources of these lines belong to a heterotic group opposite of that of VL0511298. Tester, TZEI17 derived from TZE COMP5-Y population, showed a positive SCA effects with 6 IITA lines (TZEI 151, TZEI 146, TZEI 8, TZEI 158, , TZEI 148 and TZEI 161) derived from the same population as tester TZEI10, TZE-Y Pop DT STR. It produced hybrids that yielded between 0.5 and 1t/ha better than TZEI17 x TZEI10 (Table 7.9), suggesting that the two source populations could be two opposite heterotic groups. The white and yellow INERA lines extracted from the same source, FBC6, showed diverse heterotic responses (SCA effects) with both CIMMYT testers as well as both IITA testers (Table 7.9).

Table 7. 9 Mean Grain yield and Specific combining ability (SCA) effects of the inbred lines across environments.

SET1						**SET2**					
Testers	VL0511298 (A)		VL054881 (B)		HG	Testers	TZEI17 (A)		TZEI10 (B)		HG
Lines	GY	SCA	GY	SCA		Lines	GY	SCA	GY	SCA	
ELN41111	1800	-180	1805	178	NA	ELN39382	2013	227	1618	-215	antiA
ELN41112	1725	-109	1589	107	NA	ELN39427	1948	-304	2570	271	antiB
ELN41114	1061	-276	1265	281	NA	ELN402213	1909	-189	2316	171	antiB
ELN41115	1965	180	1251	-181	NA	ELN40791	2349	102	2204	-91	antiA
ELN41271	2036	-121	1924	119	NA	ELN40823	1994	-53	2170	77	antiB
ELN41272	2280	-11	1947	10	NA	ELN40941	2543	215	2171	-204	antiA
ELN42441	2514	104	1951	-106	antiA	ELN431251	2458	147	2222	-136	antiA
ELN42442	2544	-78	2346	77	antiB	ELN43453	1617	-64	1798	69	antiB
ELN42444	2203	-262	2372	260	antiB	ELN43574	2327	56	2273	-45	antiA
ELN42445	2331	52	1871	-54	antiA	ELN45111	2807	264	2338	-252	antiA
ELN48392	2376	428 x	1165	-430*	antiA	ELN462121	1634	-187	2066	198	antiB
T02058	2749	147	2101	-149	antiA	ELN464171	2389	139	2170	-128	antiA
VL0511247	2369	-36	2113	62	antiB	ELN47132	1805	-164	2191	175	antiB
VL0512593	1876	-75	1671	73	NA	FBML10	2167	-160	2672	297	antiB
VL054794	2830	303	1869	-305	antiA	TZEI124	2054	-276	2645	267	antiB
VL05615	2375	97	1826	-99	antiA	TZEI146	2390	314	1820	-303	antiA
VL05616	2421	-6	2077	4	antiB	TZEI148	2052	161	1789	-149	antiA
VL057903	2063	-94	1897	92	NA	TZEI149	1801	-199	2258	211	antiB
VL057967	2734	125	2129	-127	antiA	TZEI151	2602	318	2082	-249	antiA
VL058014	2491	98	1940	-100	antiA	TZEI158	2081	209	1722	-197	antiA
VL058025	2531	102	1973	-104	antiA	TZEI16	1245	-296	1895	307	antiB
VL058589	2025	-35	1741	33	NA	TZEI161	2043	110	1882	-99	antiA
VL081464	2082	-115	1958	113	NA	TZEI177	1758	-94	2005	106	antiB
VL081466	1711	-206	1767	204	NA	TZEI23	1907	-248	2461	259	antiB
VLA x VLB	2154		2154			TZEI8	2103	187	1788	-176	antiA
SE	163	203	163	203		TZI18	2112	-179	2529	191	antiB
						TZA*TZB	1553		1553		
						SE	158	199	158	199	

VLA x VLB=VL0511298 x VL054881; GY= Grain yield (kg/ha); HG= Heterotic group ; antiA=anti-VL0511298; antiB=VL054881; NA= Not assigned; S.E= Standard error

7.3.6 Classification of inbreds into heterotic groups

Grouping of white mid-altitude and lowland tropical inbred lines

Based on SCA effects estimated across environments (Table 7.9) and the methodology of classifying lines into heterotic group described in materials and methods, 3 INERA and 6

CIMMYT lines were assigned in the anti-VL0511298 (antiA) heterotic group while 2 INERA and 2 CIMMYT lines were placed into anti-VL054881 (antiB) heterotic group. Eleven lines could not classified. The grouping within each of the three growing conditions showed that one ELN41111 was constantly placed with anti VL054881 heterotic group and four lines (ELN48392, T02058, VL054794 and VL058025) with anti VL0511298 heterotic group (Table 7.10). Two lines (ELN42442 and VL05616) produced hybrids with testers VL0511298 that yielded about 40% higher than the mean yield of VL0511298 x VL054881 and was placed into anti-VL0511298 group under drought stress. However, these lines were assigned to the anti-VL054881 group under well-watered environments and across environments. This implied that these lines should be considered in anti-VL0511298 heterotic group in development of drought tolerant inbred lines.

Grouping of yellow lowland tropical inbred lines

All the 26 yellow inbred lines were classified into heterotic group. Thirteen lines were placed each into anti-TZEI17 and anti-TZEI10 groups, with 7 INERA and 6 IITA lines in each group (Table 7.9). The following inbred lines, ELN43453; ELN462121; TZEI124; TZEI149; TZEI16 and TZ18 were anti-TZEI10 while ELN40791; ELN45111; TZEI146 and TZEI8 were anti-TZEI17 in each of the three growing environments (Table 7.11).

7.3.7 Heterosis , Selection tolerance index and yield stability

The average value of mid-parent heterosis (MPH), high parent heterosis (HPH), selection tolerance index (STI), relative decrease in yield (RDY), AMMI stability value (ASV) and yield stability index (YSI) of the hybrids in both sets are summarized in Table 7.15

Table 7. 10 Mean Grain yield and Specific combining ability (SCA) effects of CIMMYT and INERA white inbred lines evaluated under three growing environments

	WW					**WS**					**WWHPD**				
Testers	VL0511298 (A)		VL054881 (B)		**HG**	VL0511298 (A)		VL054881 (B)		**HG**	VL0511298 (A)		VL054881 (B)		**HG**
Lines	GY	SCA	GY	SCA		GY	SCA	GY	SCA		GY	SCA	GY	SCA	
ELN41111	2034	-219	2132	214	antiB	1483	-186	1399	185	antiB	1964	-85	1962	85	antiB
ELN41112	2197	62	1733	-67	antiA	1349	-148	1187	146	NA	1533	-370	2102	370	antiB
ELN41114	1538	-60	1297	34	NA	742	-443	1171	442	NA	746	-412	1399	412	antiB
ELN41115	2034	131	1433	-135	NA	1917	284	893	-285	antiA	1922	75	1600	-75	antiA
ELN41271	2415	-64	2204	59	antiB	2277	189	1442	-190	antiA	796	-851	2326	851	antiB
ELN41272	2593	53	2148	-58	antiA	2067	74	1462	-75	antiA	2077	-306	2518	306	antiB
ELN42441	2929	117	2356	-122	antiA	1716	-21	1302	20	NA	3280	333	2442	-333	antiA
ELN42442	2512	-525	3223	520	antiB	2082	85	1456	-86	antiA	3532	493	2374	-493	antiA
ELN42444	2157	-546	2909	541	antiB	1632	-326	1828	325	antiB	3440	440	2388	-440	antiA
ELN42445	2738	209	1980	-214	antiA	1830	-40	1452	38	antiB	2518	-72	2491	72	antiB
ELN48392	2816	743	991	-748	antiA	1573	196	723	-197	antiA	3103	267	2397	-267	antiA
T02058	2732	169	2054	-174	antiA	2266	104	1601	-105	antiA	3748	192	3192	-192	antiA
VL0511247	2236	-535	2905	469	antiB	1935	52	1467	40	antiAB	3168	587	1822	-587	antiA
VL0512593	2111	-15	1802	10	NA	1408	-81	1114	80	NA	2341	-178	2526	178	antiB
VL054794	3001	367	1928	-371	antiA	2484	222	1583	-223	antiA	3184	346	2321	-346	antiA
VL05615	2924	175	2235	-179	antiA	1496	-26	1092	25	NA	3038	196	2474	-196	antiA
VL05616	2471	-267	2667	263	antiB	2227	259	1253	-260	antiA	2707	-5	2545	5	antiB
VL057903	2554	-202	2620	198	antiB	1368	-99	1110	98	NA	2470	138	2023	-138	antiA
VL057967	3171	177	2477	-182	antiA	2495	223	1591	-224	antiA	2339	-171	2509	171	antiB
VL058014	3059	154	2411	-159	antiA	1478	-49	1119	48	NA	3381	284	2641	-284	antiA
VL058025	3244	133	2638	-138	antiA	1589	88	955	-90	antiA	2992	71	2678	-71	antiA
VL058589	2512	84	2004	-89	antiA	1427	-2	974	0	NA	2247	-336	2748	336	antiB
VL081464	2457	21	2075	-26	antiA	1746	-143	1575	142	antiB	2007	-327	2489	327	antiB
VL081466	1969	-211	2052	207	NA	1288	-148	1126	147	NA	2040	-306	2480	306	antiB
VLA x VLB	2342		2342			1549		1549			1719		1719		
S.E	287	380	287	380		216	252	216	252		387	422	387	422	

VLA x VLB = VL0512298 x VL054881 GY= Grain yield (kg/ha); HG= Heterotic group ; antiA=anti-VL0511298; antiB=VL054881; NA= Not assigned; S.E= Standard error,

Table 7. 11 Mean Grain yield and Specific combining ability (SCA) effects of IITA and INERA yellow inbred lines evaluated under three growing environments

	WW					WS				HG	WWHPD				
Testers	TZEI17 (A)		TZEI10 (B)		HG	TZEI17 (A)		TZEI10 (B)			TZEI17 (A)		TZEI10 (B)		HG
Lines	GY	SCA	GY	SCA		GY	SCA	GY	SCA		GY	SCA	GY	SCA	
ELN 39382	2797	532	1802	-529	antiA	1476	222	873	-229	antiA	1519	-418	2741	418	antiB
ELN 39427	1845	-506	2835	419	antiB	1677	-320	2118	273	antiB	2570	8	2940	-8	antiA
ELN 402213	1781	-620	2980	514	antiB	1663	99	1337	-76	antiA	2533	-14	2948	14	antiB
ELN 40791	2729	5	2788	-2	antiA	1899	74	1592	-81	antiA	2488	308	2258	-308	antiA
ELN 40823	1926	-52	2117	73	antiB	1203	-370	1841	420	antiB	3710	645	2807	-645	antiA
ELN 40941	3067	227	2682	-224	antiA	1653	-44	1583	37	antiB	3274	668	2325	-668	antiA
ELN 431251	3364	358	2716	-355	antiA	1373	-32	1279	25	antiB	2815	40	3121	-40	antiA
ELN 43453	2035	-27	2157	30	antiB	856	-207	1090	179	antiB	2050	-29	2495	29	antiB
ELN 43574	3196	256	2753	-253	antiA	2366	203	1802	-210	antiA	510	-678	2253	678	antiB
ELN 45111	3414	291	2901	-288	antiA	1839	153	1375	-160	antiA	3527	388	3139	-388	antiA
ELN 462121	2194	-40	2342	43	antiB	936	-88	953	81	antiB	1773	-371	2902	371	antiB
ELN 464171	2743	-94	2999	97	antiB	1788	339	952	-346	antiA	2885	163	2945	-163	antiA
ELN 47132	1964	-382	2795	385	antiB	1185	-3	1033	-4	NA	2267	-46	2745	46	antiB
FBML 10	2578	-305	3595	647	antiB	1509	36	1163	-159	antiA	2661	-104	3257	104	antiB
TZEI 124	2511	-393	3365	396	antiB	1149	-318	1588	271	antiB	2648	-143	3321	143	antiB
TZEI 146	2868	237	2463	-234	antiA	1829	386	898	-393	antiA	2556	282	2379	-282	antiA
TZEI 148	2637	315	2075	-312	antiA	1351	-99	1390	91	antiB	2284	327	2017	-327	antiA
TZEI 149	2223	-133	2558	136	antiB	1249	-225	1540	218	antiB	2062	-323	3094	323	antiB
TZEI 151	2794	323	2312	-224	antiA	2409	257	1736	-265	antiA					
TZEI 158	2215	38	2208	-35	antiA	1730	474	623	-481	antiA	2516	-23	2949	23	antiB
TZEI 16	1969	-167	2370	170	antiB	603	-411	1267	404	antiB	1081	-366	2199	366	antiB
TZEI 161	2786	287	2280	-284	antiA	934	-126	1027	118	antiB	2777	184	2795	-184	antiA
TZEI 177	1971	-207	2453	210	antiB	1247	-64	1216	57	antiB	2354	28	2684	-28	antiA
TZEI 23	2217	-188	2661	191	antiB	1346	-80	1347	73	antiB	2410	-745*	4286	745*	antiB
TZEI 8	2668	91	2554	-88	antiA	1315	113	930	-120	antiA	2551	483	1971	-483	antiA
TZI 18	2995	-126	3315	129	antiB	838	-84	848	77	antiB	2503	-268	3425	268	antiB
TZA x TZB	2123		2123			1055		1055			1408		1408		
S.E.	248	331	248	331		254	289	254	289		347	356	347	356	

TZA x TZB = TZEI17 x TZEI10; GY= Grain yield (kg/ha); HG= Heterotic group ; NA= Not assigned; antiA= anti-TZEI17; antiB= anti-TZEI10; S.E= Standard error

7.3.7.1 Mid-parent and high-parent heterosis for grain yield

In general, testcross hybrids with IITA testers crossed with INERA and IITA lines were less vigorous (low heterosis) compared to the hybrids with mid-altitude and lowland tropical inbred lines from CIMMYT and INERA (Figure 7.2). The average mid-parent heterosis (MPH) for grain yield estimated across well-watered and water stressed environments for all the hybrids ranged from 446% for white hybrids to 221% for yellow hybrids; and high parent heterosis (HPH) ranged from 297% for white hybrids to 161% for yellow hybrids. Crosses with highest MPH and HPH values were : VL054881 x T02058 for white hybrids and TZEI17 x ELN43574 for yellow hybrids (Table 7.15). Of the 48 white hybrids, 17 had 20 % HPH higher than the percentage HPH of VL0511298 x VL054881 and 41 (of the 45) yellow hybrids had 50% HPH greater than TZEI17 x TZEI10. Among the four white hybrid groups including VL0511298 x INERA, VL0511298 x CIMMYT, VL054881 x INERA and VL054881 x CIMMYT, the high average heterosis across water regimes was shown by VL054881 x INERA followed by VL054881 x CIMMYT. The hybrid group, TZEI17 x INERA was significantly higher than the three others, TZEI17 x IITA, TZEI10 x INERA and TZEI10 x IITA for mean HPH across water regimes (Figure 7.2).

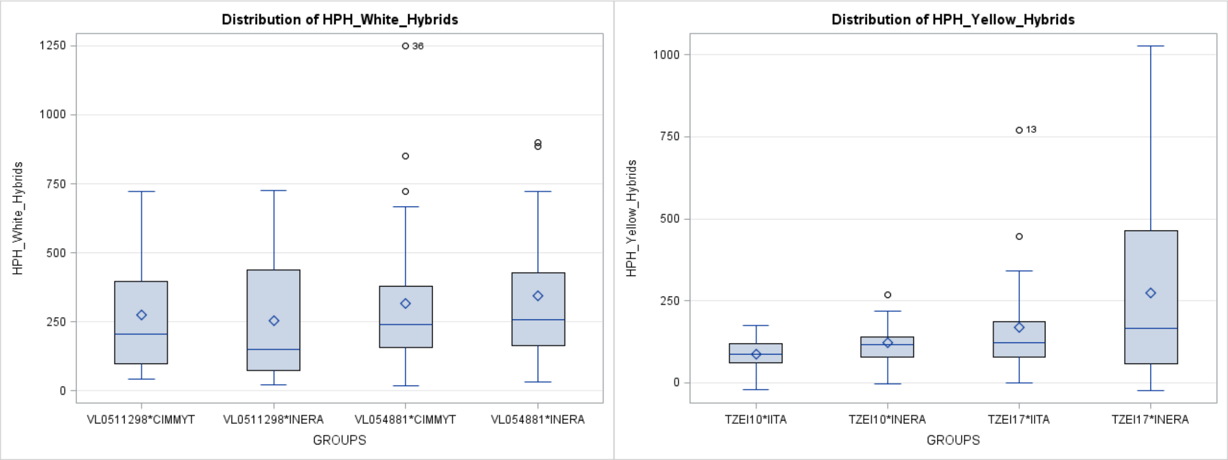


Figure 7. 2 Average percent of high-parent heterosis (HPH) for white and yellow hybrid groups across water regimes

7.3.7.2 Selection for drought tolerance

The integrated selection criterion for drought tolerance (STI) proposed by Hao *et al* (2011a) and used in this study involved grain yield and different secondary traits which were discussed in Chapter 6. Based on the selection tolerance index (STI), the hybrids were classified into four groups based on the degree of drought tolerance : highly drought tolerant "HT", drought tolerant "T", drought susceptible "S" and highly drought-susceptible "HS". The selection tolerance index (STI) does not consider the relative yield reduction under drought stress, thus a genotype classified as highly tolerant based on STI value may have high percent of yield reduction under drought stress which is not desirable. Therefore, the classification of the hybrids into four categories of drought tolerance based on STI value was improved by incorporating the relative decrease in yield under drought (RDY) index. The selection procedure involving the two selection criteria, STI and RDY is presented in Table 7.12.

Table 7. 12 Procedure for classifying hybrids into four categories of degree of drought resistance

STI classification	Ranged of RDY	SELECTION
"HT"	≤ 25 %	Highly drought tolerant
	> 25 ≤ 50%	Tolerant
	> 50 ≤75%	Susceptible
	> 75 ≤ 100%	Highly susceptible
"T"	≤ 25 %	Tolerant
	> 25 ≤ 50%	Susceptible
	> 50 ≤75%	Highly susceptible
	> 75 ≤ 100%	Highly susceptible
"S"	≤ 25 %	Susceptible
	> 25 ≤ 50%	Highly susceptible
	> 50 ≤75%	Highly susceptible
	> 75 ≤ 100%	Highly susceptible
"HS"	≤ 25 %	Highly susceptible
	> 25 ≤ 50%	Highly susceptible
	> 50 ≤75%	Highly susceptible
	> 75 ≤ 100%	Highly susceptible

"HT"=Highly drought tolerant; "R"=Drought tolerant; "S"=drought susceptible; "HS"=Highly drought susceptible

Based on this selection procedure, of the 49 white hybrids (set 1), 6 were highly drought tolerant, 19 drought tolerant, 10 drought susceptible and 14 highly drought susceptible. The hybrid between the two testers was drought resistant. Six of the 24 (T02058, VL054794, VL057967, VL081464, ELN42444 and ELN42442) white inbred lines produced hybrids drought tolearnt hybrids with VL0511298 while the hybrids derived from the crosses with VL054881 were drought susceptible. The 54 yellow hybrids (set 2), were classified into four groups based on their degree of drought tolerance : 3 were highly drought tolerant, 23 were drought tolerant, 19 were drought susceptible and 9 highly drought susceptible. The hybrids TZI18 x FBML10 and TZEI17 x TZEI10 , crosses between the two testers were drought tolerant and drought susceptible, respectively. Of the 26 inbred lines crossed to both TZEI17 and TZEI10, 13 and 12 exhibited tolerance to drought when crossed with TZEI17 and TZEI10, respectively, The drought reactions of the hybrids is presented in Table 7.15 and the summary of highly drought tolerant hybrids is presented in Table 7.13

Table 7. 13. Selected highly drought tolerant hybrids in both hybrid sets

	HYBRID	**GY (kg/ha)**		**STI**	**RDY (%)**
		WW	WS		
SET 1	VL0511298 x VL057967	3170.51	2494.53	23	21
	VL0511298 x VL054794	3000.50	2483.65	20	17
	VL0511298 x T02058	2731.95	2266.45	14	17
	VL0511298 x ELN 42442	2512.11	2082.21	13	17
	VL0511298 x ELN 41271	2415.48	2277.24	8	6
	VL0511298 x VL05616	2471.16	2226.91	8	10
SET 2	TZEI17 x TZE151	2794.24	2408.87	12	14
	TZEI10 x ELN 39427	2835.36	2118.27	8	25
	TZEI10 x TZE151	2312.21	1735.57	7	25

7.3.7.3 Yield stability index (YSI)

The AMMI analysis of the two hybrid sets evaluated separately in 5 environments is presented in Table 7.14 The results of both sets of trials indicated that environments (E), genotypes (G), and the genotype x environment interaction (GEI) were highly significant (P<0.001). The partitioning of treatment sum of squares (SS) indicated that the environment effect was the predominant source of variation followed by GEI and genotype effect. The results of AMMI analysis indicated also that the first two and the first three axes in set 1 and 2, respectively, were highly significant.

Table 7. 14 AMMI analysis of variance of the two sets hybrids evaluated under drought and well-watered environments in 2011 and 2012, and well-watered high plant density environment in 2013

Source	**SET 1**				**SET 2**			
	df	SS	MS	TSS % Explained	df	SS	MS	TSS % Explained
Total	734	641799876	874387		764	728653273	953735	
Treatments	244	416153622	1705548***	64.84	254	525698165	2069678***	72.15
Genotype	48	109578668	2282889***	26.33	50	85389569	1707791***	16.24
Env	4	165423736	41355934***	39.75	4	289701642	72425410***	55.11
Rep (Env)	10	32089149	3208915***	5.00	10	29239125	2923913***	4.01
Geno x Env	192	141151217	735163***	33.92	200	150606955	753035***	28.65
IPCA1	51	56323412	1104381***	39.90	53	68591681	1294183***	45.54
IPCA2	49	43660598	891033***	30.93	51	32708266	641339***	21.72
IPCA3	47	25034337	532645	17.74	49	27371047	558593**	18.17
Residuals	45	16132870	358508	11.43	47	21935960	466723	14.57
Error	479	193557104	404086	30.16	500	173715982	347432	23.84

SS=sum of square; MS= Mean square; Geno= genotype; Env=Environment; TSS= total sum of square ** and *** significant at P <0.01 and 0.001 respectively.

AMMI Stability Value (ASV) provides a measure to quantify and rank genotypes according to their yield stability. In ASV method, a genotype with the lowest ASV score is the most stable, accordingly, VL0511298 x VL057903 and TZEI17 x ELN464171 were the most stable white and yellow hybrids, respectively. Because the most stable genotypes would not necessarily give the best yield performance (Mohammadi *et al.*, 2007), the rank of ASV and mean yield (RY) are incorporated in a single selection index namely: yield stability index (YSI). The lowest YSI is considered as the most stable with high mean yield. Based on YSI, the most outstanding white and yellow hybrids were VL0511298 x T020-58 and TZEI17 x ELN 464171, respectively.

Table 7. 15 Mean grain yield, heterosis and, yield stability and drought tolerance indices of white and yellow hybrids

SET 1 (White hybrids)									**SET 2 (Yellow hybrids)**								
HYBRID	GY (kg/ha)	MPH (%)	HPH (%)	STI	RDY (%)	DR	ASV	YSI	HYBRID	GY (kg/ha)	MPH (%)	MPH (%)	STI	RDY (%)	DR	ASV	YSI
VLA x ELN 41111	1800	365	250	5	27	T	5	45	TZA x ELN 39382	2013	-		-2	47	T	33	78
VLA x ELN 41112	1725	427	232	-7	39	HS	8	58	TZA x ELN 39427	1982	350	374	-6	9	T	23	75
VLA x ELN 41114	1061	272	96	-16	52	HS	13	77	TZA x ELN 402213	1857	396	368	-6	7	T	18	70
VLA x ELN 41115	1965	619	329	2	6	T	17	64	TZA x ELN 40791	2349	165	90	3	30	T	14	35
VLA x ELN 41271	2036	435	409	8	6	HT	39	73	TZA x ELN 40823	1994	276	264	-8	38	S	25	76
VLA x ELN 41272	2280	263	188	5	20	T	10	36	TZA x ELN 40941	2543	229	161	-2	46	T	7	17
VLA x ELN 42441	2514	429	328	6	41	T	12	32	TZA x ELN 431251	2458	312	233	-7	59	S	16	37
VLA x ELN 42442	2544	308	256	13	17	HT	28	51	TZA x ELN 43453	1561	34	-1	-15	58	HS	5	53
VLA x ELN 42444	2203	209	153	-2	24	T	7	26	TZA x ELN 43574	2327	937	592	5	26	T	85	63
VLA x ELN 42445	2331	312	255	7	33	T	15	48	TZA x ELN 45111	2807	555	476	6	46	T	10	20
VLA x ELN 48392	2376	387	297	6	44	T	15	42	TZA x ELN 462121	1344	-		-9	57	S	8	66
VLA x T02058	2749	787	420	14	17	HT	5	9	TZA x ELN 464171	2389	-		4	35	T	3	9
VLA x VL0511247	2383	401	336	2	13	T	20	51	TZA x ELN 47132	1450	312	261	-10	40	S	19	87
VLA x VL0512593	1876	252	211	-7	33	S	16	70	TZA x FBML10	2167	259	198	-2	41	T	6	28
VLA x VL054794	2830	362	289	20	17	HT	10	20	TZA x TZE124	2080	336	275	-10	54	S	19	62
VLA x VL05615	2375	237	193	9	49	T	8	24	TZA x TZE146	2390	467	451	4	36	T	7	21
VLA x VL05616	2421	468	402	8	10	HT	10	26	TZA x TZE148	2052	225	162	-14	49	S	12	48
VLA x VL057903	2063	277	250	0	46	S	3	24	TZA x TZE149	1801	63	9	-15	44	S	9	56
VLA x VL057967	2734	423	360	23	21	HT	17	41	TZA x TZE151	2602			12	14	HT		
VLA x VL058014	2491	243	210	5	52	S	15	40	TZA x TZE158	2081	159	92	-1	22	T	12	49
VLA x VL058025	2531	305	269	8	51	T	14	36	TZA x TZE16	1245	180	123	-21	69	HS	19	90
VLA x VL058589	2025	612	259	1	43	T	12	52	TZA x TZE161	2043	207	164	-9	66	S	15	57
VLA x VL081464	2082	225	168	5	29	T	11	45	TZA x TZE177	1758	167	115	-9	37	S	6	51
VLA x VL081466	1711	392	212	-8	35	HS	4	45	TZA x TZE23	1907	252	211	-2	39	T	8	49
VLB x ELN 41111	1805	690	565	-2	34	S	7	47	TZA x TZE8	2103	197	140	-1	51	S	4	24
VLB x ELN 41112	1589	759	534	-7	31	HS	25	91	TZA x TZI18	1771	155	114	-18	72	HS	18	76
VLB x ELN 41114	1334	1045	548	-14	10	S	17	85	TZB x ELN 39382	1618	-		-20	52	HS	15	74
VLB x ELN 41115	1251	657	447	-14	38	HS	4	50	TZB x ELN 39427	2570	283	193	8	25	HT	17	35
VLB x ELN 41271	1924	457	311	-7	35	HS	4	36	TZB x ELN 402213	2316	231	131	0	55	T	12	35
VLB x ELN 41272	1947	267	138	-4	32	S	7	38	TZB x ELN 40791	2204	103	75	4	43	T	22	58

continued

SET 1 (White hybrids)									SET 2 (Yellow hybrids)								
HYBRID	GY (kg/ha)	MPH (%)	HPH (%)	STI	RDY (%)	DR	ASV	YSI	HYBRID	GY (kg/ha)	MPH (%)	MPH (%)	STI	RDY (%)	DR	ASV	YSI
VLB x ELN 42441	1951	545	438	-2	45	S	9	46	TZB x ELN 40823	2140	242	141	-2	13	R	15	49
VLB x ELN 42442	2346	356	196	8	55	T	14	43	TZB x ELN 40941	2171	140	138	-6	41	S	4	21
VLB x ELN 42444	2372	405	232	7	37	T	19	53	TZB x ELN 431251	2222	169	116	-7	53	S	19	55
VLB x ELN 42445	1871	351	210	-2	27	S	12	60	TZB x ELN 43453	1798	37	13	-16	49	S	5	46
VLB x ELN 48392	1165	229	170	-22	27	HS	20	91	TZB x ELN 43574	2273	361	163	1	35	R	23	58
VLB x T02058	2101	1173	757	1	22	T	23	64	TZB x ELN 45111	2338	244	131	-6	53	S	10	30
VLB x VL0511247	2113	540	393	-1	50	S	24	64	TZB x ELN 462121	1636	-		-14	59	HS	28	93
VLB x VL0512593	1671	332	223	-11	38	HS	8	58	TZB x ELN 464171	2170	-		-5	68	S	19	54
VLB x VL054794	1869	289	148	3	18	T	4	41	TZB x ELN 47132	1817	188	98	-13	63	HS	20	78
VLB x VL05615	1826	247	129	-9	51	HS	7	48	TZB x FBML10	2292	157	131	-2	68	S	27	59
VLB x VL05616	2077	479	341	-1	53	HS	11	45	TZB x TZE124	2645	300	168	2	53	R	16	32
VLB x VL057903	1897	403	272	-2	58	HS	16	68	TZB x TZE146	1820	130	73	-2	64	S	7	46
VLB x VL057967	2129	395	234	1	36	T	7	30	TZB x TZE148	1789	126	101	-3	33	R	12	63
VLB x VL058014	1940	263	138	-7	54	HS	10	51	TZB x TZE149	2258	67	30	1	40	R	5	19
VLB x VL058025	1973	297	175	-5	64	HS	11	48	TZB x TZE151	2082			7	25	HT		
VLB x VL058589	1741	1164	549	-14	51	HS	14	70	TZB x TZE158	1722	29	22	-26	72	HS	28	93
VLB x VL081464	1958	277	138	-3	24	T	18	68	TZB x TZE16	1895	212	101	-4	47	R	7	46
VLB x VL081466	1767	786	619	-6	45	S	8	55	TZB x TZE161	1882	117	77	-11	55	HS	7	45
VLA x VLB	2154	432	274	5	34	T	28	65	TZB x TZE177	2005	115	100	-3	50	R	7	41
Mean	2038	446	297	0	35		13	50	TZB x TZE23	2461	159	120	-4	49	R	53	56
									TZB x TZE8	1788	86	77	-6	64	S	14	67
									TZB x TZI18	2087	121	102	-10	74	HS	37	74
									TZI18 x FBML10	2101	122	99	-1	41	R	9	41
									TZA x TZB	1553	68	45	-5	50	S	22	91
									Mean	2039	221	161	-5	46		17	53

VLA=VL0511298, VLB=VL054881; TZA=TZEI17, TZB=TZEI10; GY= Adjusted mean of grain yield across 5 environments; MPH=mid-parent heterosis; HPH=high parent heterosis STI=selection tolerance index; RDY= relative decrease in yield index (RDY); ASV= AMMI stability value; YSI=yield stability index; DR=drought reaction; HT, T, S and HS for highly tolerantt, tolerant, highly susceptible and susceptible to drought stress respectively.

In general, the stability and the yield performance of the hybrids derived from crosses between the two testers in both hybrid sets was low. Based on YSI, they were ranked 40th (out of 49) and 49th (out of 51) for VL0511298 x VL054881 and TZEI17 x TZEI10, respectively.

7.3.8 Principal Component Analysis (PCA)

To better understand how MPH, STI, RDY and YSI characterized the variation among the hybrids, principal component analysis was used. The pattern of the hybrids based on these four parameters is graphically displayed in a biplot of PCA1 and PCA2 (Figure 7.3).

The PCA1 and PCA2 axes accounted for 74 and 78% of total variation in hybrid set 1 and 2 respectively. The first PC separated the hybrids based on yield stability index (YSI) and selection tolerance index (STI) in hybrid set 1 while, the same PC1 divided the hybrids for set 2 mainly based on selection tolerance index STI and mid-parent heterosis (MPH), and to some extent relative yield reduction under drought index (RDY) and yield stability index (YSI). The second PC characterized the hybrids according MPH and RDY in hybrid set 1 whereas in set 2 it separated the hybrids based on MPH and YSI

To explain 100% of the variation among the hybrids more than two axes was needed. However the two first axes (PCA1 and PCA2) elucidated six and five groups of hybrids in set 1 and 2, respectively. The best hybrids combining drought resistance, high yield and stability, and high mid-parent heterosis were clustered in groups 2 and 1 for set 1 and 2, respectively. The interpretation of each group in both hybrid sets is presented in Table 7.16

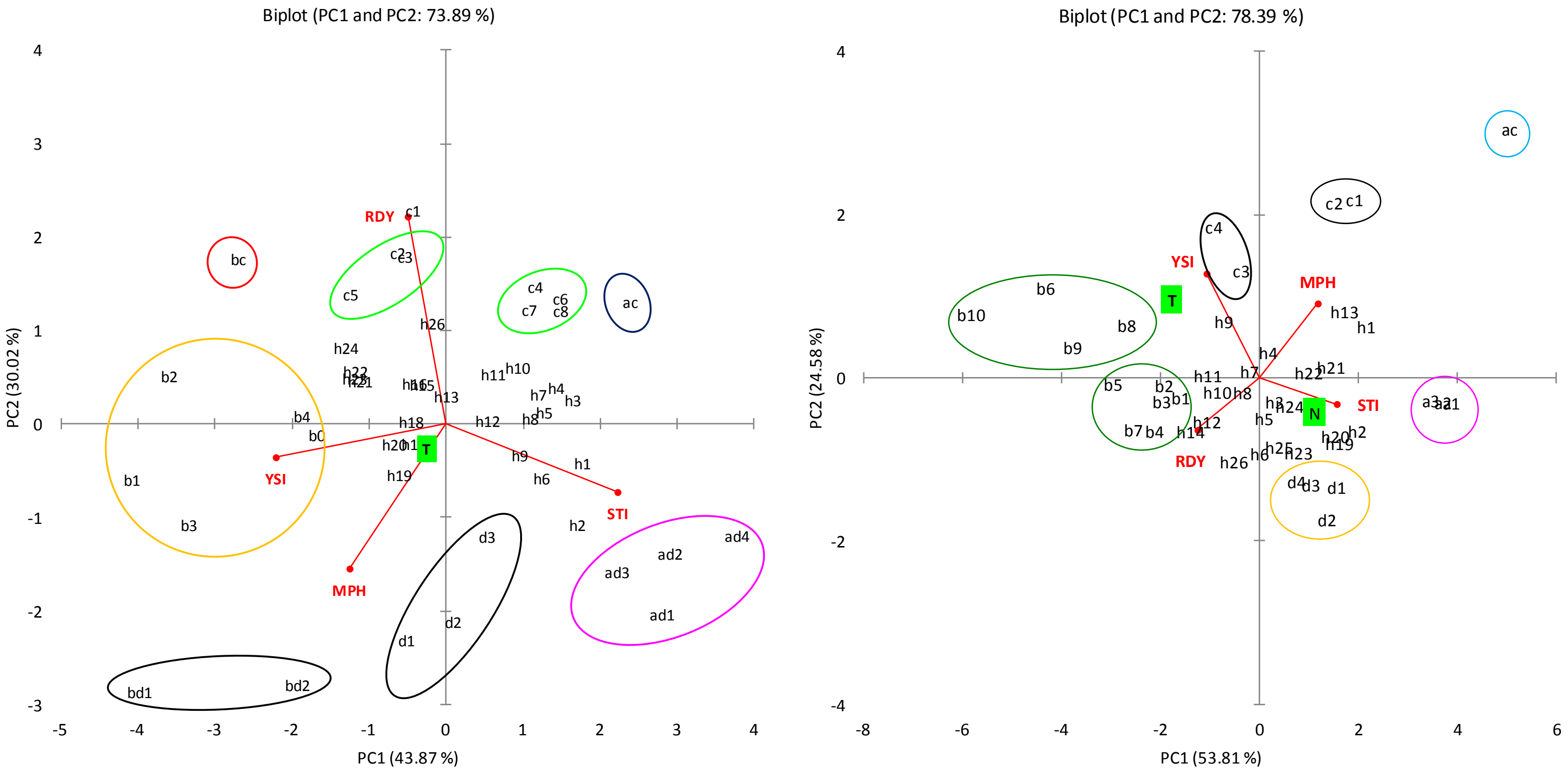
Table 7. 16 Group of hybrids constituted based on PCA results.

a- white hybrids (set 1)

Group	Hybrid code	Hybrid	Interpretation
1	ac	VL0511298 x VL05615	Drought tolerant, high yielding and stable but low mid-parent heterosis
2	ad1	VL0511298 x T02058	Drought tolerant, high yielding and stable, and high mid-parent heterosis
	ad2	VL0511298 x VL057967	
	ad3	VL0511298 x VL05616	
	ad4	VL0511298 x VL054794	
3	b1	VL054881 x VL058589	Drought susceptible and yield no stable
	b2	VL054881 x ELN 48392	
	b3	VL054881 x ELN 41112	
	b4	VL054881 x ELN 41115	
4	bc	VL0511298 x ELN 41114	Drought susceptible, yield no stable and low mid-parent heterosis
5	bd1	VL054881 x ELN 41114	High mid-parent heterosis and low percent of yield reduction under drought but yield no stable
	bd2	VL054881 x T02058	
6	c1	VL054881 x VL058025	Low mid-parent heterosis and high percent of yield reduction under drought
	c2	VL054881 x VL058014	
	c3	VL054881 x VL05615	
	c4	VL0511298 x VL058014	
	c5	VL054881 x VL057903	
	c6	VL0511298 x VL057903	
	c7	VL054881 x ELN 42442	
	c8	VL0511298 x VL058025	
7	d1	VL0511298 x ELN 41115	High mid-parent heterosis and low percent yield reduction under drought
	d2	VL0511298 x ELN 41271	
	d3	VL0511298 x VL0511247	

b Yellow hybrids (set 2)

Group	Hybrid code	Hybrid	Description
1	a1	TZEI17 x ELN 45111	Drought tolerant, high mid-parent heterosis and, high yielding and stable
	a2	TZEI17 x TZEI 146	
	a3	TZEI10 x ELN 39427	
2	ac	TZEI17 x ELN 43574	Drought tolerant, high mid-parent heterosis but low yielding and no stable
3	b1	TZEI17 x TZEI 161	Drought susceptible, low mid-parent heterosis and yield no stable
	b2	TZEI17 x TZEI 149	
	b3	TZEI10 x TZEI 8	
	b4	TZEI10 x ELN 43453	
	b5	TZEI10 x TZI 18	
	b6	TZEI17 x TZEI 16	
	b7	TZEI17 x ELN 43453	
	b8	TZEI10 x ELN 47132	
	b9	TZEI17 x TZI 18	
	b10	TZEI10 x TZEI 158	
4	c1	TZEI17 x ELN 402213	High mid-parent heterosis and low percent of yield reduction under drought but low yielding and no stable
	c2	TZEI17 x ELN 39427	
	c3	TZEI17 x ELN 40823	High mid-parent heterosis and high percent of yield reduction under drought but low yielding and no stable
	c4	TZEI17 x ELN 47132	
5	d1	TZEI17 x ELN 40941	High yielding and stable but low mid-parent heterosis
	d2	TZEI10 x TZEI 149	
	d3	TZEI17 x TZEI 8	
	d4	TZEI10 x ELN 40941	



SET 1 : White hybrids **SET 2 : Yellow hybrids**

T=VL0511298 x VL054881 and TZEI17 x TZEI10 in set 1 and 2 respectively; N=TZI18 x FBML10; h serial designed hybrids which cannot be significantly explained by the PCA1 and PCA2; MPH=mid-parent heterosis, STI=selection tolerance index; RDY= relative decrease in yield index; YSI= yield stability index

Figure 7. 3 Biplot of white and yellow hybrids characterized by four parameters (YSI, STI, RDY and MPH)

7.4 Discussion

The contribution of GCA sum of squares, which accounted for 72% and 64 % for total genetic variance in hybrid set 1 and 2 respectively, was higher than that of SCA sum of squares for grain yield averaged across environments. The high contribution of GCA over SCA was similar to that reported in temperate (Hallauer and Miranda, 1988) and tropical (Betrán *et al.*, 2003b) maize. GCA effects have been reported to be important for maize grain yield under drought stress. Our results confirm that, although SCA effects, indicative of non-additive gene effects contributed to grain yield across well-watered and water-stressed conditions, the relative contribution of GCA effects, indicative of additive gene effects, was more important than SCA effects under drought stress. The contribution of GCA effects was higher than SCA effects for most of the secondary traits under the three growing conditions, suggesting that additive gene action played a major role in the inheritance of the traits in the testcrosses evaluated. Thus, selection for secondary traits could be carried out at the early stages of the inbred line development. This is in agreement with the results of Betrán *et al*. (2003c) who indicated that inbred line performance *per se* for ASI and EPP could be predictive of hybrid performance for these traits across drought and low N stress, and non-stress environments. In addition, Lafitte and Edmeades (1995) also found line performance *per se* to be a reasonable indicator of hybrid performance for ASI and EPP under low N. General combining ability (GCA) was the main component accounting for the difference among the hybrids for grain yield and secondary traits. However, SCA effects were more important than GCA effects for EPP in one hybrid set and for leaf rolling in both hybrid sets under drought stress conditions, which was not consistent with the earlier report from Betrán *et al*. (2003c) but was in agreement with Worku *et al*. (2008), who showed that the contribution of SCA effects of EPP was more important that GCA effects under low-N conditions. These results demonstrate the importance of evaluation of hybrid combinations under stress and non-stress conditions to identify hybrids with higher EPP under stress conditions.

The four testers, 2 each from CIMMYT and IITA from opposite heterotic groups, used in the present study exhibited contrasting GCA effects that were consistent across the three growing conditions. The two CIMMYT testers classified 100% of the 24 CIMMYT and INERA lines into complementary heterotic groups based on SCA effects and grain yield of the testcrosses in well-watered and high plant density (WWHPD) and; 83% and 63% of these inbred lines were separated in well-watered and water-stressed conditions, respectively. The low percentage under drought stress was explained by the high performance of the hybrid between the two testers in that condition. On the other hand, the two IITA testers were able to separate all (26) IITA and INERA inbred lines into two contrasting heterotic groups. These percentages were higher compared to about 60% and 30% of the inbred lines successfully grouped into two groups reported by Menkir *et al*. (2003) and Agbaje *et al*. (2008). In their studies, the same testers, 1368 and 9071, were used to classify 34 lowland late/intermediate maturing inbreds in the first study and 42 early maturing in the second, evaluated under three growing conditions (Well-watered, water-stressed and wet seasons) and two level of *Striga* (*Striga* free and *striga* infested), respectively. The diversity of the inbred lines used in the present study within each hybrid set could explained the relative high performance of the testers to classify almost all the lines evaluated. For each hybrid set, the relative percentage of inbred lines from CIMMYT and INERA classified in each of the two heterotic groups (anti A and anti B) was fairly the same in the three growing conditions and across environments. These results indicate that the grouping of the inbreds was not based on the origin (breeding program) of the inbreds but on the interaction of the testers and the inbred lines. This finding implies that the testers used in the present study could be used in INERA maize breeding program to evaluate local as well as CIMMYT and IITA lines for combining ability estimates and heterotic patterns.

Heterosis is an expression of the phenomena of hybrid vigor resulting from the crossing of genetically different genotypes. Most crosses with desirable heterosis for yellow germplam were combinations of IITA (TZEI17 tester) and INERA inbred lines while in white germplam the average heterosis was fairly the same in the hybrid combinations between CIMMYT x INERA and CIMMYT x CIMMYT lines. Gissa *et al*. (2007) estimated mid and high parent heterosis among crosses of CIMMYT and Ethiopian origin maize inbred lines and reported that the most desirable combinations with high heterosis were CIMMYT x Ethiopian inbred lines. This result indicates that INERA lines were quite diverse in the heterotic response with CIMMYT testers.

Breeding implications and recommendations

Based on these results, heterotic groups could be created from these germplams for an efficient hybrid breeding program. For white germplasm, 7 lines (ELN41111, ELN41271, ELN42442, ELN42444, VL0511247, VL05616 and VL081464) may be recombined to form anti-VL054881 heterotic group source population with VL054881 as tester; and 11 lines (ELN41115, ELN41272, ELN42441, ELN42445, ELN48392, T02058, VL054794, VL05615, VL057967, VL058014 and VL058025) could be recombined to form anti VL0511298 heterotic group source population with VL0511298 as tester. In practical point of view, tester VL054881should be replaced by T02058, because VL054881 was not a good pollen shedder. Moreover hybrid, VL0511298 x T02058 produced the highest mean yield with desirable expression of heterosis.

The hybrid performance between the two IITA testers, TZEI17 and TZEI10 was low. On the other hand the current testers used in INERA, TZI18 and FBML10 were both classified into anti-TZEI17 heterotic group. This implies the need to identify new testers for yellow lines. Based on grain yield, GCA and SCA effects, and high parent heterosis, the lines TZEI17 and ELN45111 should be the potential testers for yellow germplam. The two lines were in opposite heterotic groups. Line ELN45111 had the highest GCA effects (463 kg/ha), and the cross TZEI17 x ELN45111 produced the highest grain yield of 3 t/ha and the second highest of both mid and high parent heterosis (555 % and 476%, respectively) across environments. However the classification based on TZEI17 and TZEI10 could be used to form the source population for each heterotic group. Thus, 13 lines (ELN39382, ELN40791, ELN40941, ELN431251, ELN43574, ELN464171, TZEI146, TZEI148, TZEI151, TZEI158, TZEI161, TZEI8 and TZEI10) could be recombined to form anti-TZEI17 heterotic group source populations with TZEI17 as tester, and 13 lines (ELN39427, ELN402213, ELN40823, ELN43453, ELN462121, ELN47132, FBML10, TZEI124, TZEI149, TZEI16, TZEI177, TZEI23 and TZI18) could be recombined to form anti - ELN45111 heterotic source populations with ELN45111 as tester. A preliminary screening to discard lines with poor agronomic characteristics should be carried out. Another option in source populations development, would be to select within each heterotic group lines having specific traits of interest that the tester and other lines lack. Each of these lines could, then, be crossed in pair wise combinations to produce source populations and new lines selected for combinations of desirable traits.

According to Pswarayi and Vivek (2008) and Badu-Apraku *et al*. (2013), identification of a single-cross hybrid as a potential tester for use in three-way and double cross hybrid development should be based on: 1) a good expression of GCA effects of the inbred lines constituting the single cross, 2) the two inbred lines should be in the same heterotic group and 3) the potential single-cross tester should display reasonably good yielding ability. The single-cross hybrid, VL0511298 x ELN42442 had grain yield of 2544 kg/ha (Table 7.9) across environments with the parental inbreds, VL0511298 and ELN42442 characterized by high positive and significant GCA effects for grain yield (177 and 412 kg ha^{-1}, respectively) (Table 7.8), and with the two inbreds placed in the same heterotic group (Table 7.9). This single-cross hybrid was therefore identified as a potential tester for the white three way hybrids. A further study would be required to identify a good single-cross tester for yellow three way hybrids.

Based on results from principal component analysis, 4 white hybrids (VL0511298 x T02058, VL0511298 x VL057967, VL0511298 x VL05616, VL0511298 x VL054794) and 3 yellow hybrids (TZEI17 x ELN45111, TZEI17 x TZEI146, TZEI10 x ELN39427) were identified as the best promising hybrids combining drought tolerance and high yield potential and stability.

7.5 Conclusion

The results indicate that the relative contribution of GCA effects to genetic variation observed among progeny performance was high compared to the SCA effects under drought and non-drought conditions. The exotic testers showed consistent and contrasting GCA effects across environments and were able to separate adapted and exotic inbred lines into complementary heterotic groups. The heterotic patterns observed in this study suggests that the INERA inbred lines would produce high yielding hybrid under drought and non-drought conditions when crossed with CIMMYT or IITA inbred lines of the opposite heterotic group. Based on the results of this study, a number of complementary heterotic group source populations with an appropriate tester constituted with INERA, CIMMYT and IITA inbred lines could be developed for a hybrid breeding program. Considering the results obtained by principal component analysis, the biplot analysis of drought tolerance and yield stability indices and heterosis information is proposed for use to determine high yielding and stable drought tolerant genotype with a consistent heterotic reaction.

CHAPTER EIGHT

8.0 General Overview

8.1 Introduction

The present study was designed to explore genetic diversity among local and exotic germplams and exploit the potential of this diversity to identify genetic variation associated with drought tolerance; to establish heterotic patterns for development of high yielding hybrids adapted to a wide range of environments including drought condition, and which meet farmers' preferences. The results are presented and discussed in different chapters, the purpose of this chapter is to review and conclude the complete research, and draw out some of the implications for breeding. The following research hypotheses were tested across environments in Burkina Faso: 1) Farmers have their own ways to identify, select maize varieties and perceive the constraints from which breeding strategy can be formulated. 2) There is sufficient genetic diversity among local maize inbred lines and genetic differences between local and exotic lines which can be exploited to develop new varieties; 3) The genetic variation present in the tropical inbred lines tested can be used to identify genes associated with drought tolerance; 4) Yield and its associated traits of testcross hybrids are related and heritable under drought and non-drought conditions; 5) Maize inbred lines including local and tropical exotic lines shows combining ability in testcrosses and the testers can separate the lines into heterotic groups; 6) The additive gene action of maize grain yield is more predominant than non-additive gene action under drought and non-drought conditions; 7 High yielding drought tolerant hybrids can be selected from testcross hybrids.

8.2 Findings of the Study and Their Implications

The diversity of the germplam used in this study could be exploited to meet the farmers' demand for early maturing maize varieties that tolerate the main maize constraints, drought and *Striga*, in the major maize producing zone in Burkina Faso. CIMMYT and IITA germplasm evaluated were early maturing and were previously selected for drought tolerance. In addition, IITA inbreds were developed from two *Striga* resistant populations, TZE Y Pop DT STR and TZE COMP5-Y. This germplasm should contribute favorable alleles for drought, *Striga* and/or earliness. The results of this study indicate that selection for earliness would be effective. This is because heritability was high for both days to 50 % anthesis and silking. In addition, the general combining ability effects, based mostly on additive gene action, was predominant for both grain yield and flowering traits. Selection for earliness has produced cultivars that perform well in environments with short rainy seasons, or where frequent mid-season droughts tend to coincide with flowering of late-maturing cultivars. With adequate rainfall, grain yield of maize is usually positively correlated with maturity and season length. This might prevent breeders from selecting for earliness because of 'yield penalty'. However, combining high yield and earliness was successfully achieved in temperate maize by selecting for late senescence or the "stay-green trait" (Duvick, 1997; Tollenaar and Wu, 1999). Delaying leaf senescence in early hybrids would enhance grain yield through increased duration of leaf area exposure for photosynthetic activity, especially during favorable seasons. Results from the current study for yellow hybrids showed that both low rate of leaf senescence and unrolled leaf score were significantly correlated with high grain yield under drought.

Published research suggested the use of molecular markers to determine heterotic patterns and predict hybrid performance. The present study found that high yielding hybrids were not generated by inbreds parents that were genetically distant, thus the results of this study add to the literature an example in which molecular markers were not useful for predicting heterosis and high yielding crosses. However, the study showed that the two CIMMYT testers produced high yielding hybrids in crosses with ELN42441, ELN42442 and ELN42444 but not with ELN41111, ELN41112, ELN41114 and ELN41115. The three first lines belonged to a group which was genetically distant from the group of the last four lines. Results of molecular genetic diversity studies showed that lines developed from the same source tended to be grouped together. Thus, INERA maize inbred lines, extracted from two OPVs sources (FBC6 and ESPOIR), were genetically constituted of 4 distinct groups including 2 groups of inbred lines derived from FBC6 , 1 group derived from ESPOIR and 1 mixed group composed of lines from the two sources. FBMS1 was involved in the cross with both FBC6 and ESPOIR in the development of the inbred lines which formed the mixed group. In addition, 6 IITA inbred lines (TZEI 23, TZEI 149, TZEI 8, TZEI 158, TZEI 161 and TZEI 148) developed from the same population, TZE-Y Pop DT STR, were genetically separated by PCA analysis from 18 lines derived from FBC6. Among the CIMMYT lines, some were developed from a broad base population either with Tuxpeño or ETO background and others from biparental crosses or others sources, consequently no clear groupings were found in this germplasm. VL05616, which had a temperate inbred line (FR812) as one of its two parents, tended to be grouped with temperate lines. The genetic characterization indicated that local germplam could be favorably improved using exotic germplam. In this regard, temperate and CIMMYT subtropical lines should be sought to improve or increase genetic variability within INERA germplasm. This is because the current study identified 100 missing alleles which existed predominantly in Temperate and CIMMYT or in both germplasms across the 10 maize chromosomes in INERA germplam. In addition, 28 unique alleles were identified which existed only in Temperate germplam, 17 in INERA, 7 in CIMMYT and 3 in IITA. A single nucleotide change within a gene sequence may change the end product of that gene, thus these unique alleles could contribute favorably or adversely to the expression of a character in a line or in the combination with another line. It might be interesting to point out that both CIMMYT testers, VL0511298 and VL054881, carried an unique allele G on chromosome 3 that located in different positions that were far apart from172353835 bp, VL054881 carried also, on chromosome 4, another unique allele G. In general, inbred lines which produce high yield with these CIMMYT testers also carried one or two unique alleles.

The present investigation identified 580 polymorphic SNPs which could be used routinely for genetic diversity studies with low cost. Of the 580 SNPs, 327 and 278 were previously reported by Lu *et al*. (2009) and Semagn *et al*. (2012), respectively. Important research on drought tolerant maize development using marker assisted selection and genetic engineering is ongoing in sub-Saharan Africa. Results of this study add to the scientific knowledge the potential of tropical maize inbred lines for association studies to identify loci associated with drought tolerance. Using a panel of CIMMYT, IITA and INERA inbred lines, this study discovered 22 SNPs which underlied genetic variation associated with drought tolerance traits. Some of these SNPs were also found in studies on QTls/gene detection by meta-analysis or association mapping elsewhere.

CIMMYT has established a selection index method based on yield and secondary traits (Bänzinger *et al*., 2000), an algorithm of such formula proposed by Hao *et al*. (2011a) was found to be very effective in this study. This is because, the current association mapping study, found the strongest association between the SNPs variation and this index. In addition, this index was significantly correlated to grain yield and yield stability index (data not shown).

Earlier hybrid breeding programs in Burkina Faso did not make much progress because breeders emphasized on open pollinated varieties development and also because research on maize improvement was conducted by international institutes, specially IITA and IRAT ('Institut de Recherche en Agriculture Tropicale, lead by French researchers). IITA was relocated to Nigeria and IRAT program stopped with African independence. Consequently, the inbred lines and heterotic patterns were not maintained. The current INERA maize breeding program developed a large number of lines but there is a gap in heterotic patterns establishment. Results from the present study provide scientific bases from which a sustainable heterotic relationships for maize breeding program in Burkina Faso could be designed.

PRA results showed that both white and yellow maize are fairly important. So to meet farmers' need, heterotic grouping should be established for both white and yellow germplasms. The study identified VL0511298 and T02058 as testers for two opposite heterotic groups in white maize germplasm while TZEI17 and ELN45111 were suggested for yellow maize testers. A subgroup of inbred lines including local and exotic lines was formed for use in creating source populations for each heterotic group. Another important finding from PRA study was that farmers recycle seed from local and improved varieties for many years, and the low use of improved varieties was mostly due to high seed cost. Therefore, three way and double cross hybrids should be developed for short and medium term with movement to single cross in the long-term. A hybrid, VL0511298 x ELN42442 was identified as a potential tester for three way and double cross hybrid breeding.

The Burkina government has supported the cultivation of Bondofa (maize varietal hybrid) during the off-season in the irrigated land. The off-season is divided into dry and cold season, and dry and hot season. The hybrid breeding program should target also these seasons in developing inbred lines and hybrids. The current study showed that local lines were sensitive to cold weather which extended their flowering time suggesting that it would be desirable to screen the lines in cold and hot seasons during the preliminary evaluation. Another important finding from this study was the relative predominance of general combining ability effects compared to specific combining ability for grain yield and secondary traits under drought stress, suggesting that selection for grain yield and secondary traits could be achieved at an early stage of inbred line development. This also implies that a single representative inbred line tester could be used to select promising lines to advances them to the next generation. Then, the more advanced lines would be tested by a broad base genetic tester to identify lines with high GCA for use in three-ways hybrids.

8.3 Conclusion and Future direction

The Geographic Information System (GIS) analysis of PRA data showed that farmers' demand for drought tolerance and early maturing varieties is not limited only to dry areas and also that drought and *Striga* are major constraints to maize production across all districts regardless of rainfall conditions. This implies that the future research on maize should consider stress tolerance and earliness with wide adaption.

Surveys on farmers' perceptions showed they were not informed about yield potential, stress tolerance or others characteristics of a cultivar he /she buys or receives from NGOs or the government. This demonstrates the need for an integrated framework where breeders, seed companies, seed resellers, and extension agents can work together to ensure that the farmer grows the cultivar which fits his/her preferences and is adapted to his/her environment.

The findings from the present study suggest that exotic germplasm could be used to improve local materials for stress tolerances and creating heterotic patterns. Future research should exploit the diversity of this germplam to develop cultivars which meet farmers preferences.

Although drought is a recurrent major constraint to production in sub-saharan African countries and still needs a lot of research, *Striga* should also be considered as a priority, especially in Burkina Faso, where there is limited knowledge of the resistance level of local inbred lines and the genetic analysis of *Striga hermonthica* stress.

The study identified complementary heterotic source populations along with appropriate testers. The advanced lines not included in this study should also be classified into heterotic groups and breeding effort and resources should be allocated to keep and refine these heterotic patterns for a sustainable hybrid breeding program in INERA.

High yield and stable, drought tolerant hybrids identified in this study which performed better than standard checks under drought and non-drought conditions will be recommended for release and for use as source of drought resistance in breeding programs.

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APPENDICES

Table S 1**.** Summary of the 96 maize inbred lines used in the present study**.**

CIM=CIMMYT/Zimbabwe ; US= United State; EU=European Union; C=Kernel color; W=white, Y=yellow; Reaction to Drought (DR) and *Striga hermonthica* (SR) stresses; T=tolerant; Susceptible; R=resistant Het=Heterozigosity. Inbred lines with * at the end of the name were used in the field trial.

No	**Brief Name**	**Sample Name**	**Origin**	**C**	**DR**	**SR**	**HET**	**Pedigree**
1	CML444	CML444	CIM	W	T		0.10	P43C9-1-1-1-1-1-BBBB
2	T02058	T02058*	CIM	W	T		0.01	[CML389/CML176]-B-29-2-2-B*6-B
3	VL0511247	VL0511247*	CIM	W	T		0.02	[INTA-2-1-3/INTA-60-1-2]-X-11-6-3-BBB
4	VL0511298	VL0511298*	CIM	W			0.01	MAS[MSR/312]-117-2-2-1-B*4-2-14-BB
5	VL0512593	VL0512593*	CIM	W	T		0.02	Syn01E2-64-2-B-2-BB
6	VL054794	VL054794*	CIM	W	T		0.00	[[[K64R/G16SR]-39-1/[K64R/G16SR]-20-2]-5-1-2-B*4/CML390]-B-38-1-B-2-#-1-B-2
7	VL054881	VL054881*	CIM	W			0.14	[Ent2:92SEW1-EarlySel-22/[DMRESR-W]EarlySel-#1-3-2-B/CML390]-B-26-1B-1-#-1-BB-3-1
8	VL05615	VL05615*	CIM	W	T		0.01	ZEWBc1F2-216-2-2-B-2-B*4-4-2-8-B
9	VL05616	VL05616*	CIM	W	S		0.01	[SC/CML204//FR812]-X-30-2-3-2-1-B*6
10	VL057903	VL057903*	CIM	W	T		0.20	ZEWAc1F2-151-6-1-B-1-BBB-2-2-BB
11	VL057967	VL057967*	CIM	W	T		0.01	ZEWAc1F2-219-4-3-B-1-B*4-1-3-BB
12	VL058014	VL058014*	CIM	W	S		0.01	ZEWAc1F2-254-2-1-B-1-BB-1-3
13	VL058025	VL058025*	CIM	W	T		0.00	ZEWAc1F2-164-3-2-B-1-BBB-2-2-BB
14	VL058589	VL058589*	CIM	W	T		0.01	INTA-F2-192-2-1-1-1-B*7-2-B-3
15	VL081464	VL081464*	CIM	W	T		0.03	ZEWBc2F2-101-2-BB
16	VL081466	VL081466*	CIM	W	T		0.04	ZEWBc2F2-110-1-BBB
17	TZEI10	TZEI10*	IITA	Y	T	R	0.04	TZE-Y Pop STR Co S6 Inbred 152
18	TZEI124	TZEI124*	IITA	Y	S		0.01	TZE-Y Pop STR Co S6 Inbred 3-1-3
19	TZEI146	TZEI146*	IITA	Y	T	R	0.02	TZE-Y Pop STR Co S7 Inbred 49-3-3
20	TZEI148	TZEI148*	IITA	Y	T	R	0.01	TZE-Y Pop STR Co S6 Inbred 62-1-3
21	TZEI149	TZEI149*	IITA	Y	T	R	0.04	TZE-Y Pop STR Co S6 Inbred 66-2-2
22	TZEI158	TZEI158*	IITA	Y	T	R	0.01	TZE-Y Pop STR Co S6 Inbred 102-2-2
23	TZEI16	TZEI16*	IITA	Y	T	R	0.03	TZE Comp5-YS C6 S6 Inbred 31
24	TZEI161	TZEI161*	IITA	Y	T	R	0.03	TZE-Y Pop STR Co S6 Inbred 103-2-3
25	TZEI17	TZEI17*	IITA	Y	T	T	0.09	TZE Comp5-Y C6 S6 Inbred 35
26	TZEI177	TZEI177*	IITA	Y	T	R	0.07	TZE Comp5-Y C6 S6 Inbred 62-1-2
27	TZEI23	TZEI23*	IITA	Y	T	R	0.02	TZE-Y Pop STR Co S6 Inbred 62-2-3
28	TZEI8	TZEI8*	IITA	Y	T	R	0.12	TZE-Y Pop STR Co S6 Inbred 62-3-3
29	TZI3	TZI3	IITA	Y			0.00	
30	TZI18	TZI18*	IITA	Y			0.02	
31	TZI35	TZI35	IITA	Y			0.02	
32	ELN391	ELN39111	INERA	Y			0.02	FBC6xFBMS1
33	ELN3931	ELN39381	INERA	Y			0.03	FBC6xFBMS1
34	ELN3932	ELN39382*	INERA	Y			0.01	FBC6xFBMS1
35	ELN3933	ELN39391	INERA	Y			0.04	FBC6xFBMS1
36	ELN3934	ELN39392	INERA	Y			0.05	FBC6xFBMS1
37	ELN394	ELN39427*	INERA	Y			0.02	FBC6xFBMS1
38	ELN401	ELN401172	INERA	Y			0.01	FBC6xFBMS1
39	ELN402	ELN402213*	INERA	Y			0.09	FBC6xFBMS1
40	ELN404	ELN40441	INERA	Y			0.02	FBC6xFBMS1
41	ELN407	ELN40791*	INERA	Y			0.03	FBC6xFBMS1
42	ELN408	ELN40823*	INERA	Y			0.07	FBC6xFBMS1
43	ELN409	ELN40941*	INERA	Y			0.05	FBC6xFBMS1
44	ELN4111	ELN41111*	INERA	W			0.07	FBC6xFBMS1
45	ELN4112	ELN41112*	INERA	W			0.07	FBC6xFBMS1

continued

No	Brief Name	Sample Name	Origin	C	DR	SR	HET	Pedigree
46	ELN4113	ELN41113*	INERA	W			0.05	FBC6xFBMS1
47	ELN4114	ELN41114*	INERA	W			0.03	FBC6xFBMS1
48	ELN4115	ELN41115*	INERA	W			0.03	FBC6xFBMS1
49	ELN4121	ELN41271*	INERA	W			0.02	FBC6xFBMS1
50	ELN4122	ELN41272*	INERA	W			0.03	FBC6xFBMS1
51	ELN4123	ELN41273*	INERA	W			0.02	FBC6xFBMS1
52	ELN4241	ELN424101*	INERA	W			0.04	FBC6xFBMS1
53	ELN4242	ELN424103	INERA	W			0.02	FBC6xFBMS1
54	ELN4243	ELN42441*	INERA	W			0.03	FBC6xFBMS1
55	ELN4244	ELN42442*	INERA	W			0.04	FBC6xFBMS1
56	ELN4245	ELN42444*	INERA	W			0.01	FBC6xFBMS1
57	ELN4246	ELN42445*	INERA	W			0.03	FBC6xFBMS1
58	ELN431	ELN431251*	INERA	Y			0.22	FBC6xFBMS1
59	ELN434	ELN43453*	INERA	Y			0.12	FBC6xFBMS1
60	ELN435	ELN43574*	INERA	Y			0.01	FBC6xFBMS1
61	ELN443	ELN443101	INERA	Y			0.01	FBC6xFBMS1
62	ELN451	ELN45111*	INERA	Y			0.02	FBC6xFBMS1
63	ELN452	ELN45272	INERA	Y			0.03	FBC6xFBMS1
64	ELN471	ELN47132*	INERA	Y			0.05	FBC6xFBMS1
65	ELN483	ELN48392*	INERA	W			0.02	FBC6xFBMS1
66	ERL21	ERL21811	INERA	Y			0.01	ESPOIRxFBMS1
67	ERL2211	ERL221712	INERA	Y			0.04	ESPOIRxFBMS1
68	ERL2212	ERL221813	INERA	Y			0.07	ESPOIRxFBMS1
69	ERL222	ERL222722	INERA	Y			0.03	ESPOIRxFBMS1
70	ERL225	ERL225132	INERA	Y			0.02	ESPOIRxFBMS1
71	ERL227	ERL227213	INERA	Y			0.03	ESPOIRxFBMS1
72	ERL2301	ERL23025	INERA	Y			0.02	ESPOIRxFBMS1
73	ERL2302	ERL230212	INERA	Y			0.03	ESPOIRxFBMS1
74	ERL2421	ERL242152	INERA	Y			0.03	ESPOIRxFBMS1
75	ERL2422	ERL242153	INERA	Y			0.03	ESPOIRxFBMS1
76	ERL2423	ERL242154	INERA	Y			0.04	ESPOIRxFBMS1
77	ERL2424	ERL242157	INERA	Y				ESPOIRxFBMS1
78	ERL241	ERL24132	INERA	Y			0.05	ESPOIRxFBMS1
79	ERL242	ERL24211	INERA	Y			0.09	ESPOIRxFBMS1
80	ERL243	ERL24212	INERA	Y			0.11	ESPOIRxFBMS1
81	ERL2511	ERL251323	INERA	Y			0.04	ESPOIRxFBMS1
82	ERL2512	ERL251352	INERA	Y			0.02	ESPOIRxFBMS1
83	ERL2571	ERL257112	INERA	Y			0.02	ESPOIRxFBMS1
84	ERL2572	ERL257118	INERA	Y			0.20	ESPOIRxFBMS1
85	ERL263	ERL263222	INERA	Y			0.02	ESPOIRxFBMS1
86	ERL264	ERL264411	INERA	Y			0.13	ESPOIRxFBMS1
87	ESPOIR	ESPOIR	INERA	Y				OPV/Pop 66 SR
88	FBC6	FBC6	INERA	Y				OPV/ mixing of 8 varieties
89	FBML10	FBML10	INERA	Y			0.02	Extracted from Tzi35
90	FBMS1	FBMS1	INERA	Y				OPV/mixing of different sources of sweet corn
91	B73	B73	U.S	Y			0.01	BSSS
92	C103	C103	U.S	Y			0.01	Lancaster Surecrop (from Noah Hershey)
93	DE811	DE811	U.S	Y			0.01	((C103 x Mp3204) x B73) x B68
94	Mo17	Mo17	U.S	Y			0.01	C103 × 187-2
95	OH43	OH43	U.S	Y			0.01	OH40BXW8
96	W182E	W182E	U.S	Y			0.00	WD (Wisconsin 25 population) x W22
97	EP1	EP1	E.U	Y			0.00	Lizargarote population (Spain)
98	F2	F2	E.U	Y			0.01	Lacaune population (France)
99	F7	F7	E.U	Y			0.01	Lacaune population (France)
100	F252	F252	E.U	Y			0.01	F186 x Co125

Table S 2. Summary statistics for the 1057 informative SNP markers identified from the 1151-SNPs**.**

Chr=Chromosome; NA=unknown; MAF=minor allele frequency; G.Div=Gene diversity; Het= heterozygosity; PIC=Polymorphism Information Content; High Quality SNP Markers identified in the present study (a) including SNPs also previously recommended by Lu *et al*. (2009) (b) and Semagn *et al*. (2012) (c).

No	SNP name	SNP	Chr	Position	MAF	G.Div	Het	PIC	No	SNP name	SNP	Chr	Position	MAF	G.Div	Het	PIC
1	PZA01271.1abc	G/C	1	1947984	0.48	0.49	0.06	0.37	36	PZA00240.6abc	A/G	1	41231748	0.41	0.48	0.08	0.37
2	PZA03613.1	A/T	1	2914171	0.16	0.27	0.06	0.24	37	PHM2177.85	G/A	1	41232121	0.16	0.26	0.03	0.23
3	PHM175.25a	A/C	1	3527613	0.21	0.33	0.06	0.28	38	PHM3726.129abc	A/G	1	41390404	0.25	0.37	0.06	0.30
4	PZA02032.1	T/A	1	4490461	0.05	0.10	0.00	0.10	39	PHM11000.37	A/C	1	43711880	0.03	0.05	0.01	0.05
5	PZA02372.1a	G/A	1	6220547	0.27	0.39	0.22	0.31	40	PHM11000.21a	G/A	1	43712019	0.22	0.33	0.03	0.28
6	PHM4997.17	A/G	1	6229998	0.09	0.16	0.02	0.15	41	PZA02376.1a	C/G	1	44506999	0.26	0.38	0.03	0.31
7	PHM4997.11	C/A	1	6230096	0.10	0.18	0.00	0.17	42	PZA03742.1ab	A/C	1	44535423	0.30	0.41	0.00	0.33
8	PZA00181.2	A/C	1	8366411	0.13	0.23	0.02	0.20	43	PZA00081.18	G/C	1	45675182	0.13	0.22	0.00	0.20
9	PZA00528.1	C/A	1	8367944	0.08	0.15	0.00	0.14	44	PHM4313.17	G/A	1	45788920	0.03	0.06	0.00	0.06
10	PHM13094.8a	G/A	1	8368054	0.24	0.36	0.05	0.30	45	umc13.1	A/G	1	46064387	0.04	0.08	0.00	0.08
11	PZA00175.2abc	T/A	1	8510027	0.30	0.41	0.00	0.33	46	PZA03183.5	G/A	1	46065084	0.15	0.25	0.03	0.22
12	PZA00447.8a	G/A	1	9024005	0.39	0.47	0.07	0.36	47	PZB00872.3	G/A	1	46255059	0.05	0.10	0.00	0.10
13	PZA02284.1abc	A/G	1	9273299	0.22	0.34	0.03	0.28	48	PHM4913.18	C/A	1	50197162	0.18	0.29	0.09	0.25
14	PZA03521.1	T/A	1	10068726	0.15	0.25	0.04	0.22	49	PZA02292.1	C/G	1	51407226	0.12	0.21	0.02	0.19
15	PZA00887.1abc	C/G	1	10934676	0.44	0.49	0.04	0.37	50	PZA03168.5	C/A	1	51514741	0.15	0.25	0.02	0.22
16	PZA03551.1abc	G/A	1	12208404	0.49	0.49	0.05	0.37	51	PZA01866.1abc	A/C	1	51576563	0.37	0.46	0.00	0.36
17	csu1171.2a	G/A	1	14578606	0.28	0.40	0.09	0.32	52	PHM12323.17abc	G/C	1	53357797	0.43	0.48	0.06	0.37
18	PZA01497.1abc	A/G	1	14578834	0.27	0.39	0.04	0.32	53	PZA02737.1ab	G/A	1	54049220	0.35	0.45	0.09	0.35
19	PHM1653.32a	A/C	1	14898823	0.28	0.40	0.03	0.32	54	PZB01062.3abc	G/A	1	56846728	0.34	0.44	0.10	0.35
20	PHM1653.31a	G/A	1	14898863	0.39	0.47	0.03	0.36	55	PZA01315.1abc	A/G	1	60230382	0.22	0.34	0.04	0.28
21	PZA02094.9a	A/T	1	15725672	0.28	0.40	0.00	0.32	56	PZA03189.4a	C/A	1	64261105	0.23	0.35	0.07	0.29
22	PZB00648.5abc	G/A	1	17595139	0.31	0.42	0.10	0.34	57	PHM5098.25	G/A	1	65825628	0.09	0.16	0.05	0.15
23	PHM13619.5	C/A	1	22281738	0.09	0.15	0.00	0.14	58	PHM5306.16a	A/G	1	76053490	0.31	0.42	0.08	0.33
24	PZA02487.1ab	G/A	1	22601426	0.23	0.35	0.00	0.29	59	PZA01267.3abc	G/A	1	76054042	0.29	0.41	0.03	0.33
25	PHM4531.46	C/G	1	22918337	0.10	0.19	0.03	0.17	60	PZA00752.1	G/A	1	80803012	0.08	0.15	0.05	0.14
26	PZA02279.1	G/A	1	23520113	0.12	0.21	0.00	0.19	61	PZA01135.1	A/G	1	82563962	0.02	0.04	0.00	0.04
27	PZB01957.1	C/A	1	26283293	0.02	0.04	0.00	0.04	62	PZB01235.4	G/A	1	88380865	0.01	0.02	0.00	0.02
28	PZB02058.1a	G/A	1	28526738	0.42	0.48	0.10	0.37	63	PZA03240.1a	A/G	1	90772226	0.26	0.38	0.05	0.31
29	PHM3951.25	G/A	1	31928634	0.05	0.10	0.00	0.10	64	PZA03240.2a	C/G	1	90772275	0.21	0.33	0.03	0.28
30	PZA01455.1	A/G	1	34388212	0.19	0.30	0.03	0.26	65	PZA03465.1	G/C	1	91279351	0.02	0.04	0.00	0.04
31	PZA02686.1	G/A	1	34724094	0.02	0.04	0.00	0.04	66	PZA00944.1ab	A/G	1	91429024	0.23	0.35	0.04	0.29
32	PZA02271.1	G/A	1	38607726	0.02	0.04	0.00	0.04	67	PHM9418.11	G/A	1	96545939	0.08	0.14	0.00	0.13
33	PHM4597.14abc	G/A	1	38608156	0.43	0.49	0.18	0.37	68	PHM10621.29	G/C	1	101421468	0.10	0.18	0.00	0.17
34	PZA02195.1	C/A	1	39288623	0.12	0.21	0.00	0.19	69	PZA02763.1abc	G/A	1	101421538	0.44	0.49	0.09	0.37
35	PHM4359.21	A/G	1	39922358	0.03	0.06	0.07	0.06	70	PHM12633.15	A/C	1	102664092	0.10	0.17	0.04	0.16

No	SNP name	SNP	Chr	Position	MAF	G.Div	Het	PIC
71	PZA01254.2abc	A/G	1	106204446	0.39	0.47	0.16	0.36
72	PHM3463.18a	G/A	1	106204545	0.37	0.46	0.02	0.36
73	PHM1932.51a	A/G	1	118875639	0.29	0.41	0.06	0.33
74	csu1138.4a	G/A	1	119018556	0.23	0.35	0.06	0.29
75	csu1138.3a	G/A	1	119018614	0.49	0.49	0.04	0.37
76	PHM4695.5	G/A	1	143891365	0.04	0.08	0.02	0.08
77	PZA03200.2abc	A/G	1	148694799	0.31	0.42	0.04	0.34
78	PHM1725.34abc	A/C	1	150544945	0.39	0.47	0.00	0.36
79	PHM2187.34	C/G	1	157176065	0.18	0.29	0.02	0.25
80	PZA02741.1a	T/C	1	161072169	0.35	0.45	0.07	0.35
81	an1.5	G/A	1	175504926	0.13	0.22	0.02	0.19
82	PHM4053.15a	A/G	1	176705478	0.31	0.42	0.02	0.34
83	PZA00455.16a	A/C	1	179851941	0.26	0.38	0.07	0.31
84	PZA00455.14abc	A/G	1	179852026	0.37	0.46	0.00	0.36
85	PHM3147.18abc	A/G	1	181940662	0.21	0.33	0.00	0.28
86	PZA02191.1	G/A	1	182386577	0.14	0.24	0.05	0.21
87	PHM1809.18	G/A	1	183295965	0.18	0.29	0.01	0.25
88	PHM1968.22a	G/A	1	183647544	0.44	0.49	0.05	0.37
89	PZA00068.1	A/G	1	183831591	0.06	0.12	0.00	0.11
90	PHM5622.21abc	A/T	1	183831657	0.21	0.33	0.00	0.28
91	PHM4300.6	A/G	1	189467932	0.08	0.14	0.00	0.13
92	PHM5727.5	G/A	1	192367988	0.04	0.07	0.01	0.07
93	PZA00619.3	G/A	1	195425179	0.05	0.10	0.02	0.10
94	PZA02467.10ab	C/G	1	196927608	0.33	0.44	0.07	0.34
95	PZA03074.27	G/A	1	201295774	0.04	0.08	0.00	0.08
96	PHM5597.15	A/C	1	203035169	0.03	0.06	0.00	0.06
97	PZA01216.1abc	A/G	1	203035464	0.38	0.47	0.07	0.36
98	PZA01963.15	C/G	1	203714543	0.12	0.21	0.00	0.19
99	PZA01391.1	G/A	1	204199305	0.15	0.25	0.08	0.22
100	PHM14614.22	C/A	1	205581478	0.18	0.29	0.05	0.25
101	PZA01019.1	C/G	1	208143724	0.13	0.22	0.00	0.20
102	PZA03193.2	G/A	1	212307461	0.09	0.16	0.00	0.15
103	PHM12706.14abc	A/G	1	212356401	0.28	0.40	0.00	0.32
104	PHM1438.34a	G/A	1	212389447	0.23	0.35	0.03	0.29
105	PZA02014.3a	A/G	1	212557676	0.21	0.33	0.02	0.28
106	PZA03265.3	A/C	1	214745681	0.10	0.18	0.00	0.16
107	PHM6043.19	A/G	1	216927159	0.07	0.13	0.02	0.13
108	PZA03741.1abc	G/A	1	216989646	0.39	0.47	0.04	0.36
109	PZA02655.9	G/C	1	217504762	0.05	0.10	0.00	0.10
110	PHM15871.11	A/G	1	222427156	0.05	0.10	0.00	0.10
111	PZA02117.1abc	A/G	1	223466480	0.45	0.49	0.05	0.37
112	PHM12693.8	A/G	1	223488426	0.12	0.21	0.00	0.19
113	PHM759.24a	G/A	1	225562216	0.25	0.37	0.05	0.31
114	PHM4942.12abc	A/G	1	226461786	0.44	0.49	0.06	0.37
115	PHM2478.22	G/A	1	226781494	0.04	0.08	0.00	0.08
116	PZA03001.15a	A/G	1	231037305	0.23	0.35	0.04	0.29
117	PZB01647.1	A/G	1	231039372	0.20	0.31	0.03	0.27
118	PZA00381.4	C/G	1	237639037	0.05	0.10	0.00	0.10
119	PHM297.18	T/A	1	239313773	0.04	0.08	0.00	0.08
120	PHM4926.16	C/G	1	240498919	0.17	0.28	0.03	0.24
121	PZA03301.2abc	A/G	1	240574247	0.22	0.34	0.02	0.29
122	PHM5293.11	C/A	1	243339423	0.04	0.08	0.00	0.08
123	PZA03064.6abc	A/G	1	248503300	0.49	0.49	0.08	0.38
124	PZA03404.1abc	A/G	1	249259484	0.44	0.49	0.03	0.37
125	PHM16605.19	G/A	1	251142538	0.02	0.04	0.00	0.04
126	PZA03578.1a	G/A	1	252219631	0.27	0.39	0.00	0.32
127	PZA02269.4abc	A/C	1	252721946	0.34	0.45	0.07	0.35
128	PZA02269.3abc	T/C	1	252722026	0.39	0.47	0.02	0.36
129	PHM3034.3	A/G	1	255553191	0.18	0.30	0.00	0.26
130	kip1.3abc	A/G	1	255577993	0.26	0.38	0.05	0.31
131	PHM14475.7abc	G/A	1	256245118	0.45	0.49	0.11	0.37
132	glb1.2a	A/T	1	257415499	0.36	0.46	0.09	0.35
133	PZA01588.1abc	G/A	1	258495441	0.46	0.49	0.07	0.37
134	PZA01921.20a	A/G	1	261313627	0.37	0.46	0.06	0.36
135	PZA01921.19	G/A	1	261313982	0.19	0.30	0.03	0.26
136	PZA02985.5ab	G/A	1	262339533	0.29	0.40	0.01	0.33
137	PZA03457.1	A/T	1	262715518	0.13	0.22	0.00	0.20
138	PZB00008.1abc	G/A	1	268371949	0.45	0.49	0.00	0.37
139	PZB00063.1a	C/A	1	268373631	0.48	0.49	0.02	0.37
140	PZA02278.1	G/A	1	270002291	0.13	0.23	0.02	0.20
141	PZA02698.3a	A/G	1	272318990	0.21	0.33	0.00	0.28
142	PZA00030.11a	T/A	1	275076662	0.34	0.44	0.04	0.35
143	PZB00114.1abc	A/G	1	275983631	0.40	0.47	0.05	0.36
144	PHM13362.3	G/A	1	275984322	0.07	0.13	0.03	0.12
145	PZA01978.23abc	A/G	1	276645374	0.28	0.40	0.03	0.32
146	PHM595.30	A/C	1	281818347	0.15	0.25	0.00	0.22
147	PZA03020.8a	A/T	1	282044048	0.31	0.42	0.31	0.34
148	PZA00978.1	A/T	1	282929327	0.14	0.24	0.00	0.21
149	PZA01246.1a	A/C	1	284043812	0.36	0.45	0.00	0.35
150	PZA02087.2	A/G	1	284056172	0.11	0.20	0.03	0.18

No	SNP name	SNP	Chr	Position	MAF	G.Div	Het	PIC
151	PZA00245.20a	A/G	1	284407240	0.22	0.34	0.00	0.29
152	PHM18705.23a	G/A	1	284564734	0.39	0.47	0.56	0.36
153	PZB01403.1a	A/C	1	285273845	0.41	0.48	0.00	0.37
154	PZA03037.2a	C/A	1	285977386	0.25	0.37	0.02	0.30
155	PZA03305.7	C/G	1	286642714	0.08	0.15	0.02	0.14
156	PZB01227.6a	A/T	1	288441343	0.28	0.40	0.00	0.32
157	PZA02044.1	A/G	1	290807362	0.13	0.22	0.02	0.20
158	PHM13191.6a	G/A	1	291461055	0.23	0.35	0.07	0.29
159	PZA00276.18	A/G	1	292092865	0.10	0.18	0.01	0.16
160	PZA00991.2	C/A	1	292560596	0.11	0.20	0.05	0.18
161	PZA00235.9	A/C	1	292728976	0.02	0.04	0.02	0.04
162	PZA00623.3	G/A	1	293632633	0.13	0.22	0.00	0.20
163	PZA02359.10abc	G/A	1	293907804	0.39	0.47	0.00	0.36
164	PHM9807.9	G/C	1	294311411	0.18	0.30	0.05	0.26
165	PZA01238.2a	G/A	1	294333956	0.38	0.46	0.00	0.36
166	PZA01238.1	G/A	1	294334019	0.09	0.16	0.00	0.15
167	PZA01068.1ab	A/G	1	294472123	0.22	0.34	0.08	0.28
168	PHM174.13	G/A	1	294904503	0.14	0.24	0.04	0.21
169	PHM1275.22abc	C/A	1	294904743	0.36	0.46	0.04	0.36
170	PHM673.33	G/A	1	295656485	0.07	0.14	0.00	0.13
171	PZA00856.2a	G/A	1	295785198	0.44	0.49	0.08	0.37
172	PHM7616.35a	G/A	1	298082533	0.21	0.33	0.08	0.28
173	PHM4752.14abc	G/A	1	298874066	0.42	0.48	0.06	0.37
174	PZA00680.3abc	C/A	2	1081791	0.28	0.40	0.07	0.32
175	PHM4951.8	A/G	2	1174438	0.10	0.18	0.03	0.17
176	PHM5817.15a	G/A	2	1220908	0.27	0.39	0.04	0.32
177	PZA00365.2abc	G/C	2	1221385	0.32	0.43	0.10	0.34
178	PZA02133.10ab	A/G	2	2483787	0.29	0.41	0.04	0.33
179	PHM1511.14abc	G/A	2	2483832	0.26	0.38	0.10	0.31
180	PHM13440.13abc	A/G	2	2527344	0.27	0.39	0.05	0.32
181	PZA02681.8	A/G	2	2795242	0.10	0.18	0.00	0.16
182	PZA00902.1	A/G	2	3069068	0.03	0.06	0.00	0.06
183	PHM4429.7a	G/A	2	3167148	0.34	0.45	0.64	0.35
184	PZA02264.5	G/A	2	3167435	0.20	0.32	0.05	0.27
185	PZA01211.1	A/G	2	3593488	0.05	0.10	0.00	0.10
186	PHM5535.8ab	G/A	2	4043425	0.26	0.38	0.00	0.31
187	PZA00172.12a	C/A	2	4177515	0.31	0.42	0.04	0.34
188	PZA00613.22a	A/G	2	4180789	0.41	0.48	0.11	0.37
189	PHM2601.5	G/A	2	4624088	0.07	0.14	0.00	0.13
190	PHM12952.13	A/G	2	4883487	0.15	0.25	0.01	0.22
191	PHM3334.6	A/G	2	5053798	0.03	0.06	0.00	0.06
192	PHM3334.4	G/A	2	5053894	0.10	0.17	0.00	0.16
193	PZA02081.1	G/A	2	5923120	0.03	0.06	0.00	0.06
194	PZA01935.10	G/A	2	6470320	0.15	0.26	0.04	0.22
195	PZA03747.1a	A/G	2	7473644	0.26	0.38	0.01	0.31
196	PHM12979.9	A/G	2	9037148	0.11	0.19	0.02	0.18
197	PZB00901.4abc	A/C	2	9401003	0.29	0.40	0.07	0.32
198	PZB00901.3ab	A/G	2	9401317	0.28	0.40	0.05	0.32
199	PZA02272.3abc	A/G	2	9917183	0.26	0.38	0.00	0.31
200	PHM14783.16	A/G	2	9939631	0.03	0.06	0.00	0.06
201	PHM5822.15ab	A/T	2	10429405	0.50	0.49	0.03	0.38
202	PZA00620.3ab	A/T	2	10429405	0.45	0.49	0.04	0.37
203	PHM482.23	A/G	2	11103489	0.07	0.12	0.00	0.12
204	zfl2.9a	C/A	2	12544790	0.21	0.32	0.05	0.27
205	PZA00108.4abc	T/A	2	13779970	0.30	0.42	0.07	0.33
206	PZA01753.1	C/A	2	14751136	0.16	0.27	0.01	0.24
207	PHM4425.25	A/G	2	19837545	0.10	0.17	0.02	0.16
208	PHM6111.5abc	G/A	2	21990814	0.35	0.45	0.00	0.35
209	PZA00590.1	A/G	2	21991241	0.10	0.17	0.00	0.16
210	PZA01755.1abc	T/A	2	25230561	0.46	0.49	0.03	0.37
211	PZA03568.1	G/A	2	27790383	0.11	0.20	0.05	0.18
212	PZA01374.1	G/A	2	28316042	0.18	0.29	0.08	0.25
213	PHM4586.12a	A/G	2	30113086	0.24	0.37	0.04	0.30
214	PZA01336.1	G/A	2	31394426	0.04	0.08	0.00	0.08
215	PZA02058.1a	A/T	2	32790351	0.37	0.46	0.43	0.36
216	PZA02378.7a	G/A	2	35040818	0.35	0.45	0.07	0.35
217	PZA02496.1abc	A/C	2	40814083	0.41	0.48	0.03	0.37
218	PHM10404.8a	C/G	2	40967991	0.49	0.50	0.18	0.38
219	PZB00183.4a	A/G	2	43923473	0.33	0.44	0.06	0.35
220	PZA02450.1a	C/A	2	47575949	0.35	0.45	0.06	0.35
221	PZA01820.1ab	G/A	2	48526239	0.28	0.40	0.07	0.32
222	PZA03629.1	C/G	2	51784790	0.11	0.19	0.01	0.18
223	PHM10321.11abc	G/C	2	62802971	0.32	0.43	0.05	0.34
224	PHM3457.6abc	A/T	2	62804122	0.29	0.41	0.06	0.33
225	PZA02549.3	A/G	2	103493947	0.13	0.22	0.04	0.20
226	PHM13360.13a	G/A	2	107146579	0.45	0.49	0.05	0.37
227	PHM3626.3ab	A/G	2	125642617	0.49	0.49	0.07	0.38
228	PHM4620.24	A/G	2	141942553	0.12	0.21	0.03	0.19
229	PHM13648.11a	G/A	2	144527713	0.39	0.47	0.06	0.36
230	PZA02626.1	C/G	2	144527921	0.19	0.31	0.05	0.26

No	SNP name	SNP	Chr	Position	MAF	G.Div	Het	PIC
231	PZA00029.17	C/A	2	144528184	0.10	0.17	0.02	0.16
232	PZA03211.6a	C/G	2	148837605	0.32	0.43	0.08	0.34
233	PZA01280.2a	G/A	2	149428645	0.41	0.48	0.08	0.37
234	PZA01537.2a	A/G	2	150984061	0.32	0.43	0.08	0.34
235	PZA01232.1	G/A	2	155868024	0.09	0.17	0.05	0.16
236	PZA02939.10	A/G	2	157152464	0.05	0.09	0.00	0.08
237	PHM1613.19a	C/A	2	158767674	0.27	0.39	0.10	0.32
238	PZA02465.1	C/G	2	161878465	0.08	0.14	0.00	0.13
239	PZA01321.1abc	A/G	2	164561951	0.25	0.37	0.05	0.30
240	PZA02371.6abc	A/C	2	165837357	0.44	0.49	0.06	0.37
241	PZA03692.1a	A/G	2	166659759	0.43	0.49	0.06	0.37
242	PHM635.23	C/A	2	167349862	0.02	0.05	0.00	0.04
243	PZA01638.1	G/A	2	168860136	0.03	0.05	0.01	0.05
244	PZA00515.10abc	G/A	2	169265278	0.41	0.48	0.10	0.37
245	PZA00637.6abc	A/G	2	169524491	0.49	0.49	0.11	0.38
246	PZA00495.5a	G/T	2	170377814	0.40	0.48	0.14	0.37
247	PZA03644.1abc	G/A	2	170899849	0.30	0.42	0.14	0.33
248	vdac1a.1abc	G/C	2	173767689	0.31	0.42	0.00	0.34
249	PZA03659.1abc	G/A	2	173848095	0.32	0.43	0.04	0.34
250	PZA00224.4a	C/A	2	176000581	0.36	0.46	0.08	0.36
251	PZA02890.4a	A/C	2	187225134	0.24	0.36	0.03	0.30
252	PHM5060.12a	A/G	2	190717664	0.25	0.37	0.05	0.31
253	PHM3598.20	A/T	2	193476369	0.06	0.12	0.00	0.11
254	PZA00824.2	A/G	2	193996904	0.17	0.28	0.00	0.25
255	PHM793.25abc	G/C	2	194696039	0.45	0.49	0.09	0.37
256	PHM3668.12abc	A/T	2	195555350	0.48	0.49	0.00	0.37
257	PHM7953.11a	G/A	2	195938696	0.34	0.44	0.12	0.35
258	PZA00803.3a	A/C	2	196001003	0.30	0.42	0.00	0.33
259	PZA02731.1a	A/G	2	197109533	0.27	0.39	0.14	0.32
260	PHM16125.47	C/G	2	199411414	0.08	0.14	0.00	0.13
261	PZB01103.2abc	A/G	2	200020987	0.36	0.45	0.00	0.35
262	PZA02077.1	G/A	2	206554635	0.15	0.25	0.00	0.22
263	PZA01885.2abc	G/A	2	206881202	0.43	0.48	0.09	0.37
264	PZA00390.7a	A/G	2	206974778	0.28	0.40	0.09	0.32
265	PZA02964.7a	A/G	2	208276510	0.23	0.35	0.08	0.29
266	PHM2094.34	A/G	2	208384148	0.02	0.04	0.00	0.04
267	PZB00772.7	G/A	2	208388467	0.15	0.25	0.02	0.22
268	PZA03602.1a	T/A	2	209504204	0.48	0.49	0.06	0.37
269	PZA02456.1	G/A	2	210924384	0.02	0.04	0.00	0.04
270	PZA00804.1	G/A	2	212078562	0.14	0.24	0.01	0.21
271	PZA02680.1	G/C	2	213668709	0.04	0.08	0.00	0.08
272	PZA02471.5	C/A	2	214640685	0.04	0.08	0.00	0.08
273	PZA02418.2abc	G/A	2	214647155	0.26	0.38	0.05	0.31
274	PZA00527.10abc	T/C	2	216833071	0.44	0.49	0.03	0.37
275	PHM499.19	G/A	2	217788377	0.15	0.25	0.05	0.22
276	PZA02012.7	G/A	2	218280503	0.20	0.32	0.03	0.27
277	PZA02453.1	A/G	2	219554914	0.16	0.27	0.05	0.24
278	PZA01991.3ab	A/G	2	220397345	0.42	0.48	0.03	0.37
279	PZA02564.2	A/G	2	220938475	0.10	0.18	0.03	0.17
280	PZB01013.1	C/A	2	222280720	0.09	0.15	0.02	0.14
281	PZA01895.1	C/A	2	225848026	0.03	0.06	0.00	0.06
282	PZA01352.5abc	A/G	2	226450168	0.24	0.36	0.06	0.30
283	PZA02170.1a	A/C	2	231190390	0.24	0.36	0.00	0.30
284	PHM3094.23	A/G	2	231709772	0.11	0.19	0.00	0.18
285	PZD00022.5abc	A/C	2	233128511	0.44	0.49	0.16	0.37
286	PZA03577.1ab	A/G	2	233876337	0.26	0.38	0.07	0.31
287	PZA03321.4	C/A	2	234574991	0.02	0.04	0.00	0.04
288	PHM343.7	G/A	3	1233789	0.02	0.04	0.00	0.04
289	PZA00309.1	A/G	3	1240310	0.11	0.20	0.00	0.18
290	PHM4259.5a	A/G	3	1699893	0.29	0.41	0.06	0.33
291	PZA02090.1abc	T/A	3	4138512	0.37	0.46	0.07	0.36
292	PZD00038.2a	G/A	3	4824781	0.25	0.37	0.05	0.30
293	PZA02678.1a	G/A	3	5232441	0.40	0.47	0.08	0.36
294	PZA00100.10	A/G	3	5480277	0.09	0.15	0.00	0.14
295	PZA03527.1a	A/G	3	5748473	0.37	0.46	0.05	0.36
296	PZA03212.3	C/G	3	6547051	0.12	0.20	0.02	0.19
297	PZA00749.1ab	C/G	3	6733476	0.30	0.42	0.04	0.33
298	PHM15475.27abc	C/G	3	7218902	0.27	0.39	0.00	0.32
299	PHM7672.7	C/A	3	9124219	0.05	0.10	0.00	0.09
300	PZA01765.1a	A/G	3	9598936	0.27	0.39	0.08	0.32
301	PZA00508.2abc	A/G	3	11895613	0.44	0.49	0.06	0.37
302	PHM4145.18	G/A	3	21098404	0.13	0.23	0.08	0.20
303	PHM2343.25a	G/A	3	27981649	0.47	0.49	0.15	0.37
304	PZA00210.9	G/A	3	29693390	0.05	0.09	0.00	0.09
305	PHM2766.14	G/C	3	31062080	0.03	0.06	0.00	0.06
306	PZA03054.5	A/G	3	31695534	0.02	0.04	0.00	0.04
307	PZA00348.11a	C/G	3	32449584	0.31	0.43	0.08	0.34
308	PZA02255.2	A/C	3	33226251	0.19	0.31	0.05	0.26
309	zb21.1abc	G/A	3	37536931	0.31	0.43	0.03	0.34
310	PZA00297.2a	G/C	3	39992968	0.35	0.45	0.15	0.35

No	SNP name	SNP	Chr	Position	MAF	G.Div	Het	PIC
311	PZA01114.2	A/C	3	40198671	0.19	0.30	0.08	0.26
312	PHM13473.12	A/G	3	42534072	0.01	0.02	0.00	0.02
313	PZA03070.9abc	C/A	3	43868043	0.44	0.49	0.15	0.37
314	PHM1960.37	G/A	3	45731544	0.17	0.28	0.15	0.25
315	PZA00581.3abc	G/A	3	48747913	0.40	0.47	0.00	0.36
316	PZA00279.2abc	G/A	3	52804070	0.39	0.47	0.03	0.36
317	PHM15474.5abc	A/G	3	53548967	0.49	0.49	0.06	0.38
318	PZA01447.1abc	G/A	3	53549251	0.34	0.45	0.13	0.35
319	PZA03119.1a	A/G	3	54127608	0.41	0.48	0.28	0.37
320	PZA00265.6abc	A/G	3	55946581	0.48	0.49	0.05	0.37
321	PZA02589.1abc	A/G	3	57360226	0.35	0.45	0.02	0.35
322	PHM12153.9	G/A	3	57612577	0.02	0.04	0.00	0.04
323	PZA00509.1	G/A	3	57966892	0.03	0.05	0.01	0.05
324	PZA02699.1abc	A/G	3	63847564	0.32	0.43	0.10	0.34
325	PHM5502.31a	A/G	3	67284067	0.46	0.49	0.08	0.37
326	PHM2439.25	A/G	3	69892464	0.02	0.04	0.00	0.04
327	PZA00109.4abc	T/A	3	82173052	0.26	0.38	0.03	0.31
328	PZA02742.1abc	G/C	3	97441783	0.46	0.49	0.08	0.37
329	PZA02299.16abc	G/A	3	103386170	0.34	0.45	0.07	0.35
330	PZA00707.9a	T/A	3	110715954	0.39	0.47	0.23	0.36
331	PZA02619.1	A/G	3	120046370	0.04	0.07	0.01	0.07
332	PZA03198.3abc	G/C	3	123785072	0.40	0.47	0.10	0.36
333	PHM15449.10abc	A/G	3	125077922	0.44	0.49	0.10	0.37
334	PZA00413.20abc	C/A	3	125192432	0.43	0.49	0.07	0.37
335	PZA00363.7abc	G/A	3	132192069	0.49	0.49	0.08	0.37
336	PZD00016.4	A/G	3	133480598	0.01	0.02	0.00	0.02
337	PZD00015.5	A/G	3	133481473	0.01	0.02	0.00	0.02
338	PZB02002.1	A/G	3	133483395	0.02	0.04	0.00	0.04
339	PHM1745.16abc	A/G	3	140079003	0.35	0.45	0.12	0.35
340	PHM4339.79	C/A	3	141920024	0.05	0.10	0.02	0.10
341	PZA00920.1abc	A/G	3	142821031	0.39	0.47	0.07	0.36
342	PHM4955.12abc	A/G	3	150256198	0.21	0.32	0.00	0.27
343	PZA00827.1	C/A	3	155566732	0.09	0.16	0.00	0.15
344	PZB02179.1a	A/G	3	157640380	0.35	0.45	0.08	0.35
345	PZA00828.2	A/T	3	157646430	0.02	0.04	0.00	0.04
346	PHM9914.11abc	A/G	3	159996138	0.39	0.47	0.07	0.36
347	PZA00667.2a	G/A	3	161516227	0.28	0.40	0.08	0.32
348	PZA01396.1abc	A/G	3	164833462	0.50	0.49	0.05	0.38
349	PZA00186.4a	G/A	3	165800340	0.41	0.48	0.17	0.37
350	PHM2885.31	G/A	3	165901548	0.13	0.22	0.00	0.20
351	PZD00027.2a	A/G	3	169757334	0.42	0.48	0.11	0.37
352	PZA02402.1abc	G/A	3	169771952	0.32	0.43	0.09	0.34
353	PZA03032.19	G/C	3	171364540	0.13	0.22	0.00	0.19
354	PZA00783.1	A/G	3	171821889	0.19	0.30	0.06	0.26
355	PZA02212.1abc	A/G	3	174550726	0.49	0.49	0.10	0.37
356	PHM17210.5abc	A/C	3	178229653	0.38	0.46	0.02	0.36
357	PZA01962.12abc	T/A	3	178229838	0.36	0.45	0.10	0.35
358	PZA02654.3	T/A	3	178773618	0.04	0.08	0.02	0.08
359	PHM1675.29a	A/G	3	180341088	0.22	0.34	0.09	0.29
360	PZA03735.1abc	A/G	3	180533618	0.50	0.49	0.07	0.38
361	PZA03191.1abc	G/A	3	185290072	0.22	0.33	0.00	0.28
362	PZA03191.4	G/A	3	185290390	0.14	0.23	0.03	0.21
363	zb27.1	G/C	3	185290956	0.04	0.08	0.00	0.08
364	PZA03647.1abc	C/A	3	185318331	0.46	0.49	0.06	0.37
365	PZA00494.2abc	A/G	3	186179390	0.23	0.35	0.06	0.29
366	PHM351.36	A/G	3	189553290	0.11	0.20	0.04	0.18
367	PHM351.40	A/G	3	189553357	0.10	0.17	0.01	0.16
368	PHM3075.15	A/G	3	189861169	0.01	0.02	0.00	0.02
369	PZA01228.2	A/T	3	189861328	0.03	0.05	0.01	0.05
370	PHM824.17	A/T	3	190451804	0.02	0.04	0.00	0.04
371	PZA03743.1	G/C	3	191863818	0.04	0.08	0.00	0.08
372	PZA03744.1	C/A	3	191864249	0.05	0.09	0.05	0.09
373	PHM13673.53ab	A/G	3	192236275	0.21	0.33	0.02	0.28
374	PZA00071.2a	C/G	3	194292587	0.24	0.36	0.00	0.30
375	PZB01109.1	G/A	3	194643731	0.20	0.32	0.03	0.27
376	PZA00308.24	A/C	3	196191461	0.02	0.04	0.00	0.04
377	PZA01457.1	C/G	3	196870325	0.08	0.15	0.00	0.14
378	PZA02122.9	G/A	3	197797720	0.10	0.17	0.02	0.16
379	PHM5716.60	G/A	3	198309265	0.10	0.17	0.00	0.16
380	PZA01501.1	G/A	3	199272204	0.08	0.14	0.00	0.13
381	PZA00892.5	G/A	3	199886201	0.11	0.19	0.04	0.18
382	PHM2919.23ab	C/A	3	199886409	0.27	0.39	0.05	0.32
383	PZA03154.4a	A/G	3	202364110	0.25	0.37	0.05	0.30
384	PZA03458.1	G/A	3	203318583	0.14	0.24	0.09	0.21
385	PZA00538.18ab	G/A	3	206889455	0.40	0.48	0.09	0.36
386	PZA02516.1	C/A	3	208180034	0.18	0.29	0.06	0.25
387	PHM3352.19ab	T/A	3	208711808	0.42	0.48	0.11	0.37
388	PHM3352.21ab	G/A	3	208711875	0.40	0.47	0.13	0.36
389	PZA02616.1abc	A/G	3	210161536	0.39	0.47	0.12	0.36
390	PHM13742.5	A/G	3	213606180	0.13	0.22	0.04	0.20

No	SNP name	SNP	Chr	Position	MAF	G.Div	Het	PIC
391	PHM1600.31	A/G	3	214887907	0.02	0.03	0.01	0.03
392	PZA01154.1abc	G/A	3	216028207	0.45	0.49	0.03	0.37
393	PHM3342.31a	G/A	3	216028374	0.21	0.33	0.00	0.28
394	PZA02665.2abc	A/C	3	216624570	0.43	0.49	0.17	0.37
395	PZA02514.1	C/A	3	216915474	0.02	0.04	0.00	0.04
396	PZA02824.4	A/G	3	217329672	0.13	0.23	0.00	0.20
397	PZA02668.2	T/A	3	218119861	0.04	0.08	0.00	0.08
398	PZA00219.7abc	G/A	3	219309085	0.39	0.47	0.09	0.36
399	PZA03391.1a	G/A	3	219859920	0.45	0.49	0.10	0.37
400	PHM15964.16a	A/G	3	221986592	0.39	0.47	0.08	0.36
401	PZA00316.10ab	G/C	3	223513639	0.22	0.33	0.00	0.28
402	PZA01688.3a	G/A	3	223670423	0.31	0.42	0.03	0.33
403	PZA01931.17a	A/C	3	227682081	0.28	0.40	0.16	0.32
404	PHM2423.33a	C/A	3	227682146	0.39	0.47	0.07	0.36
405	PZA00088.3a	C/G	3	228614270	0.32	0.43	0.06	0.34
406	PZA02423.1abc	A/C	3	229178560	0.28	0.40	0.02	0.32
407	PHM1971.20abc	G/C	4	2265349	0.42	0.48	0.13	0.37
408	PHM1184.26	A/G	4	2637083	0.07	0.13	0.01	0.13
409	PHM14235.15	A/G	4	3069114	0.08	0.14	0.00	0.13
410	PHM14235.16	G/A	4	3069135	0.12	0.21	0.00	0.19
411	PHM14235.17	A/G	4	3069171	0.11	0.19	0.00	0.18
412	PHM2438.28abc	A/G	4	3548007	0.37	0.46	0.00	0.36
413	PHM3963.33a	G/C	4	5459125	0.27	0.39	0.00	0.32
414	PHM3301.28	C/G	4	5659518	0.08	0.14	0.00	0.13
415	PZA00436.7abc	G/A	4	6401332	0.47	0.49	0.06	0.37
416	PZA02358.1abc	G/A	4	11329241	0.30	0.41	0.00	0.33
417	PHM2518.28	G/C	4	12618115	0.09	0.16	0.02	0.15
418	PZA01122.1	G/C	4	12618115	0.10	0.17	0.04	0.16
419	PHM16788.6ab	A/C	4	13581955	0.28	0.40	0.07	0.32
420	PHM2524.4	C/A	4	13585212	0.08	0.15	0.03	0.14
421	PHM259.7	A/G	4	14326036	0.03	0.06	0.00	0.06
422	PHM259.11	G/A	4	14326091	0.14	0.24	0.00	0.21
423	PHM8527.2	A/T	4	17307041	0.16	0.27	0.04	0.24
424	PZA01422.3	C/A	4	24475394	0.16	0.27	0.04	0.23
425	PHM2159.8	A/G	4	28981480	0.16	0.27	0.01	0.24
426	PZA02457.1a	G/A	4	29031200	0.43	0.49	0.05	0.37
427	PHM3112.9	G/C	4	32968646	0.07	0.12	0.00	0.12
428	PHM3112.5	G/A	4	32968749	0.06	0.11	0.01	0.10
429	PZA02705.1	C/A	4	33860915	0.05	0.09	0.00	0.09
430	PHM15427.11a	G/A	4	33862684	0.42	0.48	0.05	0.37
431	PHM13623.14	A/C	4	35051574	0.06	0.11	0.01	0.10
432	PZA01106.3	A/G	4	35051622	0.07	0.12	0.02	0.11
433	PHM5572.19abc	A/G	4	35384118	0.32	0.43	0.00	0.34
434	PZA01713.4abc	G/C	4	35384247	0.23	0.35	0.00	0.29
435	PZA00541.1	A/G	4	35594464	0.12	0.21	0.05	0.19
436	PZA03247.1a	G/C	4	35769506	0.26	0.38	0.10	0.31
437	PZA03385.1a	C/A	4	37067373	0.24	0.37	0.05	0.30
438	PZA01751.2a	T/A	4	37185517	0.23	0.35	0.03	0.29
439	PHM14717.2	G/A	4	40517665	0.10	0.18	0.02	0.16
440	PHM1283.14	A/C	4	41198528	0.10	0.18	0.03	0.17
441	PZA00445.22	T/A	4	49714016	0.12	0.21	0.00	0.19
442	PHM3587.6	G/A	4	59448994	0.16	0.27	0.03	0.24
443	PZA01759.1	C/A	4	60429503	0.18	0.29	0.03	0.25
444	PZA00726.10a	A/C	4	60768063	0.21	0.33	0.05	0.28
445	PHM14055.6	A/G	4	60768111	0.20	0.32	0.04	0.27
446	bt2.4	A/G	4	66288635	0.11	0.19	0.01	0.18
447	bt2.7a	G/A	4	66290994	0.28	0.40	0.01	0.32
448	PZA03254.1	A/G	4	73176932	0.07	0.13	0.03	0.12
449	PZA03587.1	G/A	4	73311260	0.07	0.12	0.02	0.11
450	PZA03597.1a	G/A	4	74239902	0.44	0.49	0.05	0.37
451	PZA03270.2	G/A	4	76122802	0.02	0.05	0.00	0.05
452	PZA00218.1abc	G/A	4	78946415	0.31	0.42	0.03	0.34
453	PZA03564.1abc	A/C	4	86797748	0.40	0.48	0.09	0.37
454	PZA03203.2abc	G/A	4	90203822	0.36	0.46	0.03	0.36
455	PZA00104.1	C/G	4	93812507	0.03	0.06	0.00	0.06
456	PZA03231.1	A/G	4	104160525	0.20	0.32	0.00	0.27
457	PZA03536.1	G/A	4	107751353	0.17	0.27	0.00	0.24
458	PZA03409.1a	A/C	4	128632208	0.43	0.48	0.07	0.37
459	PZA02027.1a	G/A	4	132975471	0.43	0.48	0.08	0.37
460	PZA03459.1	A/G	4	134292522	0.13	0.22	0.19	0.20
461	PHM1505.31a	G/A	4	143162745	0.38	0.46	0.06	0.36
462	PZA03152.3	A/G	4	147044805	0.08	0.14	0.01	0.13
463	PZA02982.7	G/A	4	148401277	0.08	0.14	0.00	0.13
464	PZA03317.1	G/A	4	149531093	0.03	0.06	0.00	0.06
465	PZA02992.15	G/A	4	149588842	0.07	0.12	0.00	0.11
466	PHM15864.8	A/G	4	150662732	0.05	0.10	0.00	0.10
467	PZA00057.2	G/A	4	155043539	0.14	0.24	0.00	0.21
468	PZA01926.1	G/A	4	158126191	0.18	0.29	0.04	0.25
469	PZA03116.1a	A/G	4	166002318	0.40	0.48	0.10	0.37
470	PZA00453.2ab	G/A	4	166281188	0.48	0.49	0.10	0.37

No	SNP name	SNP	Chr	Position	MAF	G.Div	Het	PIC
471	PZA01289.1	A/T	4	169608659	0.17	0.28	0.27	0.24
472	PHM3155.14abc	A/G	4	170467673	0.38	0.47	0.14	0.36
473	PZA00271.1	A/G	4	171613440	0.02	0.04	0.00	0.04
474	PZA01658.1	A/C	4	172269192	0.19	0.31	0.03	0.26
475	PZA01477.3abc	T/C	4	172301064	0.47	0.49	0.08	0.37
476	PZD00030.2	G/A	4	177976082	0.13	0.22	0.04	0.20
477	PHM3637.14a	C/A	4	179758341	0.22	0.35	0.29	0.29
478	PZA02194.1	G/A	4	180309373	0.07	0.13	0.02	0.13
479	PHM14618.11a	A/C	4	180315663	0.48	0.49	0.06	0.37
480	PZA02289.2	G/A	4	180316143	0.18	0.29	0.01	0.25
481	PZA01766.1a	A/G	4	183610048	0.48	0.49	0.08	0.37
482	PZA01954.1ab	G/C	4	184373943	0.42	0.49	0.44	0.37
483	PZA00941.2abc	C/G	4	185562016	0.25	0.37	0.05	0.31
484	PZB01461.1a	A/G	4	194092995	0.24	0.36	0.00	0.30
485	PZA00332.5	A/G	4	194749287	0.05	0.09	0.01	0.09
486	PHM4348.16abc	C/T	4	197459875	0.40	0.48	0.03	0.37
487	PZA00193.2a	A/G	4	197964459	0.36	0.46	0.00	0.36
488	PZA01790.1	C/G	4	202245921	0.08	0.14	0.00	0.13
489	PZA03205.1a	G/A	4	202883367	0.27	0.39	0.04	0.32
490	PZA01810.2abc	G/A	4	203767481	0.44	0.49	0.06	0.37
491	PZA02421.1abc	A/C	4	205427981	0.39	0.47	0.03	0.36
492	PZA02614.2a	A/G	4	206188976	0.50	0.49	0.03	0.38
493	PZA02779.1abc	A/G	4	207114208	0.38	0.47	0.00	0.36
494	PHM1684.20	A/T	4	209047447	0.18	0.29	0.02	0.25
495	PHM4117.14abc	A/C	4	215393158	0.43	0.49	0.06	0.37
496	PZA03081.1	C/G	4	215393350	0.09	0.16	0.00	0.15
497	PZB01021.1a	A/G	4	216310538	0.27	0.39	0.02	0.32
498	PZA02479.1ab	G/A	4	218367682	0.44	0.49	0.03	0.37
499	PZA00155.1	G/A	4	220885121	0.06	0.10	0.00	0.10
500	PZA03155.3	A/G	4	222233297	0.07	0.12	0.00	0.12
501	PZA00878.2abc	G/C	4	226223649	0.30	0.41	0.05	0.33
502	PZA00636.7abc	A/G	4	226884613	0.43	0.48	0.14	0.37
503	PZA00399.11abc	G/A	4	229644826	0.33	0.44	0.06	0.34
504	PZA00521.3	C/A	4	234783765	0.09	0.16	0.00	0.15
505	PZA00694.6	A/G	4	235779015	0.08	0.14	0.00	0.13
506	PZA02155.1	G/A	4	237336600	0.10	0.18	0.02	0.16
507	PHM5780.15	A/G	4	237583793	0.04	0.07	0.01	0.07
508	PZA00513.1	G/A	4	238553534	0.01	0.02	0.00	0.02
509	PHM5599.20abc	G/A	4	239237605	0.31	0.42	0.06	0.34
510	PZA02585.2a	T/A	4	239453847	0.48	0.49	0.00	0.37
511	PHM18386.29a	G/A	4	240105203	0.30	0.42	0.07	0.33
512	PZA00529.4abc	G/A	4	240769260	0.26	0.38	0.06	0.31
513	PZA00936.1a	G/A	4	241050436	0.40	0.47	0.08	0.36
514	PZA03322.5abc	G/C	4	242019440	0.44	0.49	0.00	0.37
515	PZA03322.3	A/T	4	242019760	0.15	0.26	0.06	0.23
516	PHM4125.11a	C/G	4	242313290	0.29	0.40	0.03	0.33
517	PZA01905.12abc	G/A	4	244087450	0.32	0.43	0.11	0.34
518	PHM4310.112abc	A/C	4	244087971	0.27	0.39	0.00	0.32
519	PZA02239.12	A/G	4	244721608	0.19	0.31	0.02	0.26
520	PZA00682.17	G/A	4	244992721	0.01	0.02	0.00	0.02
521	PZA00282.19	C/G	4	245123514	0.15	0.25	0.02	0.22
522	PZA01887.1	G/A	5	656148	0.15	0.26	0.01	0.23
523	PZA02367.1	A/G	5	862416	0.13	0.22	0.02	0.19
524	PZA01983.1abc	C/A	5	1037243	0.39	0.47	0.01	0.36
525	PHM662.27a	A/C	5	1046780	0.37	0.46	0.05	0.36
526	PHM3061.315	T/A	5	1621791	0.10	0.17	0.00	0.16
527	PZA00191.5abc	G/A	5	2123914	0.42	0.48	0.00	0.37
528	PZA01438.1	A/T	5	2690742	0.14	0.24	0.03	0.21
529	PHM3714.12	G/A	5	2930514	0.05	0.09	0.00	0.08
530	PHM5359.10a	G/A	5	3376550	0.30	0.42	0.08	0.33
531	PZA01570.1abc	G/A	5	3533995	0.22	0.34	0.00	0.29
532	PHM13122.43	C/G	5	4190804	0.07	0.12	0.09	0.12
533	PHM3762.18abc	G/A	5	5917407	0.34	0.45	0.00	0.35
534	PZA02653.12	A/G	5	5917587	0.10	0.18	0.00	0.16
535	PHM789.16	A/G	5	6784311	0.10	0.17	0.02	0.16
536	PZA02462.1abc	C/A	5	6820571	0.45	0.49	0.07	0.37
537	PHM15223.38	T/A	5	6961253	0.06	0.12	0.00	0.11
538	PZB00054.3	G/C	5	7086096	0.09	0.16	0.00	0.15
539	PHM3137.17	G/A	5	7487050	0.14	0.24	0.03	0.21
540	PZA03036.6a	C/G	5	7656748	0.40	0.47	0.09	0.36
541	PZB00094.1a	G/C	5	7946002	0.35	0.45	0.05	0.35
542	PZA02029.21	A/G	5	7956551	0.04	0.08	0.00	0.08
543	PHM533.46ab	A/G	5	7956824	0.34	0.44	0.10	0.35
544	PZA01371.1a	G/A	5	8220010	0.38	0.47	0.02	0.36
545	PZA01925.1	G/A	5	8598835	0.17	0.28	0.00	0.24
546	PZA00865.1	G/A	5	9279777	0.19	0.30	0.05	0.26
547	PZB00079.4	A/G	5	11737636	0.07	0.13	0.03	0.12
548	PZA01284.6	A/T	5	11990631	0.08	0.14	0.01	0.13
549	PZA03092.7abc	G/A	5	11992705	0.32	0.43	0.02	0.34
550	PZA01327.1abc	A/G	5	15056528	0.22	0.34	0.00	0.28

No	SNP name	SNP	Chr	Position	MAF	G.Div	Het	PIC
551	PZA01523.1	A/G	5	16208240	0.03	0.06	0.02	0.06
552	PZA00517.7	A/G	5	18240488	0.01	0.02	0.00	0.02
553	PZA03226.3a	C/A	5	20089946	0.23	0.35	0.00	0.29
554	PZA03340.2	G/C	5	20201009	0.12	0.21	0.01	0.19
555	PHM5484.22a	C/A	5	21449633	0.45	0.49	0.05	0.37
556	PHM13942.9abc	A/G	5	21461663	0.34	0.44	0.03	0.35
557	PHM13942.8abc	A/G	5	21461728	0.35	0.45	0.03	0.35
558	PHM13942.7abc	G/A	5	21461779	0.33	0.44	0.03	0.35
559	PZA02792.25ab	C/A	5	21771015	0.24	0.37	0.06	0.30
560	PZA01427.1a	C/A	5	23135578	0.32	0.43	0.01	0.34
561	PHM565.31abc	A/G	5	24242002	0.39	0.47	0.08	0.36
562	PZA02113.1	C/G	5	30239292	0.19	0.30	0.01	0.26
563	PZA00801.1	G/C	5	31528260	0.17	0.28	0.00	0.24
564	PZB00869.4a	A/G	5	32366193	0.32	0.43	0.04	0.34
565	PZA01563.1	C/A	5	32599139	0.03	0.06	0.00	0.06
566	PHM4647.8a	C/G	5	32599447	0.31	0.43	0.09	0.34
567	PHM16854.3abc	C/G	5	34587029	0.26	0.38	0.07	0.31
568	PZA00981.3a	G/A	5	37030384	0.25	0.37	0.02	0.30
569	PZA00996.1abc	A/G	5	37788653	0.29	0.41	0.04	0.33
570	PHM3402.11	A/G	5	47757634	0.09	0.16	0.00	0.15
571	PZA02207.1abc	G/A	5	49203492	0.40	0.47	0.02	0.36
572	PZA00805.1	A/T	5	57507670	0.09	0.17	0.06	0.16
573	PHM2769.43abc	A/G	5	57831576	0.35	0.45	0.06	0.35
574	PZA00522.12a	G/A	5	57933548	0.25	0.37	0.00	0.31
575	PZA00222.7ab	A/G	5	58576757	0.26	0.38	0.12	0.31
576	PHM2614.14	G/A	5	58734149	0.06	0.11	0.01	0.10
577	PHM1870.20abc	A/G	5	59299993	0.48	0.49	0.02	0.37
578	PZA02676.2abc	A/G	5	60072336	0.43	0.49	0.07	0.37
579	PZB01115.3abc	G/A	5	60964334	0.26	0.38	0.04	0.31
580	PZA01050.1abc	G/A	5	63415425	0.25	0.37	0.04	0.30
581	PHM4165.14abc	A/T	5	65741535	0.30	0.42	0.00	0.33
582	PHM13675.17	T/A	5	66811586	0.08	0.14	0.02	0.13
583	PHM13675.18	T/A	5	66811651	0.08	0.15	0.02	0.14
584	PZB01112.1	G/C	5	68419652	0.05	0.10	0.00	0.09
585	PZA01349.2	G/A	5	69319346	0.07	0.14	0.00	0.13
586	PZA03677.1abc	A/G	5	71093631	0.46	0.49	0.12	0.37
587	PHM5798.39abc	G/A	5	71095193	0.37	0.46	0.07	0.36
588	PZA01303.1	C/A	5	72408251	0.02	0.04	0.00	0.04
589	PZA02818.6	A/G	5	77679565	0.15	0.25	0.06	0.22
590	PZA01779.1	G/A	5	81227637	0.07	0.13	0.01	0.12
591	PZA00273.5	C/A	5	83357444	0.18	0.30	0.05	0.26
592	PZA01693.1a	A/T	5	83544273	0.30	0.41	0.04	0.33
593	PZA03049.24	A/C	5	88604977	0.03	0.06	0.00	0.06
594	PZA00643.13	G/T	5	91096945	0.20	0.31	0.00	0.27
595	PZA02164.16a	G/A	5	112179855	0.43	0.49	0.04	0.37
596	PZA01608.1	A/G	5	158599491	0.20	0.32	0.05	0.27
597	PZA02981.2abc	A/T	5	166468431	0.24	0.36	0.00	0.30
598	ae1.7abc	G/A	5	167873309	0.49	0.49	0.04	0.37
599	ae1.8	G/A	5	167884630	0.09	0.16	0.00	0.15
600	PZA01763.2	A/G	5	168080654	0.06	0.10	0.00	0.10
601	PZA02641.2	C/A	5	168924728	0.08	0.15	0.00	0.14
602	PZA00255.14	A/G	5	169719641	0.09	0.16	0.00	0.15
603	PHM6910.187abc	C/A	5	169853895	0.33	0.43	0.00	0.34
604	PZA02040.2abc	G/A	5	171389174	0.37	0.46	0.07	0.36
605	PZA00300.14abc	A/G	5	171688876	0.28	0.40	0.02	0.32
606	PZA00987.1	A/G	5	172230823	0.13	0.22	0.00	0.20
607	PZA01410.1	G/A	5	172682963	0.09	0.16	0.04	0.15
608	PHM13696.9a	A/G	5	175367030	0.32	0.43	0.08	0.34
609	PHM13696.11a	G/A	5	175367080	0.28	0.40	0.03	0.32
610	PZA03714.1	G/A	5	175463023	0.17	0.28	0.00	0.25
611	PZA01304.1	G/A	5	178584058	0.05	0.10	0.00	0.10
612	PHM1899.157abc	A/G	5	179060561	0.35	0.45	0.08	0.35
613	PZA02633.4a	G/A	5	179953106	0.36	0.46	0.03	0.35
614	PZA02408.2ab	G/A	5	180413515	0.21	0.32	0.02	0.27
615	PZA02209.2abc	A/C	5	180428220	0.39	0.47	0.09	0.36
616	PZA02356.7abc	A/C	5	180603220	0.37	0.46	0.04	0.36
617	PHM5296.6ab	A/G	5	180603557	0.39	0.47	0.08	0.36
618	PHM5337.18	G/A	5	180611413	0.07	0.12	0.00	0.11
619	PZA03324.1	A/T	5	181256363	0.03	0.06	0.00	0.06
620	PZA01575.1	G/A	5	182337304	0.01	0.02	0.00	0.02
621	PZA03452.6	A/G	5	182823983	0.13	0.22	0.03	0.20
622	PHM6386.11	G/A	5	190026183	0.18	0.29	0.02	0.25
623	PHM7908.20	A/C	5	191075278	0.18	0.29	0.05	0.25
624	PHM7908.25a	C/A	5	191075472	0.23	0.35	0.07	0.29
625	PZA00352.23abc	G/A	5	191075557	0.36	0.45	0.04	0.35
626	PZA02426.1	G/A	5	191642443	0.02	0.04	0.00	0.04
627	PZA02411.3a	G/A	5	192326110	0.28	0.40	0.02	0.32
628	PHM532.23	A/C	5	193208321	0.15	0.25	0.00	0.22
629	PHM4616.8	A/G	5	197018389	0.05	0.10	0.00	0.10
630	PZA03024.16abc	G/A	5	198534153	0.29	0.41	0.01	0.33

No	SNP name	SNP	Chr	Position	MAF	G.Div	Het	PIC
631	PZA01142.4a	G/A	5	199697937	0.25	0.37	0.05	0.30
632	PZA02820.17	A/G	5	200813183	0.10	0.18	0.00	0.16
633	PZA01265.1	G/A	5	201139510	0.05	0.10	0.00	0.10
634	PZB00765.1abc	C/G	5	202174585	0.35	0.45	0.06	0.35
635	PZA02060.1abc	G/A	5	202342720	0.37	0.46	0.00	0.36
636	PZA02667.1	A/G	5	202777978	0.02	0.04	0.00	0.04
637	PZA00395.2abc	C/A	5	202847197	0.46	0.49	0.07	0.37
638	PHM3512.186a	A/G	5	203434263	0.42	0.48	0.01	0.37
639	PZA00980.1ab	G/A	5	203778507	0.38	0.46	0.03	0.36
640	PZA02068.1	A/G	5	204198466	0.20	0.31	0.02	0.27
641	PHM563.9	A/G	5	204993639	0.13	0.22	0.01	0.20
642	PHM4349.3	G/A	5	206329214	0.02	0.04	0.00	0.04
643	PZA02099.3	A/G	5	206329295	0.07	0.13	0.00	0.12
644	PZA00545.26	G/C	5	206665095	0.14	0.24	0.02	0.21
645	PZA00963.3abc	G/A	5	207273479	0.30	0.42	0.01	0.33
646	PZA02015.11	A/G	5	207464707	0.20	0.31	0.04	0.27
647	PZA03167.5abc	A/G	5	207603495	0.27	0.39	0.06	0.32
648	PZA01680.3	G/A	5	208901002	0.07	0.13	0.04	0.12
649	PZA03339.2abc	A/C	5	210890886	0.26	0.38	0.08	0.31
650	PZA02390.1	G/A	5	211884165	0.13	0.22	0.05	0.19
651	PHM3612.19	C/A	5	212483471	0.07	0.13	0.00	0.12
652	PZA02480.1abc	G/A	5	214953055	0.22	0.33	0.03	0.28
653	PHM13639.13	A/G	5	214953143	0.12	0.21	0.03	0.19
654	PZA02769.1abc	T/A	5	215505432	0.47	0.49	0.02	0.37
655	PZA00606.4	C/A	6	2379238	0.14	0.23	0.00	0.21
656	PHM15961.13a	G/C	6	9561803	0.34	0.44	0.01	0.35
657	PZA03047.12a	G/A	6	20837504	0.28	0.40	0.00	0.32
658	PZA00440.15a	A/G	6	22403926	0.23	0.35	0.05	0.29
659	PZA00440.1abc	C/G	6	22404308	0.27	0.39	0.03	0.32
660	PZA02948.24	A/G	6	25035450	0.10	0.18	0.00	0.17
661	PZA01901.1	G/A	6	30864632	0.04	0.08	0.02	0.08
662	PZA03120.1a	C/A	6	57774238	0.38	0.46	0.02	0.36
663	PZA00355.2a	G/C	6	78756133	0.33	0.44	0.09	0.34
664	PZA00427.3	A/T	6	79815961	0.17	0.28	0.03	0.25
665	PHM14522.5a	A/G	6	79816260	0.44	0.49	0.03	0.37
666	PZA00543.12a	A/C	6	80534129	0.35	0.45	0.03	0.35
667	PZA03069.4abc	A/G	6	81808364	0.47	0.49	0.03	0.37
668	PZA03069.8a	G/A	6	81808446	0.48	0.49	0.02	0.37
669	PZB01009.1a	A/G	6	84664840	0.43	0.48	0.08	0.37
670	PZB01009.2abc	A/C	6	84665035	0.24	0.36	0.15	0.30
671	PHM2551.31a	A/G	6	85125455	0.45	0.49	0.06	0.37
672	PHM12904.7	G/A	6	88691499	0.12	0.21	0.02	0.19
673	PZA00214.1a	T/A	6	91704092	0.45	0.49	0.10	0.37
674	PHM8909.12abc	G/A	6	91883155	0.27	0.39	0.06	0.32
675	PZA03488.1	G/A	6	95789142	0.05	0.10	0.00	0.10
676	PZA00942.2abc	A/C	6	102566000	0.22	0.34	0.00	0.28
677	PZB01658.1abc	T/A	6	102953833	0.41	0.48	0.00	0.37
678	PZA01029.1abc	G/A	6	114031392	0.33	0.44	0.02	0.34
679	PZA01589.2	G/A	6	117238344	0.08	0.15	0.01	0.14
680	PZA00382.17	A/G	6	118741714	0.18	0.29	0.00	0.25
681	PHM13451.15	A/G	6	119239746	0.09	0.16	0.01	0.15
682	PZA01055.1	G/A	6	120059553	0.02	0.04	0.00	0.04
683	lac1.3abc	A/G	6	120230802	0.26	0.38	0.03	0.31
684	PHM1190.3	A/G	6	120234344	0.16	0.26	0.00	0.23
685	PZA00571.1	G/A	6	120679943	0.17	0.28	0.14	0.24
686	PZA01729.1	G/A	6	123998706	0.16	0.27	0.03	0.24
687	PHM13020.10a	A/G	6	124020185	0.22	0.34	0.00	0.28
688	PZA00473.5abc	A/G	6	124407006	0.43	0.49	0.00	0.37
689	PZA01591.1abc	G/A	6	125113941	0.25	0.37	0.02	0.31
690	PHM4904.16	T/A	6	125725871	0.07	0.13	0.05	0.12
691	PHM12794.48	G/A	6	128476428	0.19	0.31	0.00	0.26
692	PZA01618.2abc	A/G	6	129927781	0.48	0.49	0.16	0.37
693	PZB00414.2a	G/A	6	131405570	0.23	0.35	0.02	0.29
694	PZA01884.1abc	A/G	6	132316835	0.45	0.49	0.01	0.37
695	PHM3438.2	G/A	6	133843693	0.02	0.04	0.00	0.04
696	PZA02328.5a	C/G	6	137254358	0.29	0.41	0.00	0.33
697	PZA01072.1	C/A	6	137479485	0.15	0.26	0.03	0.23
698	PZA02187.2	A/T	6	139105889	0.02	0.04	0.00	0.04
699	PZA02187.1ab	A/C	6	139106115	0.42	0.48	0.07	0.37
700	PZA02478.7abc	A/G	6	141111650	0.35	0.45	0.04	0.35
701	PZA02673.1a	G/A	6	142648706	0.48	0.49	0.11	0.37
702	PZA02148.1a	G/A	6	143875945	0.34	0.44	0.01	0.35
703	PZB00942.1	A/G	6	144744222	0.17	0.28	0.07	0.24
704	PZB01308.1	A/G	6	144744486	0.10	0.18	0.02	0.16
705	PZA02247.1abc	A/G	6	146544292	0.22	0.34	0.02	0.29
706	PHM11985.27a	A/G	6	147389036	0.39	0.47	0.00	0.36
707	PZA01342.2	G/A	6	148798630	0.09	0.16	0.00	0.14
708	PZA02436.1abc	C/G	6	149251173	0.43	0.49	0.05	0.37
709	PZA02472.2	A/C	6	149648334	0.05	0.09	0.03	0.09
710	PHM15251.5a	A/T	6	153123234	0.30	0.42	0.11	0.33

No	SNP name	SNP	Chr	Position	MAF	G.Div	Het	PIC
711	PHM15251.3abc	A/G	6	153123339	0.27	0.39	0.04	0.32
712	PZA01672.1abc	G/A	6	154495634	0.44	0.49	0.02	0.37
713	PHM4662.153	A/G	6	155390787	0.17	0.28	0.12	0.25
714	PZA03027.12	A/G	6	156645239	0.17	0.28	0.01	0.24
715	PHM5794.13abc	G/A	6	156740198	0.45	0.49	0.08	0.37
716	PZA00223.4abc	G/A	6	156740239	0.46	0.49	0.13	0.37
717	PHM597.18a	A/G	6	157943848	0.35	0.45	0.09	0.35
718	PHM597.12	A/G	6	157944068	0.18	0.30	0.07	0.26
719	PHM16607.11a	A/G	6	160156483	0.22	0.34	0.08	0.29
720	PZB01569.7	T/A	6	160749024	0.05	0.10	0.00	0.09
721	PHM4503.25a	C/A	6	161137975	0.38	0.47	0.03	0.36
722	PZA00266.7	A/G	6	161570136	0.20	0.32	0.03	0.27
723	PZA01468.1abc	G/A	6	161663671	0.35	0.45	0.09	0.35
724	PHM2108.61	G/C	6	161663814	0.03	0.06	0.00	0.06
725	PHM3056.28	T/A	6	162996794	0.06	0.12	0.02	0.11
726	PHM7922.8	C/A	6	163120296	0.18	0.29	0.04	0.25
727	PZA00889.2abc	A/C	6	163201925	0.39	0.47	0.05	0.36
728	PZA00821.1	C/A	6	163933232	0.05	0.10	0.00	0.10
729	PZA02688.2abc	A/G	6	164081101	0.27	0.39	0.04	0.32
730	PZB01222.1	C/G	6	164419435	0.16	0.27	0.04	0.23
731	PHM2658.129abc	A/G	6	164999578	0.45	0.49	0.11	0.37
732	PHM5361.13	G/A	6	166687965	0.06	0.12	0.00	0.11
733	PZA00910.1a	A/G	6	166688213	0.34	0.44	0.06	0.35
734	PHM5529.7	C/A	6	167120728	0.12	0.21	0.00	0.19
735	PHM5529.4	C/A	6	167120845	0.09	0.16	0.00	0.15
736	PHM3466.69	A/G	6	167148384	0.10	0.19	0.05	0.17
737	PHM4468.13abc	A/G	6	167527305	0.47	0.49	0.10	0.37
738	PZA02815.25abc	A/G	6	167883450	0.22	0.34	0.03	0.29
739	PZA02035.5	G/A	7	2585198	0.02	0.04	0.00	0.04
740	PZA01909.2	A/G	7	6437808	0.11	0.19	0.06	0.17
741	PHM4135.15	G/C	7	6437899	0.20	0.32	0.03	0.27
742	PHM2691.32a	C/A	7	7947901	0.25	0.37	0.07	0.30
743	PHM2691.31ab	G/A	7	7947949	0.31	0.42	0.07	0.33
744	PZA02872.1abc	A/G	7	13058813	0.28	0.40	0.04	0.32
745	PZA00256.27abc	G/A	7	17397414	0.23	0.35	0.05	0.29
746	PHM4080.15abc	A/G	7	20240404	0.32	0.43	0.09	0.34
747	PZA03344.2	G/A	7	22002659	0.18	0.30	0.01	0.25
748	PHM4285.20	G/C	7	29199495	0.08	0.14	0.00	0.13
749	PHM15501.9	A/G	7	32876504	0.19	0.31	0.03	0.26
750	PZA00132.17	A/C	7	33979080	0.07	0.13	0.00	0.12
751	PHM4353.31abc	A/C	7	36392983	0.29	0.41	0.03	0.33
752	PZA03687.1a	A/G	7	40409237	0.21	0.33	0.02	0.28
753	PZA00084.2	C/T	7	43948264	0.15	0.25	0.00	0.22
754	PZA01230.1	G/A	7	46238189	0.09	0.15	0.00	0.14
755	PZA02612.1a	A/C	7	48612205	0.30	0.42	0.09	0.33
756	PZA03363.1abc	A/G	7	49538583	0.39	0.47	0.03	0.36
757	PZA01445.1	C/A	7	53160767	0.09	0.16	0.04	0.14
758	PZA01607.1abc	G/C	7	68051112	0.44	0.49	0.09	0.37
759	PHM4818.15a	G/C	7	68051404	0.33	0.43	0.07	0.34
760	PHM904.21abc	G/C	7	69232215	0.30	0.42	0.02	0.33
761	PZA00418.2abc	G/A	7	71717601	0.47	0.49	0.04	0.37
762	PHM12830.14a	G/A	7	71717792	0.47	0.49	0.13	0.37
763	PZA03645.1abc	T/C	7	73892322	0.45	0.49	0.01	0.37
764	PZA01210.2	G/A	7	75098939	0.02	0.04	0.00	0.04
765	PZA01210.1a	G/A	7	75099046	0.40	0.47	0.03	0.36
766	PZA02018.1	C/A	7	86401923	0.18	0.29	0.05	0.25
767	PZA01933.3a	A/G	7	98070498	0.26	0.38	0.07	0.31
768	PZA00616.13	C/G	7	122626201	0.10	0.18	0.15	0.17
769	PZA01690.7	G/A	7	123122705	0.08	0.14	0.00	0.13
770	PZA01946.7abc	C/A	7	123601837	0.30	0.41	0.01	0.33
771	PZA00986.1	G/A	7	123609597	0.08	0.14	0.03	0.13
772	PZA02352.1	C/G	7	124598905	0.12	0.21	0.02	0.19
773	PZA02643.1	C/A	7	128365318	0.08	0.14	0.00	0.13
774	PZA03583.1a	G/A	7	128404558	0.38	0.47	0.09	0.36
775	PZA01542.1abc	G/A	7	129793873	0.25	0.37	0.09	0.31
776	PZA02449.13	C/A	7	134847920	0.19	0.30	0.00	0.26
777	PZA03728.1	A/G	7	137207838	0.03	0.07	0.00	0.06
778	PZA03166.1abc	A/G	7	137632654	0.22	0.34	0.06	0.29
779	PZA02854.13a	G/A	7	137834376	0.47	0.49	0.08	0.37
780	PHM9162.135abc	A/G	7	137834663	0.37	0.46	0.00	0.36
781	PZA00405.7	G/A	7	138551298	0.15	0.25	0.04	0.22
782	PZA00405.6abc	G/C	7	138551416	0.35	0.45	0.09	0.35
783	PZA02722.1	A/G	7	143285700	0.09	0.16	0.00	0.15
784	PZA02386.2	A/G	7	154594107	0.06	0.11	0.08	0.11
785	PHM1912.23a	G/C	7	155970264	0.23	0.35	0.07	0.29
786	PHM1912.20a	G/A	7	155970323	0.21	0.33	0.08	0.28
787	PHM424.13	A/G	7	156125140	0.15	0.25	0.03	0.22
788	PZA00795.1	G/A	7	159417489	0.17	0.28	0.02	0.24
789	PHM3435.6	G/A	7	160099445	0.09	0.15	0.00	0.14
790	PZA02373.1abc	A/G	7	161618509	0.39	0.47	0.05	0.36

No	SNP name	SNP	Chr	Position	MAF	G.Div	Het	PIC
791	PHM7898.10a	A/C	7	161993743	0.34	0.45	0.10	0.35
792	PHM10225.15	C/G	7	162173638	0.18	0.29	0.03	0.25
793	PZA01533.2a	A/G	7	162381818	0.38	0.46	0.03	0.36
794	PZA00505.6	G/A	7	162531284	0.06	0.10	0.00	0.10
795	PZA01414.1a	A/G	7	163380515	0.28	0.40	0.06	0.32
796	PZA01028.2	A/G	7	164853203	0.06	0.12	0.00	0.11
797	PZA00695.3abc	A/G	7	165518207	0.34	0.44	0.04	0.35
798	PZB00605.1	A/G	7	165995045	0.06	0.11	0.01	0.11
799	PZA01744.1a	G/C	7	168991227	0.25	0.37	0.07	0.30
800	PZA00424.1	A/G	7	169017514	0.14	0.24	0.03	0.21
801	PZA01044.1abc	G/A	7	169409684	0.48	0.49	0.06	0.37
802	PZA02274.1	G/C	7	169955290	0.03	0.06	0.00	0.06
803	PZA02388.1a	G/A	8	169137	0.46	0.49	0.06	0.37
804	PHM5218.14	A/G	8	1876323	0.06	0.12	0.00	0.11
805	PZA02174.2abc	G/T	8	4101256	0.49	0.49	0.02	0.37
806	PZA01623.3abc	A/G	8	5112203	0.34	0.44	0.06	0.35
807	PHM9126.15abc	A/G	8	5632196	0.27	0.39	0.00	0.32
808	PZA00368.1a	C/G	8	5632308	0.45	0.49	0.09	0.37
809	PZA00058.1abc	A/G	8	5966657	0.46	0.49	0.07	0.37
810	PHM2487.6abc	A/G	8	8236274	0.44	0.49	0.09	0.37
811	PZA01601.1abc	A/C	8	8404207	0.31	0.42	0.11	0.34
812	PHM5637.15	A/G	8	11620073	0.14	0.24	0.01	0.22
813	PZA01691.1a	A/G	8	11620274	0.32	0.43	0.03	0.34
814	PHM9695.8a	G/A	8	12401723	0.33	0.43	0.00	0.34
815	PZA01079.1	G/A	8	14123731	0.07	0.14	0.10	0.13
816	PZA02955.3a	A/C	8	14721554	0.22	0.34	0.00	0.29
817	PHM5158.13	C/A	8	18455269	0.16	0.27	0.00	0.24
818	PZA02454.2	A/G	8	18466403	0.19	0.30	0.02	0.26
819	PHM1978.111a	G/A	8	21859513	0.27	0.39	0.00	0.32
820	PZA01186.1a	A/G	8	22423379	0.27	0.39	0.00	0.32
821	PHM2350.17abc	C/A	8	23985819	0.42	0.48	0.00	0.37
822	PZB00145.2a	A/G	8	44553828	0.30	0.41	0.08	0.33
823	PZA00498.5abc	C/A	8	48775713	0.38	0.47	0.06	0.36
824	PZA01209.1a	A/C	8	52678845	0.39	0.47	0.05	0.36
825	PHM3856.10	A/G	8	55008368	0.15	0.25	0.03	0.22
826	PZA02203.1abc	C/A	8	55008669	0.35	0.45	0.06	0.35
827	PHM4968.10	G/C	8	63529968	0.10	0.18	0.11	0.16
828	PZA00793.2a	C/A	8	64421988	0.46	0.49	0.08	0.37
829	PZA00379.2a	G/A	8	65657055	0.48	0.49	0.06	0.37
830	PHM4552.6	C/A	8	67931578	0.20	0.32	0.06	0.27
831	PZA00717.15a	A/C	8	68476189	0.36	0.45	0.02	0.35
832	PZA01257.1abc	G/A	8	68789720	0.27	0.39	0.08	0.31
833	PHM11114.7a	A/G	8	70899841	0.46	0.49	0.11	0.37
834	PZA01470.1	G/A	8	72801790	0.11	0.20	0.03	0.18
835	PZA02522.1	C/G	8	82710229	0.03	0.06	0.00	0.06
836	PZA01297.1	A/C	8	82856261	0.03	0.06	0.00	0.06
837	PHM1534.45a	G/A	8	82856619	0.23	0.35	0.00	0.29
838	PHM5235.8a	G/A	8	94414978	0.22	0.34	0.08	0.28
839	PHM3978.104a	G/A	8	99959088	0.46	0.49	0.00	0.37
840	PZA00908.2ab	A/G	8	99959553	0.26	0.38	0.09	0.31
841	PZA02019.1	A/G	8	100932328	0.09	0.17	0.02	0.16
842	PHM4134.8abc	G/C	8	105795742	0.32	0.43	0.04	0.34
843	PZA00739.1a	G/A	8	105796017	0.26	0.38	0.04	0.31
844	PZA01972.14	G/C	8	111333947	0.10	0.17	0.02	0.16
845	PHM934.19ab	G/A	8	116786833	0.41	0.48	0.02	0.37
846	PZA02748.3a	G/A	8	117273146	0.30	0.41	0.03	0.33
847	PZA03638.1	A/T	8	118438296	0.15	0.25	0.00	0.22
848	PZA03637.1	A/G	8	118439775	0.17	0.28	0.03	0.24
849	PHM5805.19abc	C/A	8	120860173	0.24	0.36	0.02	0.30
850	PZA01038.1	A/G	8	123599285	0.14	0.23	0.03	0.21
851	PHM10525.11	A/G	8	124752481	0.18	0.29	0.10	0.25
852	PHM10525.9	C/A	8	124752624	0.18	0.30	0.07	0.26
853	PZA00118.1ab	A/G	8	126154819	0.25	0.37	0.00	0.30
854	PZA00118.5	G/A	8	126155082	0.10	0.17	0.00	0.16
855	PZA01049.1abc	G/A	8	129044327	0.49	0.49	0.06	0.37
856	PHM5468.25	G/A	8	129163361	0.08	0.15	0.00	0.14
857	PHM1668.12	C/A	8	133149270	0.02	0.04	0.00	0.04
858	PHM4203.11a	C/A	8	133529939	0.39	0.47	0.00	0.36
859	PHM448.23abc	A/G	8	133775120	0.45	0.49	0.06	0.37
860	PZA00766.1a	A/G	8	133775220	0.50	0.49	0.07	0.38
861	PZA00770.1abc	G/C	8	134140609	0.29	0.41	0.05	0.33
862	PHM15744.10a	A/G	8	134813437	0.39	0.47	0.09	0.36
863	PZA02011.1abc	G/A	8	140212456	0.40	0.48	0.06	0.37
864	PZA00429.1	G/A	8	144443084	0.05	0.10	0.02	0.09
865	PZB01454.1a	G/A	8	145713853	0.28	0.40	0.20	0.32
866	PZA03650.1abc	T/A	8	146342067	0.23	0.35	0.01	0.29
867	PZA03651.1ab	C/A	8	146342784	0.27	0.39	0.06	0.31
868	PHM4757.14	A/C	8	151452567	0.20	0.32	0.00	0.27
869	PZA03182.5abc	C/T	8	152155087	0.44	0.49	0.11	0.37
870	PZA00951.1	A/G	8	154672041	0.19	0.31	0.04	0.26

No	SNP name	SNP	Chr	Position	MAF	G.Div	Het	PIC
871	PHM12749.13abc	G/C	8	155071379	0.26	0.38	0.05	0.31
872	PZA03698.1abc	A/C	8	155571435	0.29	0.41	0.05	0.33
873	PZA01741.1a	A/C	8	156061850	0.25	0.37	0.07	0.30
874	PHM2805.37a	G/A	8	156069634	0.22	0.33	0.06	0.28
875	PZA01857.1a	G/A	8	156100505	0.29	0.41	0.03	0.33
876	PZA00838.2a	G/A	8	158949206	0.30	0.41	0.00	0.33
877	PZA00706.16	A/G	8	159897914	0.10	0.18	0.00	0.16
878	PZA00675.1	C/A	8	161460108	0.12	0.21	0.03	0.19
879	PHM1834.47ab	C/G	8	162446230	0.48	0.49	0.07	0.37
880	PZA02746.2abc	G/T	8	163067200	0.38	0.47	0.04	0.36
881	PZA00460.8abc	G/A	8	163565334	0.37	0.46	0.00	0.36
882	PHM4560.54	G/A	8	163565429	0.16	0.27	0.04	0.24
883	PZA00020.5	G/A	8	164113892	0.03	0.06	0.00	0.06
884	PHM2714.11	A/C	8	164369201	0.04	0.08	0.00	0.08
885	PZA01316.1	A/G	8	164369718	0.18	0.29	0.03	0.25
886	PHM4786.9abc	G/A	8	164795880	0.42	0.48	0.03	0.37
887	PHM13493.12	G/A	8	165809580	0.07	0.13	0.01	0.12
888	PZA00904.1a	A/G	8	165809816	0.38	0.47	0.04	0.36
889	PZA01964.29	G/A	8	166984405	0.18	0.29	0.01	0.25
890	PHM765.24	G/A	8	166984456	0.16	0.27	0.03	0.23
891	PHM14046.9	C/G	8	169471006	0.13	0.23	0.00	0.21
892	PZA00189.23	A/C	8	170014475	0.07	0.13	0.00	0.12
893	PHM14104.23	A/G	8	170632002	0.10	0.17	0.03	0.16
894	PHM3337.23	A/C	8	170750982	0.09	0.15	0.00	0.14
895	PHM3312.23abc	G/A	8	170971974	0.34	0.44	0.07	0.35
896	PZA00760.1a	C/G	8	171099906	0.45	0.49	0.06	0.37
897	PHM2749.10a	G/A	8	171703522	0.46	0.49	0.07	0.37
898	PZA01290.1ab	G/A	8	171827952	0.33	0.44	0.07	0.35
899	PZA02281.3abc	C/A	8	172057947	0.24	0.37	0.07	0.30
900	PZD00036.2abc	A/T	9	6992336	0.37	0.46	0.10	0.36
901	PZA01799.1a	A/G	9	9885916	0.38	0.47	0.08	0.36
902	PHM11946.19a	G/A	9	9886093	0.41	0.48	0.05	0.37
903	sh1.11abc	A/G	9	11339891	0.46	0.49	0.04	0.37
904	sh1.12abc	A/G	9	11340882	0.35	0.45	0.09	0.35
905	PHM1218.6	G/A	9	11342096	0.16	0.26	0.06	0.23
906	PZA01195.3	A/G	9	11345280	0.09	0.16	0.00	0.14
907	PZA00466.1	G/A	9	11972467	0.11	0.19	0.02	0.17
908	PZA01386.3	G/C	9	12214341	0.20	0.31	0.02	0.27
909	PHM5181.10abc	C/T	9	15582065	0.40	0.48	0.09	0.37
910	PZA02702.1	A/G	9	16228585	0.02	0.04	0.00	0.04
911	PZA03416.7ab	G/A	9	16980092	0.31	0.42	0.04	0.34
912	PHM9374.5abc	G/A	9	17653676	0.23	0.35	0.03	0.29
913	PHM7584.9a	A/G	9	18683737	0.25	0.37	0.13	0.30
914	PHM7584.7	C/A	9	18683799	0.01	0.02	0.00	0.02
915	PZA00860.1a	C/A	9	18684111	0.29	0.41	0.13	0.33
916	PZA03058.22	G/A	9	19293027	0.10	0.19	0.02	0.17
917	PHM697.21a	A/C	9	21993146	0.34	0.45	0.05	0.35
918	ZHD1.1abc	A/G	9	22042681	0.33	0.44	0.04	0.35
919	wx1.1	C/G	9	23215033	0.08	0.14	0.00	0.13
920	PZA01999.3	G/C	9	23217376	0.07	0.14	0.00	0.13
921	PZB01110.6abc	A/G	9	24028336	0.27	0.39	0.06	0.31
922	PZA02648.2	A/G	9	25654718	0.19	0.31	0.03	0.26
923	PHM4495.14	G/A	9	26765204	0.02	0.04	0.00	0.04
924	PZA00693.3	A/G	9	28318787	0.08	0.14	0.00	0.13
925	PZB00959.1	G/A	9	28529745	0.19	0.30	0.03	0.26
926	PHM229.15abc	A/G	9	30003189	0.48	0.49	0.11	0.37
927	PHM2101.21	G/A	9	34889555	0.05	0.09	0.03	0.09
928	PZA02878.13	G/A	9	37421825	0.08	0.15	0.03	0.14
929	PZA02861.14	G/A	9	42849080	0.03	0.06	0.04	0.06
930	PZB00014.1	A/G	9	46590628	0.07	0.12	0.02	0.11
931	PZA01791.2	T/C	9	77467426	0.18	0.29	0.00	0.25
932	PZB00761.1abc	A/G	9	83959651	0.49	0.49	0.09	0.37
933	PZA01861.1ab	C/G	9	85740054	0.29	0.41	0.08	0.33
934	PZA01062.1a	C/A	9	88057320	0.38	0.46	0.06	0.36
935	PZA03596.1abc	G/A	9	90436248	0.32	0.43	0.01	0.34
936	PZA00947.1a	C/A	9	96885738	0.31	0.42	0.05	0.34
937	PZB01899.1abc	G/A	9	98502843	0.28	0.40	0.05	0.32
938	PZA00225.8	A/G	9	104537847	0.09	0.16	0.00	0.15
939	PHM13183.12	A/G	9	104710976	0.14	0.23	0.01	0.21
940	PHM2278.86	G/A	9	108864060	0.07	0.13	0.01	0.12
941	PZA00015.5	A/G	9	109909820	0.10	0.18	0.00	0.16
942	PZA03235.1a	A/G	9	114897665	0.28	0.40	0.07	0.32
943	PZA02325.4a	T/A	9	117870773	0.28	0.40	0.05	0.32
944	PZA01819.1	G/A	9	118511506	0.10	0.18	0.05	0.16
945	PZA00840.1a	C/A	9	124112589	0.35	0.45	0.00	0.35
946	PZA00060.2abc	G/A	9	124619423	0.21	0.33	0.04	0.28
947	PZA00213.19	C/G	9	126584377	0.08	0.15	0.07	0.14
948	PHM3330.25ab	A/G	9	128515174	0.26	0.38	0.00	0.31
949	PZA02111.1	C/A	9	130251868	0.11	0.20	0.00	0.18
950	PZA03670.1a	C/A	9	130886940	0.32	0.43	0.02	0.34

No	SNP name	SNP	Chr	Position	MAF	G.Div	Het	PIC
951	PHM3502.17	G/A	9	130958297	0.14	0.24	0.00	0.21
952	PZA02235.14abc	A/G	9	132119630	0.32	0.43	0.02	0.34
953	PHM7916.4a	A/G	9	132762904	0.26	0.38	0.05	0.31
954	PZA01096.1abc	A/C	9	133450713	0.22	0.34	0.07	0.28
955	PHM4689.49	A/G	9	135151857	0.13	0.22	0.02	0.19
956	PHM1766.1a	G/C	9	136401627	0.47	0.49	0.06	0.37
957	PZA02252.2	A/G	9	136597740	0.17	0.28	0.01	0.24
958	PZA01369.1a	G/A	9	140926271	0.24	0.37	0.08	0.30
959	PHM816.29a	G/A	9	142044016	0.30	0.42	0.06	0.33
960	PZB00221.3abc	A/G	9	142271047	0.26	0.38	0.09	0.31
961	PZD00055.1abc	A/G	9	142271281	0.40	0.47	0.09	0.36
962	PZA02197.1a	C/A	9	142430556	0.32	0.43	0.00	0.34
963	PZA00323.3a	A/G	9	142499873	0.22	0.34	0.06	0.28
964	PHM4604.18	G/A	9	142925413	0.06	0.12	0.00	0.11
965	PZA01715.2abc	T/C	9	142948449	0.48	0.49	0.06	0.37
966	PZA01715.1abc	A/G	9	142948545	0.48	0.49	0.05	0.37
967	PZA02381.1	G/A	9	144723067	0.08	0.14	0.03	0.13
968	PHM15445.25abc	A/C	9	147130708	0.50	0.49	0.00	0.38
969	PZA00832.1	G/C	9	147131097	0.20	0.31	0.00	0.27
970	PZA00708.3a	G/C	9	147381231	0.36	0.46	0.07	0.35
971	PHM1911.173a	G/A	9	148570003	0.26	0.38	0.05	0.31
972	PZA03573.1	G/A	9	150138200	0.05	0.10	0.00	0.09
973	PZA02554.1	G/A	10	1939921	0.04	0.08	0.00	0.08
974	PZA02221.20abc	A/G	10	1948030	0.30	0.41	0.05	0.33
975	PZA01313.2a	G/A	10	3598262	0.43	0.49	0.04	0.37
976	PZA02095.10	C/A	10	5119007	0.04	0.08	0.00	0.08
977	PHM2828.83abc	G/A	10	6121374	0.49	0.49	0.03	0.37
978	PHM5740.9	G/A	10	8773358	0.13	0.23	0.02	0.20
979	PZA01883.2	A/G	10	8950935	0.07	0.14	0.02	0.13
980	PHM1752.36ab	A/G	10	9746552	0.21	0.33	0.04	0.28
981	PZB01301.5a	G/A	10	9748559	0.48	0.49	0.04	0.37
982	PHM15331.16a	G/A	10	10432605	0.30	0.42	0.04	0.33
983	PZA00463.3a	G/A	10	13546326	0.26	0.38	0.00	0.31
984	PHM3896.9abc	C/A	10	14703075	0.24	0.36	0.00	0.30
985	PZA01642.1a	G/A	10	14703451	0.29	0.41	0.02	0.33
986	PZA02961.6a	A/T	10	16264897	0.27	0.39	0.07	0.32
987	PHM3922.32abc	A/G	10	17722938	0.49	0.49	0.08	0.37
988	PZA00079.1	G/A	10	18903460	0.01	0.02	0.00	0.02
989	PZA02853.11	C/G	10	25966022	0.09	0.16	0.00	0.14
990	PZA02443.8	G/A	10	29194886	0.10	0.18	0.05	0.16
991	PHM4066.11	A/G	10	41187565	0.17	0.28	0.04	0.25
992	PHM3931.17	A/G	10	50501338	0.02	0.04	0.00	0.04
993	PZA00409.17	T/A	10	62062819	0.08	0.15	0.02	0.14
994	PZA01677.1	G/A	10	70797556	0.03	0.06	0.00	0.06
995	PZA02941.7ab	G/A	10	71120345	0.29	0.41	0.02	0.33
996	PHM2770.19abc	C/A	10	72565410	0.23	0.35	0.02	0.29
997	PZA01877.2	G/A	10	77492186	0.10	0.18	0.08	0.17
998	PZB00409.1	G/A	10	84002430	0.20	0.32	0.01	0.27
999	PZA00337.4abc	G/C	10	86327620	0.39	0.47	0.07	0.36
1000	PHM12990.15	G/C	10	86998232	0.08	0.15	0.00	0.14
1001	PZA00048.1a	A/G	10	98582149	0.45	0.49	0.00	0.37
1002	PZA02398.2ab	G/A	10	99471436	0.22	0.34	0.09	0.28
1003	PHM537.22	G/C	10	99471668	0.16	0.27	0.00	0.23
1004	PZA00444.1	A/C	10	106780357	0.09	0.16	0.01	0.15
1005	PZA01292.1	G/A	10	109633741	0.12	0.21	0.04	0.19
1006	PZA01919.2abc	G/C	10	111260278	0.33	0.44	0.08	0.34
1007	PZA02128.3	C/A	10	111608788	0.11	0.19	0.04	0.18
1008	PZA01089.1a	G/A	10	117372550	0.31	0.42	0.03	0.34
1009	PHM13687.14a	G/A	10	117796822	0.24	0.36	0.04	0.30
1010	PZA02219.2a	C/G	10	118308828	0.33	0.44	0.03	0.34
1011	PZA03713.1a	A/T	10	121489957	0.42	0.48	0.03	0.37
1012	PHM557.21	G/A	10	122517810	0.05	0.10	0.02	0.10
1013	PHM2270.15	G/A	10	122667719	0.01	0.02	0.00	0.02
1014	PZA03196.1a	G/A	10	124005403	0.22	0.34	0.06	0.29
1015	PHM1576.25	A/G	10	124203168	0.13	0.22	0.02	0.20
1016	PZA00866.2a	G/A	10	124203565	0.49	0.49	0.07	0.37
1017	PZA01241.2abc	A/C	10	130452676	0.46	0.49	0.09	0.37
1018	PZA02320.1a	C/A	10	132256848	0.49	0.49	0.08	0.38
1019	PZB01111.8abc	C/A	10	134034407	0.28	0.40	0.03	0.32
1020	PZA01456.2abc	G/A	10	135928293	0.47	0.49	0.02	0.37
1021	PHM18513.156abc	A/G	10	136530988	0.26	0.38	0.00	0.31
1022	PHM15868.56abc	A/G	10	137132183	0.24	0.36	0.00	0.30
1023	PZA02663.1abc	G/A	10	137627429	0.27	0.39	0.08	0.32
1024	PZA01995.2	A/G	10	139616614	0.12	0.20	0.05	0.18
1025	PZA03607.1abc	A/G	10	141828531	0.29	0.41	0.04	0.33
1026	PZA03606.1	T/A	10	141829598	0.12	0.21	0.00	0.19
1027	PZA03605.1abc	A/G	10	141830532	0.29	0.41	0.09	0.33
1028	PZA03603.1abc	T/A	10	141832715	0.27	0.39	0.05	0.32
1029	PZA00130.9abc	A/G	10	143269455	0.41	0.48	0.07	0.37
1030	PZA02969.9a	A/G	10	143676084	0.46	0.49	0.00	0.37

No	SNP name	SNP	Chr	Position	MAF	G.Div	Het	PIC
1031	PZA02049.1abc	G/C	10	144025574	0.36	0.46	0.07	0.35
1032	PZA00007.1a	A/C	10	144172316	0.37	0.46	0.07	0.36
1033	PHM5435.25a	G/A	10	144239494	0.42	0.48	0.03	0.37
1034	PZA01073.1ab	A/G	10	144549995	0.39	0.47	0.00	0.36
1035	PHM3309.8a	A/G	10	144752780	0.30	0.41	0.08	0.33
1036	PZA01001.2a	A/G	10	146538889	0.48	0.49	0.03	0.37
1037	PHM3844.14	A/T	10	146554012	0.20	0.32	0.00	0.27
1038	PZA00062.4	G/A	10	146999711	0.02	0.04	0.00	0.04
1039	PHM3736.11a	G/A	10	147762925	0.31	0.42	0.10	0.34
1040	PHM10750.26	C/G	10	148332525	0.04	0.08	0.09	0.08
1041	PZA02527.2	C/G	10	148488692	0.19	0.30	0.03	0.26
1042	PHM14519.8a	A/G	NA	NA	0.21	0.33	0.07	0.28
1043	PHM1956.90	T/A	NA	NA	0.11	0.19	0.00	0.17
1044	PHM3911.11a	G/A	NA	NA	0.45	0.49	0.09	0.37
1045	PHM4905.6abc	A/T	NA	NA	0.35	0.45	0.15	0.35
1046	PZA00311.5abc	G/A	NA	NA	0.28	0.40	0.06	0.32
1047	PZA00755.2abc	G/A	NA	NA	0.23	0.35	0.03	0.29
1048	PZA01039.1	G/A	NA	NA	0.08	0.14	0.02	0.13
1049	PZA01934.6	C/A	NA	NA	0.11	0.20	0.00	0.18
1050	PZA01993.7	A/G	NA	NA	0.17	0.28	0.02	0.25
1051	PZA02167.2abc	A/G	NA	NA	0.38	0.46	0.02	0.36
1052	PZA02296.1	A/G	NA	NA	0.17	0.28	0.00	0.24
1053	PZA02396.14ab	A/G	NA	NA	0.24	0.37	0.10	0.30
1054	PZA02397.1	C/G	NA	NA	0.20	0.31	0.02	0.27
1055	PZA02474.1a	A/G	NA	NA	0.49	0.49	0.06	0.37
1056	PZA02528.1	C/G	NA	NA	0.05	0.10	0.02	0.09
1057	PZB01944.1	G/A	NA	NA	0.18	0.29	0.00	0.25

Table S 3. Markers with missing alleles identified in INERA collection but present in CIMMYT, IITA and Temperate germplasm collections.

Chr=chromosome

SNP No	SNP Name	Chr	Position	Missing allele in INERA	Allele frequency in		
					CIMMYT	IITA	Temperate
3	PZA02032.1	1	4490461	A	0.07	0.00	0.44
28	PHM3951.25	1	31928634	A	0.00	0.00	0.50
30	PZA02686.1	1	34724094	A	0.00	0.07	0.10
44	umc13.1	1	46064387	G	0.14	0.00	0.20
60	PZA01135.1	1	82563962	G	0.00	0.00	0.20
61	PZB01235.4	1	88380865	A	0.00	0.00	0.11
64	PZA03465.1	1	91279351	C	0.00	0.00	0.20
66	PHM9418.11	1	96545939	A	0.00	0.15	0.56
88	PZA00068.1	1	183831591	G	0.19	0.07	0.20
95	PHM5597.15	1	203035169	C	0.07	0.00	0.20
106	PHM6043.19	1	216927159	G	0.06	0.33	0.10
108	PZA02655.9	1	217504762	C	0.00	0.00	0.50
114	PHM2478.22	1	226781494	A	0.19	0.00	0.11
124	PHM16605.19	1	251142538	A	0.00	0.07	0.10
169	PHM673.33	1	295656485	A	0.00	0.27	0.30
181	PZA00902.1	2	3069068	G	0.00	0.00	0.30
184	PZA01211.1	2	3593488	G	0.25	0.00	0.10
188	PHM2601.5	2	4624088	A	0.06	0.36	0.10
190	PHM3334.6	2	5053798	G	0.00	0.07	0.20
192	PZA02081.1	2	5923120	A	0.00	0.07	0.20
213	PZA01336.1	2	31394426	A	0.14	0.13	0.00
235	PZA02939.10	2	157152464	G	0.07	0.27	0.00
252	PHM3598.20	2	193476369	T	0.00	0.31	0.20
265	PHM2094.34	2	208384148	G	0.00	0.07	0.00
268	PZA02456.1	2	210924384	A	0.07	0.00	0.10
271	PZA02471.5	2	214640685	A	0.13	0.00	0.20
279	PZB01013.1	2	222280720	A	0.10	0.37	0.10
286	PZA03321.4	2	234574991	A	0.06	0.00	0.10
304	PHM2766.14	3	31062080	C	0.07	0.00	0.20
305	PZA03054.5	3	31695534	G	0.13	0.00	0.00

continued Table S3

SNP No	SNP Name	Chr	Position	Missing allele in INERA	Allele frequency in		
					CIMMYT	IITA	Temperate
311	PHM13473.12	3	42534072	G	0.06	0.00	0.00
321	PHM12153.9	3	57612577	A	0.00	0.00	0.29
325	PHM2439.25	3	69892464	G	0.00	0.00	0.20
330	PZA02619.1	3	120046370	G	0.17	0.00	0.10
335	PZD00016.4	3	133480598	G	0.00	0.00	0.11
336	PZD00015.5	3	133481473	G	0.00	0.00	0.10
337	PZB02002.1	3	133483395	G	0.06	0.00	0.10
344	PZA00828.2	3	157646430	T	0.13	0.00	0.00
362	zb27.1	3	185290956	C	0.13	0.00	0.20
367	PHM3075.15	3	189861169	G	0.00	0.00	0.10
368	PZA01228.2	3	189861328	T	0.07	0.03	0.10
369	PHM824.17	3	190451804	T	0.00	0.00	0.20
370	PZA03743.1	3	191863818	C	0.00	0.08	0.30
375	PZA00308.24	3	196191461	C	0.07	0.00	0.10
379	PZA01501.1	3	199272204	A	0.21	0.14	0.22
390	PHM1600.31	3	214887907	G	0.09	0.00	0.00
394	PZA02514.1	3	216915474	A	0.00	0.00	0.20
396	PZA02668.2	3	218119861	A	0.00	0.00	0.40
420	PHM259.7	4	14326036	G	0.13	0.00	0.10
450	PZA03270.2	4	76122802	A	0.08	0.00	0.13
463	PZA03317.1	4	149531093	A	0.00	0.00	0.43
472	PZA00271.1	4	171613440	G	0.06	0.00	0.10
484	PZA00332.5	4	194749287	G	0.06	0.04	0.30
506	PHM5780.15	4	237583793	G	0.22	0.00	0.00
507	PZA00513.1	4	238553534	A	0.00	0.00	0.10
519	PZA00682.17	4	244992721	A	0.00	0.00	0.10
525	PHM3061.315	5	1621791	A	0.07	0.50	0.30
536	PHM15223.38	5	6961253	A	0.08	0.08	0.38
541	PZA02029.21	5	7956551	G	0.07	0.00	0.30
547	PZA01284.6	5	11990631	T	0.09	0.00	0.60
551	PZA00517.7	5	18240488	G	0.00	0.07	0.00
564	PZA01563.1	5	32599139	A	0.06	0.07	0.10
583	PZB01112.1	5	68419652	C	0.06	0.07	0.30
584	PZA01349.2	5	69319346	A	0.38	0.07	0.00
587	PZA01303.1	5	72408251	A	0.06	0.00	0.10
592	PZA03049.24	5	88604977	C	0.07	0.00	0.20
599	PZA01763.2	5	168080654	G	0.13	0.07	0.25
618	PZA03324.1	5	181256363	T	0.00	0.13	0.10
619	PZA01575.1	5	182337304	A	0.00	0.07	0.00
625	PZA02426.1	5	191642443	A	0.00	0.00	0.22
632	PZA01265.1	5	201139510	A	0.00	0.08	0.40
635	PZA02667.1	5	202777978	G	0.00	0.00	0.20
674	PZA03488.1	6	95789142	A	0.00	0.00	0.56
678	PZA01589.2	6	117238344	A	0.00	0.39	0.10
681	PZA01055.1	6	120059553	A	0.00	0.00	0.20
694	PHM3438.2	6	133843693	A	0.00	0.00	0.20
697	PZA02187.2	6	139105889	T	0.06	0.00	0.10
731	PHM5361.13	6	166687965	A	0.07	0.07	0.40
738	PZA02035.5	7	2585198	A	0.07	0.00	0.14
749	PZA00132.17	7	33979080	C	0.29	0.00	0.22
763	PZA01210.2	7	75098939	A	0.13	0.00	0.00
776	PZA03728.1	7	137207838	G	0.00	0.00	0.43
834	PZA02522.1	8	82710229	G	0.07	0.00	0.20
835	PZA01297.1	8	82856261	C	0.07	0.00	0.20
840	PZA02019.1	8	100932328	G	0.16	0.23	0.30
856	PHM1668.12	8	133149270	A	0.00	0.00	0.20
863	PZA00429.1	8	144443084	A	0.13	0.07	0.20
909	PZA02702.1	9	16228585	G	0.00	0.00	0.10
913	PHM7584.7	9	18683799	A	0.06	0.00	0.00
922	PHM4495.14	9	26765204	A	0.00	0.00	0.20
928	PZA02861.14	9	42849080	A	0.03	0.11	0.13
939	PHM2278.86	9	108864060	A	0.09	0.27	0.10
940	PZA00015.5	9	109909820	G	0.08	0.43	0.22
972	PZA02554.1	10	1939921	A	0.06	0.08	0.22
993	PZA01677.1	10	70797556	A	0.00	0.00	0.30
999	PHM12990.15	10	86998232	C	0.07	0.23	0.33
1002	PHM537.22	10	99471668	C	0.13	0.70	0.50
1003	PZA00444.1	10	106780357	C	0.31	0.03	0.20
1012	PHM2270.15	10	122667719	A	0.00	0.00	0.10
1037	PZA00062.4	10	146999711	A	0.07	0.00	0.11