Genetic diversity and combining ability of selected quality protein maize (QPM) inbred lines adapted to the highland agro-ecology of Ethiopia

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Thesis Abstract

The high altitude sub-humid agro-ecology in Ethiopia is estimated to cover 20% of the land devoted to annual maize cultivation. More than 30% of small-scale farmers in this agro-ecology depend on maize production for their livelihood. This area is also characterized by high human population density necessitating maize production for food security and alleviating malnutrition. Research on highland maize improvement in Ethiopia has generally lagged behind compared to research undertaken in the other agro-ecologies until it was accelerated in 1998 as part of the Highland Maize Gene Pool Project for east and central African countries (ECA). A strategic research is required to develop nutritionally enhanced maize cultivars such as quality protein maize (QPM) to alleviate food insecurity and nutritional problem of millions of people who depend on this staple food crop. The Highland Maize Breeding Program of Ethiopia in collaboration with CIMMYT-Ethiopia has been systematically developing a pool of highland QPM source germplasm by converting non-QPM highland maize germplasm into QPM counterparts. The converted genotypes should be well studied for strategic breeding or production purposes in the highland sub-humid agro-ecologies of Ethiopia and other ECA countries with similar environments.

The objectives of this study were, therefore, to: (1) establish farmers' preferences of normal and QPM cultivars and traits of interest, and identify maize production constraints in the highlands of Ethiopian; (2) examine phenotypic variability among Tropical-highland adapted QPM inbred lines; (3) examine genotypic variability among Tropical-highland adapted QPM inbred lines using simple sequence repeat (SSR) markers; (4) estimate heterosis and combining ability for grain yield and yield related agronomic traits; and (5) determine genotype-environment interaction (G x E) and yield stability of experimental QPM hybrids.

A participatory rural appraisal (PRA) research was conducted involving 160 experienced maize farmers selected from four districts of two zones in the central highlands of Ethiopia during 2012. Results showed that few highland maize cultivars are available and adopted by farmers. A two-decade old cultivar, 'BH660', originally released for the mid-altitude agro-ecology, has been widely adopted in most

highland areas. Grain yield was considered the most important trait for maize cultivar selection. Limited access to inputs (improved maize seeds and inorganic fertilizers) and late on-set and inadequate rainfall were the primary constraints identified by farmers across the study areas.

Thirty-six maize inbred lines (30 QPM and six normal) adapted to tropical highlands were phenotyped and genotyped using 18 morpho-agronomic traits and 25 polymorphic SSR markers, respectively, in 2012. Significant phenotypic variation observed among inbred lines for all measured traits. Principal component and unweighted paired group method using arithmetic averages (UPGMA) cluster analyses of the phenotypic data revealed the presence of three distinct clusters. Seven inbred lines [KIT32Q, 142-1eQ, SRSYN20Q, FS67(BC_2), FS170Q, FS60, and F7215] with complementary phenotypic traits and relatively better yield performance were selected for further genetic analysis and breeding. Similarly, the SSR data showed that nearly 98% of the pairwise comparisons had genetic distance between 0.30 and 0.78, indicating large genetic differences among tested inbred lines. The model-based population structure, principal coordinate and neighbor-joining cluster analyses also revealed the presence of three genetic groups, which is generally consistent with pedigree information and partly with heterotic grouping. Analysis of molecular variance indicated a considerable genetic difference among heterotic groups explained by 8.6 to 15.4% of the total SSR variance.

Sixty-six experimental QPM hybrids obtained from a 12-parent diallel cross was tested for grain yield and related traits under rain-fed conditions to determine combining ability and heterosis. The ratio of dominance to additive genetic variance was relatively larger for grain yield compared to other traits, suggesting the genetic worthiness of QPM hybrid development. Inbred lines KIT32, FS60 and 142-1-EQ were good general combiners (GCA) for grain yield; while FS60 exhibited good GCA for days to anthesis, plant and ear height. The best crosses identified were KIT32 x 142-1-EQ and SRSYN20 x FS60 that yielded 9.6 t ha^{-1} and 8.8 t ha^{-1}, respectively. The hybrids can be used as potential single cross testers for the development of three-way cross QPM hybrids for the highland agro-ecologies of Ethiopia and ECA.

Genotype-environment interaction (GEI) and grain yield stability analyses were conducted using AMMI and GGE biplot models for the newly developed QPM single crosses and two commercial hybrid checks evaluated across seven environments. Hybrid 10 (KIT32 x 142-1-eQ) followed by hybrid 66 (142-1-eQ x CML144) and hybrid 59 (FS60 x 142-1-eQ) with yield levels of 10.3, 9.6 and 9.4 t ha^{-1}, respectively, were selected as the best performers and hence desirable hybrids while KUL13 (Kulumsa) was the most suitable environment identified by both analyses. The GGE analysis divided the highland test environments into two mega-environments.

Overall, the study attempted to understand the importance of highland maize and farmers' production constraints, identified useful highland adapted QPM inbred lines with good combining ability for breeding and good performing QPM single crosses to be used as testers for hybrid development in the highland sub-humid agro-ecologies of Ethiopia, in particular, and the ECA countries, in general.

Declaration

I, Demissew Abakemal, declare that

1. The research reported in this thesis, except where otherwise indicated, is my original research.
2. This thesis has not been submitted for any degree or examination at any other University.
3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.
4. This thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:

a. Their words have been re-written but the general information attributed to them has been referenced.

b. Where their exact words have been used, then their writing has been placed in italics and inside quotation marks, and referenced.

5. This thesis does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the thesis and in the references sections.

Signed

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Demissew Abakemal Ababulgu

As the candidate's supervisors, we agree to the submission of this thesis:

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Prof. Shimelis Hussein (Supervisor)

……

Prof. John Derera (Co-Supervisor)

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Dedication

This thesis is dedicated to my mother (Fanayie Tadesse), my wife (Ayelech Gurmesa) and children (Yeron and Simera).

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Introduction to Thesis

Background and importance of maize

Maize (*Zea mays* L.) is a member of the grass family *Gramineae (Poaceae)* and one of the oldest cultivated crops (Sleper and Poehlman, 2006). It is the primary food grain in Mexico, Central America, the Andean region of South America, and eastern and southern Africa (Poehlman, 1987; Sleper and Poehlman, 2006). The crop is grown at latitudes varying from the equator to 50° north and south, from sea level to over 3 000 meters elevation, under conditions ranging from heavy rainfall to semi-arid, from cool to very hot climates with growing cycles ranging from 3 to 13 months (Dowswell et al., 1996). To date, maize is widely cultivated in most parts of the world, over a wide range of environmental variables signifying its global and regional importance to millions of people who rely on the crop in pursuit of food security and livelihoods.

Maize is the third most important cereal crops widely grown throughout the world after wheat and rice. The world maize production area in 2013 was around 184.2 million hectares, and that of wheat and paddy rice was 218.4 million and 166 million hectares, respectively (FAOSTAT, 2013). Maize surpasses wheat and rice in terms of actual production. Worldwide maize production was around 1 billion tonnes in 2013 - considerably more than wheat (~713.2 million tonnes) or rice (~745.1 million tonnes). Although 70% of the world maize area was in developing countries, only 49% of the maize was produced there, and almost half of the world's harvest (~42.5%) was produced by the United States (FAOSTAT, 2013). The maize produced in the developed world is mostly used as a feed for livestock (70%) and only a small percentage (5%) for food. Conversely, in developing countries 34% of the maize produced is used for food and the remaining 62% for feed. The remaining quantity is used for varied industrial uses and as seed.

Increased production and consumption trends of maize have been observed in sub-Saharan Africa over the past years. In the region, maize is the dominant staple crop grown by the vast majority of rural households (DeVries and Toenniessen, 2001).

The share of Africa's maize production in 2013 was 71 million tonnes harvested from 34.9 million hectares. This was higher than wheat and rice each contributing at 28 and 29 million tonnes, respectively (FAOSTAT, 2013). The consumption rate of maize in Africa is 43.4 kilograms per capita per year. It contributes to 28.8% of the protein and 29.6% of the calories derived from cereal crops in the region (Banziger and Diallo, 2004; Diallo et al., 2004; FAOSTAT, 2013). In eastern and southern Africa, maize accounts for over 23% and 29% of the total calories consumed, respectively. The per capita annual consumption of maize averages more than 100 kilograms in several southern African countries while it was 56.6 in eastern Africa (CIMMYT, 1999; Hassan et al., 2001; FAOSTAT, 2013). Sub-Saharan African countries, however, do not produce enough maize to meet their needs and therefore import more than three million tonnes of maize annually (Pingali and Pandey, 2001). Accordingly, demand for maize in sub-Saharan Africa is projected to increase nearly twofold by the year 2020 (Bigirwa et al., 2003). In addition to strong demand for maize as a food staple, there is also the potential for maize to become an increasingly important non-traditional agricultural export crop.

Similarly, the demand for maize has been steadily growing in Ethiopia. It contributes to the greatest share of production and consumption along with other major cereal crops such as tef (*Eragrostis tef* (Zucc.)), wheat (*Triticum aestivum* L.) and sorghum (*Sorghum bicolor* L.) In 2006/07 main cropping season, cereals were cultivated on 8.6 million hectares, producing 13.7 million metric tonnes of grain (CSA, 2007). This represents 81.5% of the total production area and 88.2% of grain production in the country. Among the cereal crops, maize ranks second to tef in area coverage and first in total production.

The production of maize has been increasing over the years in the major maize producing regions of the Ethiopia. In the 1980s, the total production was below 2 million tonnes and the maize area was slightly more than 1 million hectares (Kebede et al., 1993). However, significant increase in production of 2.34 million tonnes was observed in the 1990s. From 1995-2000, the annual growth rates of yield per hectare, maize area, and total production were 3.1%, 7.1% and 11.3%, respectively (Worku et al., 2002). Recent reports of the Central Statistical Agency of Ethiopia

showed that maize was produced on about 2 million hectares with total production of about 6 million tonnes in 2011/12 main cropping season. During the same year, an average national yield of 2.95 t ha^{-1} was recorded (CSA, 2011). In general, the area under maize has increased by about 50%, and production by 66%, with the national average yield increasing from 1.6 to 3.0 t ha^{-1} over the last 20 years (CSA, 2011). The per capita consumption of maize is 60 kg $year^{-1}$ per annum in Ethiopia, although the level of consumption varies from place to place (Mosisa et al., 2012). Maize is, therefore, a crucial commodity crop in Ethiopia in the short and medium terms. The Growth and Transformation Plan (GTP) proposes doubling of its production by 2015 (FDRE, 2011).

Highlights of maize production, breeding efforts and gaps in Ethiopia

In Ethiopia, maize growing agro-ecologies are broadly classified into four major categories: mid-altitude sub-humid (1000-1800 metres above sea level [m.a.s.l.]), highland sub-humid (1800-2400 m.a.s.l.), lowland moisture stress areas (300-1000 m.a.s.l.) and lowland sub-humid (<1000 m.a.s.l.) (Kelemu and Mamo, 2002). Maize research and development was started in 1952 in the country to enhance its productivity targeting the needs of small-scale farmers who produce more than 90% of maize (Tolessa and Ransom, 1993; Nigusse and Tanner, 2002). In the 1970s and 1980s, locally developed improved open pollinated varieties (OPVs) were released for wide area production at different agro-ecologies of the country. In the late 1980s, the first locally developed non-conventional hybrid was released for the mid-altitude sub-humid maize growing areas. Since then, several improved OPVs and hybrids with pest and disease resistance were released for large scale production across different agro-ecologies by the National Maize Research Project of the Ethiopian Institute of Agricultural Research (EIAR). Currently, the National Maize Research Project has three main breeding stations located in the above three major agro-ecologies excluding the lowland sub-humid agro-ecology.

The mid-altitude sub-humid agro-ecology is a high potential area for maize production in Ethiopia. It is the leading maize growing agro-ecology contributing the largest share of maize produced in the country (Tolessa and Ransom, 1993; Nigusse and Tanner, 2002; Mosisa et al., 2012). However, production and productivity of

maize in this and other agro-ecologies is constrained by several factors. These include unavailability of improved varieties, limited access to improved seeds, diseases such as gray leaf spot caused by *Cercospora zeae-maydis*, *Turcicum* leaf blight (*Exserohilum turcicum*) and common rust (*Puccinia sorghi*), field and storage insect pests (e.g., maize stalk borers and the maize weevil), low soil fertility and poor market development (Worku et al., 2002; Mosisa et al., 2012). Therefore, there is a need to develop improved maize varieties and their production packages for sustainable maize production in the country.

The lowland moisture stress agro-ecology is the other maize producing agro-ecology of Ethiopia. This agro-ecology encompasses drought stressed areas occupying over 40% of area in the country and contributes to 20% of the total maize production (Mandefro et al., 2002). In addition to the above constraints, recurrent drought is the most important challenge of maize production and productivity in this agro-ecology (Tolessa and Ransom, 1993; Nigusse and Tanner, 2002; CIMMYT and IITA, 2010).

The high altitude sub-humid agro-ecology, including the highland transition and true highlands, is next to the mid-altitude agro-ecology with greater maize area and production in Ethiopia. This agro-ecology is estimated to cover 20% of the land devoted to annual maize cultivation and consisted of more than 30% of small-scale farmers who depend on maize production for their livelihoods (CSA, 1998). The Ethiopian highland maize breeding program is situated at Ambo to coordinate maize research and technology development for the highland agro-ecologies. The program was initiated in 1998 in collaboration with the International Maize and Wheat Improvement Center (CIMMYT) and National Agricultural Research Systems (NARS) of east and central African countries including Ethiopia, Kenya, Tanzania, Uganda, Rwanda, and Burundi (http://www.cimmyt.org.com). Research and variety development of highland maize has generally lagged behind when compared to other agro-ecologies before the launch of the breeding program (Twumasi et al., 2002). In the region, maize varieties popularly grown beyond 2000 m.a.s.l. have been local varieties with low yield potential (Twumasi et al., 2002; Kassa et al., 2013). In view of this constraint, the breeding program develops a pool of both highland normal and QPM improved germplasm for the region (Twumasi-Afriyie et al., 2012).

The regional highland maize breeding activities are being conducted at Ambo, Ethiopia while germplasm evaluations have been done across different environments of the member countries (Figure 1.1). Each partner country funds germplasm evaluation and development being undertaken in their specific environments in addition to grants secured from partner organizations. Since 2003, the QPM development endeavour of this program has been supported by the Canadian International Development Agency (CIDA) through Quality Protein Maize Development (QPMD) project implemented by CIMMYT. Also the program has been supported by the Staple Crops Program of the Association for Strengthening Agricultural Research in East and Central Africa (ASARECA) through grants to meet food security in the region (www.asareca.org). The synergetic research efforts of the member countries and partner institutions helped the release of some cultivars for the highland agro-ecologies of the region (Mudruma et al., 2006; Twumasi et al., 2006).

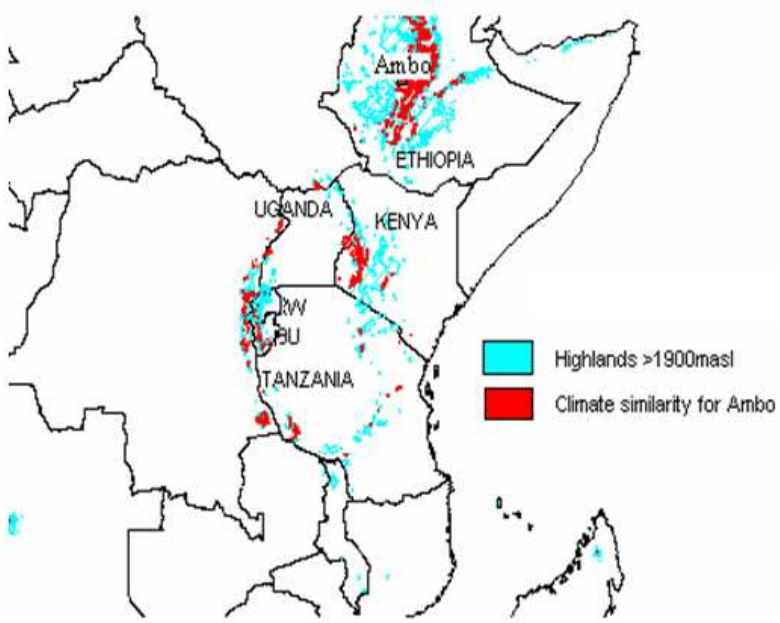

Figure1. 1 Highland zones of eastern Africa and climates similar to Ambo, the regional highland maize improvement hub (*Source: Twumasi et al., 2002*)

In Ethiopia, the highland agro-ecology was previously known for its highland crops production including barley, wheat, broad beans, and potatoes whereby maize was a homestead crop of minor importance (Demissew et al., 2013). This agro-ecology is

densely populated and limited lands are available for crop production. Deployment of nutritionally enhanced and high yielding maize cultivars in this environment would enhance food and nutritional security. Besides, introduction of improved highland maize genotypes may diversify the cropping system and significantly improve the livelihoods of poor households. Recent research developments for highland maize improvement program in Ethiopia hold out the hope for attaining this goal. The program has a good foundation of already improved normal and QPM counterparts of highland maize inbred lines useful for the development of hybrids and/or OPVs. The QPM inbred lines were developed through a backcross conversion procedure outlined by Vivek et al. (2008).

The QPM inbred lines developed by the highland breeding program need to be tested in hybrid combinations with selected lines or testers. However, prior studies aimed at classifying these lines into different heterotic groups would be useful in the development of heterotic maize hybrids and synthetic varieties (Menkir et al., 2004). These authors further pointed out that classifying the lines into distinct heterotic groups based only on the results of combining ability studies may not be reliable. Therefore, the combined use of molecular markers that allow direct comparison of the similarity of inbred lines at the DNA level with phenotypic evaluations facilitate the separation of inbred lines into well-defined heterotic groups (Menkir et al., 2004; Xia et al., 2004). Several DNA marker technologies have been developed and are available to study genetic diversity. In maize, SSRs have proved to be an important tool for diversity measurements (Warburton et al., 2002; Pinto et al., 2003). Information related to the general and specific combining abilities of the elite lines for grain yield and other desirable traits is also worth considering in this study. This can be possible through combining ability studies using different mating designs such as diallel (Griffing, 1956). When the genotypes are grown under a wide range of environments, genotype-by-environment interaction (GEI) is expected. The existence of GEI thus necessitates evaluation of genotypes in more than one environment so that repeatable rankings of genotypes are identified with narrow of broad adaptation (Hallauer and Miranda, 1988; Yan and Kang, 2003).

Quality protein maize as the noble option against malnutrition

Maize contributes over 15% of the protein and 20% of the calories derived from food crops in the world's diet (NRC, 1988; Atlin et al., 2011). According to Bressani (1992) and Prasanna et al. (2001), in many developing countries such as Latin America, Africa, and Asia, maize is a staple food and sometimes the only source of protein in diet, especially in weaning food for babies. Most of the maize is produced and consumed by resource poor farmers who have limited access to protein sources such as milk and other protein products. This leaves the majority to depend on conventional maize, which is deficient in two essential amino acids, lysine and tryptophan, leading to malnutrition and protein deficiency. Conventional/normal maize, being deficient in lysine and tryptophan, is nutritionally poor with a biological value (BV) of 40 to 57% that of milk (Bressani, 1992).

Quality protein maize is nutritionally enhanced maize developed through conventional breeding method. It contains nearly twice the quantity of lysine and tryptophan present in the normal maize (CIMMYT, 2000). QPM cultivars with homozygous embryo and endosperm for the mutant allele *opaque-2* at the *α*-zeins regulatory gene show about 60-100% increase in lysine and tryptophan and a higher BV than most conventional maize varieties. The nutritional composition of QPM is 90% of the protein quality of casein contained in cow's milk (Bressani, 1992; Bhatnagar et al., 2004). QPM cultivars are also superior to conventional maize as an animal feed for poultry and swine (Graham et al., 1980; NRC, 1988). Hence, the use of QPM in the highlands of Ethiopia in general can alleviate malnutrition, and leads to healthier population, increased productivity and higher income for better livelihoods.

Research problem statement

Shortage of protein has been a major problem related to nutrition in the world (Johnson et al., 1968; WHO, 2007; Atlin et al., 2011). Although the highland agro-ecology of Ethiopia has great potential for maize production, it is characterized by high human population density and consequently high levels of malnutrition and poverty. Alleviation of the nutritional problem of millions of people whose staple food is maize

may depend upon the improvement of the inherent nutritional quality of the maize (Johnson et al., 1968; Graham et al., 1990; Prasanna et al., 2001; Vivek et al., 2008; Sofi et al., 2009; Atlin et al., 2011). The Highland Maize Breeding Program in Ethiopia in collaboration with CIMMYT is systematically developing a pool of highland QPM source germplasm by converting non-QPM highland maize genotypes into QPM counterparts. The breeding program has developed phenotypically stable elite QPM lines with protein levels of 8-14% and significantly high tryptophan content. Detailed information is, therefore, needed on the newly generated elite highland adapted QPM inbred lines including their genetic diversity, combining ability, heterosis, and genotype-by-environment interaction for cultivar development, which constituted the main focus of the present study. Also, the study included participatory rural appraisal (PRA) in selected areas of the highland sub-humid agro-ecology of Ethiopia to assess farmers' perceptions of the maize production system and breeding priorities. In light of this background, the following research objectives were set out in the study.

Specific objectives of the research

(i) To assess the magnitude and production system of highland maize, farmers' production constraints and implications for adoption of new maize cultivars in two zones of the Oromia Regional State representing highland sub-humid agro-ecology of Ethiopia.
(ii) To determine genetic variation among quality protein and normal maize inbred lines using phenotypic traits for cultivar development.
(iii) To verify the genetic purity, determine the effect of conversions of normal maize lines to QPM on the original heterotic system and understand patterns of relationships among 36 white maize inbred lines (30 QPM and 6 non-QPM) using 25 simple sequence repeat (SSR) markers.
(iv) To determine the levels of heterosis and combining ability of elite QPM inbred lines and their hybrids for cultivar development.
(v) To determine G x E interaction and yield stability of recently developed QPM single-cross hybrids and to identify representative test and/or production environments.

Hypotheses

- There is awareness and positive perception towards normal and nutritionally enhanced highland maize cultivars in the highland agro-ecology of Ethiopia.
- There exists genetic variability among the already developed elite QPM lines both phenotypically and genotypically.
- The elite lines and their crosses could show good combining ability and heterosis.
- The performance of yield and yield related traits of the QPM single-crosses across environments is uniform.

Outline of thesis

This thesis consists of six different chapters in accordance with a number of activities related to the above objectives (see outline below). Chapters 2 to 6 are written in the form of discrete research chapters, each following the format of a stand-alone research paper (whether or not the chapter has already been published). The Journal of Crop Science system of referencing is used in the chapters of this thesis. This is the dominant thesis format adopted by the University of KwaZulu-Natal. As such, there is some unavoidable repetition of references and some introductory information between chapters. Chapter 2 is published in Journal of Agricultural Science (vol.5, No. 11; 2013) while chapter 4 is being reviewed by Euphytica.

Chapter	Title
-	Introduction to thesis
1	Review of the literature
2	Farmers' perceptions of maize production systems and breeding priorities, and their implications for the adoption of new varieties in selected areas of the highland agro-ecology of Ethiopia
3	Phenotypic variation among quality protein and normal maize inbred lines adapted to Tropical-highlands of east Africa

4	Determination of genetic purity and patterns of relationships among highland adapted quality protein and normal maize inbred lines using microsatellite markers
5	Combining ability and heterosis analyses of quality protein maize genotypes adapted to Tropical-highlands
6	Genotype-by-environment interaction and yield stability of quality protein maize hybrids adapted to Tropical-highlands
7	Overview of the research findings

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CHAPTER 1

A review of the Literature

This chapter reviews relevant literature to support the thesis research work and serving as background information for QPM breeders. The review provides general information about QPM in retrospect and prospects. This is followed by brief discussion on maize genetic diversity and analysis for selection. The last section highlights heterosis, combining ability, and genotype-environment interaction with reference to maize breeding where necessary.

1.1 Quality Protein Maize

1.1.1 Historical overview

Wheat, maize, and rice are estimated to constitute 85% of total global cereals production, which brings about 200 million tonnes of protein harvest annually and the largest proportion is utilized for human consumption (Shewry, 2007). However, malnutrition has still been a major challenge, and is particularly severe in developing nations with low per capita income (Prasanna et al., 2001). With its high content of carbohydrates, fats, proteins, some important vitamins and minerals, maize is considered as a 'poor man's' nutritionally balanced cereal grain (Prasanna et al., 2001). Several million people, particularly in developing countries, where animal protein is scarce and unaffordable, obtain their protein and calorie requirements from maize (Sofi et al., 2009). However, the conventional maize is deficient in essential amino acids such as lysine and tryptophan which are the limiting amino acids in human beings and non-ruminants. The poor quality of normal maize in storage protein is attributed to a relatively higher proportion of prolamines or zeins. Consequently, the diets of humans and other monogastric animals must be supplemented with alternate sources of lysine and tryptophan to alleviate protein deficiency (Prasanna et al., 2001; Sofi et al., 2009).

Improving the poor nutritional value of maize had been a concern for a long time by breeders (Osborne and Mendel, 1914). Efforts to improve maize nutritional value

through conventional breeding had not been simple. It necessitated heavy investments for sustained research. It is in this context that the story of QPM assumes significance. One of the milestones of the QPM breeding efforts was a successful collaboration and achievement among maize breeders that eventually resulted in the award of the 'World Food Prize' to Surinder K. Vasal and Evangelina Villegas in the year 2000 (Prasanna et al., 2001; Sofi et al., 2009).

Selection for special maize genotypes with unique amino acid profile in endosperm started early in the 1900. During that time two major problems were confronted: firstly, no specific genes responsible for improved amino acid profile of maize proteins were isolated for breeding. Secondly, the genetic systems were complicated to use backcross breeding to improve protein quality in maize (Sofi et al., 2009). However, some years later, a naturally occurring maize mutant named as opaque 2 (*o2*) with attributes of soft and opaque grains (Singleton, 1939) was identified in Connecticut maize fields in the USA. In the 1960s, Nelson and Mertz identified mutant maize lines with improved protein profile. A year after, researchers at Purdue University observed that mutant genotypes homozygous for *o2* allele had double lysine level in their endosperm compared to the normal counterparts (Mertz et al., 1964). These findings led to a successful genetic manipulation of protein quality gene for maize breeding (Nelson et al., 1965).

1.1.2 Adverse effects associated with *opaque-2* gene

After the discovery the 0_2 gene, maize breeders attempted to develop inbred lines and populations (Vasal, 2000; Prasanna et al., 2001; Sofi et al., 2009). However, it would not soon led to the development of quality protein maize. As such development of superior quality protein maize was slowed down and cultivars were not widely released and adopted (Lauderdale, 2000). The slow progress in breeding quality protein maize genotypes was associated with pleiotropic effect of the 0_2 gene. This gene is pleiotropic to soft endosperm development. This, in turn, results in damaged and poor quality kernels with increased susceptibility to field and storage pests and fungal diseases, inferior food processing, and poor harvest ability (Bjarnason and Vasal, 1992; Toro et al., 2003). There is also a corresponding decline in maize grain yield because of kernel weight reduction of genotypes with the

0_2 gene (Singh and Venkatesh, 2006). In developing countries where farmers used to grow flint and dent grained maize, the poor kernel phenotype of the *o2* genotypes was a major barrier for their wide-scale acceptance (Prasanna et al., 2001; Toro et al., 2003).

1.1.3 Genetic manipulation to ameliorate problems associated with *opaque-2*

The side effects associated with *o2* gene impacted negatively the research and development endeavours of nutritionally enhanced maize (Prasanna et al., 2001). However, CIMMYT's researchers and a few others from the University of Natal, South Africa, continued searching for maize genotypes that had high lysine and tryptophan content and improved endosperm phenotype (Sofi et al., 2009). Consequently, the discovery of the '*o2* endosperm modifier genes' was the other important milestone in the history of maize improvement to enhance protein quality (Atlin et al., 2011). The endosperm modifier genes alter the soft chalky nature of *o2* mutants into a normal hard (vitreous) phenotype. Paez et al. (1969) were the first to report on endosperm modification of kernels caring the *o2 gene* by selecting 50% translucent and 50% opaque segregants. Different endosperm modifier genes with varying effects of modification levels were subsequently discovered enabling selection of genotypes with good grain characteristics and high lysine content (Paez et al., 1969; Annapurna and Reddy, 1971; Bjarnason et al., 1976; Lodha et al., 1976). This helped in overcoming one of the major problems of popularization of quality protein maize (Prasanna et al., 2001; Sofi et al., 2009; Atlin et al., 2011). It is believed that the modifier genes interact each other to improve grain hardiness and appearance which, in turn, increase kernel weight and density (Sofi et al., 2009). However, endosperm modifiers are known for their complex nature of inheritance (Larkins et al., 1995) though efficient selection methods are available to detect genetic modification in *o2* genotypes helpful in avoiding the adverse effects of the opaque phenotype (Prasanna et al., 2001).

1.1.4 Breeding efforts and progress in QPM development and adoption

Two breeding approaches were followed to identify high frequency modified kernels in *o2* converted maize (Bjarnason and Vasal, 1992; Vivek et al., 2008). The first was

intra-population selection for genetic modifiers in *o2* backgrounds using various selection procedures such as full-sib and modified ear-to-row systems as suggested by Lonnquist (1964). Selection was practised for modified ears and modified kernels at all stages. The second approach involved recombination of pools formed from various modified *o2* sources. Selection for modified ears with high frequencies of modifier genes and good protein quality was done for three to four cycles. Eventually, a high level of kernel modification was reached in these materials in the 1970s (Bjarnason and Vasal, 1992; Prasanna et al., 2001). The materials from these two approaches were used as donor breeding stocks for conversion programmes. Through these breeding efforts a few tropical populations and highland maize composite were developed. The donor stocks were used in large scale QPM conversion programs to convert germplasm from wide genetic backgrounds found in various target environments (Prasanna et al., 2001; Sofi et al., 2009; Atlin et al., 2011).

Due to the complex inheritance of modifier genes a modified type of backcross breeding procedure was adopted to breed for quality protein maize. This breeding scheme is referred to as 'modified back crossing-cum-recurrent selection' (Vasal et al., 1980; Vasal et al., 1984). Important selection criteria during conversion include grain yield, kernel modification and appearance, reduced ear rot incidence, rapid drying and other desirable characteristics. These traits were lacking in the initial o_2 genotypes. As a result, various QPM germplasm pools and populations were developed by CIMMYT for breeding (Bjarnason and Vasal, 1992; Sofi et al., 2009). This resulted in the release of a large number of QPM versions of elite tropical, subtropical and highland lines/populations with progressive reduction in the yield gaps and concurrent increase in the average kernel modification of QPM ears (Prasanna et al., 2001; Sofi et al., 2009). The Level of protein quality in the kernels was routinely checked and superior genotypes selected during all stages of improvement. Often no differences in appearance should be observed between the QPM and normal counterparts (Villegas et al., 1984; Villegas et al., 1992).

There are foreseeable advantages of development of QPM hybrids than open pollinated varieties (OPVs) (Prasanna et al., 2001) such as (a) exploitation of heterosis, (b) ease of regulating seed purity, (c) uniformity and stability in endosperm

modification of hybrids, and (d) requirement for minimum protein quality monitoring once purity is maintained in the parental genotypes. Hybrid breeding requires knowledge on heterotic patterns and combining ability of QPM germplasm to exploit heterosis (Vasal et al., 1993a; Vasal et al., 1993b). This facilitated QPM breeding by CIMMYT and national breeding programs in sub-Saharan African countries through evaluation of introduced QPM germplasm, conversion of locally adapted genotypes and pedigree breeding (CIMMYT., 2004; Krivanek et al., 2007). Consequently, several QPM hybrids were reported to be better yielding than the local non-QPM checks in trials designed for possible release (Sofi et al., 2009).

Presently, maize growing area using QPM cultivars is estimated to be 2.5 million hectares (Sofi et al., 2009). Several developing countries such as India, China, Honduras, Bolivia, Colombia, Ethiopia, Mozambique, Tanzania, Uganda, Zimbabwe, and South Africa, are participating in QPM Research and Development Network facilitated by CIMMYT (Prasanna et al., 2001; Sofi et al., 2009). The network focused on sharing knowledge on selection, evaluation, and demonstration of on-farm QPM trials to maize growers. Several QPM cultivars have been developed, tested and disseminated in most of these countries. For instance, in China, about 30% of maize area will be expected to be under QPM cultivars by 2020 (Gill, 2008). Progress in eastern Africa on QPM varietal development and adoption has been most rapid. In Ethiopia, the normal maize hybrid cultivar ‘BH660’ was successfully converted and its QPM counterpart ‘AMH760Q’ released in 2011 (Atlin et al., 2011; Twumasi-Afriyie et al., 2012). BH660 is the most popular three-way hybrid which accounts for 60 to 70% of annual hybrid seed sales and dominates the high-potential maize growing areas of Ethiopia. It is likely that the release of QPM version of this popular hybrid will lead to a significant increase in the production area of QPM.

1.1.5 Nutritional benefits

The nutritional benefit of QPM is very significant for millions of people in developing countries who rely on maize for their protein and energy requirements, and the fact that protein malnutrition is a severe problem in most of these countries (Graham et al., 1990). As first reported by Mertz et al. (1964), the lysine content in *o2* genotypes ranged from 3.3 to 4.0 g per 100 g of endosperm protein, and it was more than

double that of normal maize endosperm (1.3 g lysine/100 g endosperm protein). Further studies confirmed the superiority of QPM in protein quality and protein digestibility over non-QPM (Graham et al., 1980; Bressani, 1995; Paes and Bicudo, 1995; Gupta et al., 2009). Most of these studies generally indicated that the QPM protein contains 55% more tryptophan, 30% more lysine and 38% less leucine than non-QPM counterparts.

'Biological value', which is at 45% and 80% in non-QPM and QPM respectively, is another important quality attribute of QPM. It refers to the amount of absorbed nitrogen needed to provide the necessary amino acids for different metabolic functions (Bjarnason and Vasal, 1992). This means that in normal maize a protein intake of only 37% is being utilized when compared to 74% of the same amount in QPM. In order to guarantee nitrogen equilibrium, at least 125 g of QPM meal per day is estimated to be enough (Graham et al., 1980; NRC, 1988). However, this could not be possible by even doubling the amount of non-QPM (Prasanna et al., 2001). The nitrogen balance index for skimmed milk and *o2* maize protein is 0.80 and 0.72, respectively, which indicates that the protein quality of QPM is 90% of that of milk (Bressani, 1992, 1995).

The nutritional and biological superiority of QPM has also been exhibited in model systems such as rats (Mertz et al., 1965), pigs (Lodha et al., 1974; Maner, 1975), infants and small children (Graham et al., 1980; Graham et al., 1990; Gunaratna et al., 2010) as well as adults (Bressani, 1992), broiler chickens (Osei et al., 1994), and dairy cattle (Glover, 1992). Children suffering from a severe protein deficiency disease were recovered by feeding on a diet containing only QPM as the source of protein (Clark et al., 1977; Graham et al., 1990). A detailed study carried out in Ghanaian babies (0-15 months) given food supplemented with QPM and normal maize further witnessed that the QPM fed children were healthier, suffered fewer fatalities, and had better growth rates (Afriyie et al., 1997). A community based study was also conducted in the eastern Wollega Zone of Ethiopia by the Ethiopian Health and Nutrition Research Institute (EHNRI). In the study, growth was monitored in 160 young children of maize-producing families. The result showed that in such areas where maize is cultivated mainly for consumption, use of QPM in children's diets could enhance growth and development (Girma et al., 2010). QPM would have

equally beneficial effects on adults, as in case of infants and children (Bressani, 1992).

Apart from the key roles in human health, QPM could also be used to significantly reduce the protein supplement in animal feed (Prasanna et al., 2001). It provides a cheaper way of obtaining a balanced animal feed and fattening with excellent monetary returns (Krivanek et al., 2007). Other rewards of QPM include its industrial use as an ingredient in the preparation of composite flours to supplement wheat flour for bread and biscuit preparation (Prasanna et al., 2001). Overall, QPM possess superior protein quality and enhances food and feed efficiency.

1.2 Genetic diversity in maize

Maize (2n=2x=20) is one of the domesticated crop species with the highest level of molecular polymorphism. Nucleotide diversity of more than 5% was reported in certain loci of the maize genome (Henry and Damerval, 1997). The average sequence divergence between any two individuals for a given locus is referred as nucleotide diversity (Buckler and Thornsberry, 2002). The polyploidy origin and the abundance of transposons in maize could make it possible to study genome size evolution (Bennetzen et al., 2005).

Maize genetic diversity studies revealed that it is highly variable both within and across populations. DNA sequencing of the *adh1* locus, in *Z. mays ssp. parviglumis* (the maize progenitor), and *Zea luxurians* (a distant maize relative), showed that maize retained 77% of the diversity of *parviglumis* and is more diverse than *Z. luxurians* (Eyre-Walker et al., 1998). The molecular diversity of maize is approximately three to tenfold higher than that of other domesticated grass crops (Buckler et al., 2001). Several factors are suggested as reasons for this diversity in maize, *viz*., (1) variability of environments, culture, production system and the type of consumption of maize (Aguirre et al., 1998); (2) the high level of out-crossing in maize favours continuous gene exchange between neighbouring plants, and in some cases, with their wild relatives; (3) chromosomal duplications in maize are extensive, providing new mutational opportunities for creating greater phenotypic variability

(Helentjaris et al., 1998); and (4) transposons and retrotransposon elements also play a pivotal role in its genetic variation (Bennetzen et al., 2005).

1.3 Analysis of genetic diversity

Genetic variation and analysis are important components of crop improvement programs (Mohammadi and Prasanna, 2003; Kitti et al., 2012). Genetic diversity analysis is essential to: (i) determine the magnitude of genetic variation present among germplasm (Smith, 1984; Cox et al., 1986; Yunbi et al., 2009), (ii) identify suitable parents for crosses and create segregating progenies with maximum genetic variability for further selection (Barrett and Kidwell, 1998; Bertan, 2007), (iii) introgress desirable genes from diverse germplasm sources into the available genetic base (Thompson et al., 1998) and (iv) conserve unique and novel germplasm. Long-term genetic gain on economically desirable traits is possible by conserving and utilizing useful genetic diversity (Hallauer et al., 1988; Goodman, 2005; Smith et al., 2005). Critical assessment of the genetic relationships that may exist within and among the inbred lines or pure lines is useful to make successful cross combination, assigning lines according to heterotic groups, and for effective varietal protection (Melchinger et al., 1990; Flint-Garcia et al., 2009; Lu et al., 2009). Besides genetic diversity analysis facilitates grouping of accessions of a germplasm collections for specific breeding purposes (Hallauer and Miranda, 1988; Semagn et al., 2012).

Variation among individuals or groups of individuals or populations is analyzed using a specific method or a combination of methods of genetic diversity study (Mohammadi and Prasanna, 2003). Usually, data from phenotypic measurements and combinations of different types of variables are involved. Thus far diverse data sets are collected to analyze genetic diversity in crop plants. These include pedigree data (Bernardo, 1993; Van Hintum and Haalman, 1994), morphological data (Bar-Hen et al., 1995), biochemical data obtained from isozymes analysis (Hamrick and Godt, 1997), analyses of storage proteins (Smith et al., 1987), and DNA-based marker data that allow more reliable differentiation of genotypes (Melchinger, 1999; Mohammadi and Prasanna, 2003; Demissew et al., 2012). The choice of a particular

technique(s) depends on the aim(s) of the study, the level of resolution required, and availability of facilities (Mohammadi and Prasanna, 2003).

1.4 The use of simple sequence repeat markers in diversity analysis of maize

DNA based molecular markers such as simple sequence repeats (SSR) or microsatellites are used to identify specific sequence variation between two or more individuals (Lorz and Wenzel, 2008). They are not influenced by environmental factors and are also fast, efficient and more sensitive than field testing to detect large numbers of distinct differences between genotypes (Melchinger, 1999). The choice of the most appropriate marker system depends on the species, the objective and resources available (Lorz and Wenzel, 2008). Several DNA marker technologies have been developed and are available to study genetic diversity. Microsatellites or simple sequence repeats are one of the molecular marker systems which detect variation in the number of short repeat sequences, usually two or three base repeats (Lorz and Wenzel, 2008). They are widely preferred markers for genotyping, genome analysis and gene mapping in diverse crop species, including maize. These markers are PCR-based, co-dominant, locus-specific, highly reproducible, hypervariable, informative and relatively simple to use (Powell et al., 1996; Hamblin et al., 2007; Van-Inghelandt et al., 2010).

In both normal maize and QPM, SSRs have been proved to be a valuable tool for diversity measurements (Warburton et al., 2002; Kassahun and Prasanna, 2003; Liu et al., 2003; Legesse et al., 2007; Dhliwayo et al., 2009; Makumbi et al., 2011; Abera et al., 2012) and to assign inbred lines into heterotic groups (Enoki et al., 2002; Reif et al., 2003). SSR data from a number of loci have the potential to provide unique allelic profiles or DNA fingerprints for precisely establishing genotypic identity. They also have greater discriminatory power than Restricted Fragment Length Polymorphisms (RFLPs) markers, and can exhibit genetic relations that are reflective of the pedigree of the inbred lines (Smith et al., 1997; Senior et al., 1998). Genotyping of inbred lines using SSRs is a reliable way of germplasm characterization which, together with morphological descriptions, leads to unambiguous differentiation of genotypes that can be utilized for hybrid breeding program (Kassahun and Prasanna, 2003; Karanja et al., 2009; Dagne et al., 2013).

1.5 Heterosis, combining ability, and genotype-by-environment interaction

1.5.1 Heterosis

The term heterosis was coined by Shull (1952). It is defined as "*the difference between the hybrid value for one trait and the mean value of the two parents for the same trait*" (Falconer and Mackay, 1996). According to Miranda (1999), heterosis is the genetic expression of the superiority of a hybrid in relation to its parents. Two definitions of heterosis are reported in literature; namely, mid-parent or average heterosis, which is the increased vigour of the F_1 over the mean of two parents; and high-parent or better parent heterosis, which is the increased vigour of the F_1 over the better parent (Sinha and Khanna, 1975; Jinks, 1983). Assigning of maize inbred lines into heterotic groups is helpful to exploit heterosis or hybrid vigour (Stuber, 1994; Troyer, 2006; Flint-Garcia et al., 2009) particularly for grain yield (Osorno and Carena, 2008). Although several economically important crops benefit from the manifestation of heterosis, both the genetic and physiological mechanisms underlying this phenomenon are still unexplained (Hallauer and Miranda, 1988; Tollenaar et al., 2004; Osorno and Carena, 2008; Hallauer et al., 2010). Three major theories have been proposed including dominance, over-dominance and epistasis, to explain mechanisms underlying the phenomena of heterosis (Hallauer and Miranda, 1988; Singh, 2005). However, it is generally accepted that heterosis, to a large extent, is due to dominance gene action (Singh, 2005). To overcome many of the difficulties that are encountered in the interpretation of heterosis for complex traits, yield component analysis approaches have been applied used to study the effect of heterosis on grain yield (Sinha and Khanna, 1975). Grain yield has several components, for instance, number of ears per plant, number of kernels per cob, and kernel weight in an attempt to understand how heterosis influences grain yield (Sinha and Khanna, 1975).

Heterosis is dependent on level of dominance and differences in gene frequency. It has been extensively exploited in maize breeding (Troyer, 2004). The manifestation of heterosis depends on genetic divergence of the two parents (Hallauer and Miranda, 1988). Low grain yield heterosis is observed for crosses among genetically similar germplasm and for crosses among broad genetic base germplasm (Hallauer

and Miranda, 1981; Beck et al., 1991; Vasal et al., 1993a). High-level of heterosis was observed with increased divergence within a certain range, but that heterosis declined in extremely divergent crosses (Moll et al., 1965; Prasad and Singh, 1986; Melchinger, 1999; Hallauer et al., 2010). Genetic divergence of the parents is inferred from the heterotic patterns manifested in a series of crosses (Moll et al., 1965; Hallauer and Miranda, 1988; Miranda, 1999).

1.5.2 Combining ability

The concept of general combining ability (GCA) and specific combining ability (SCA) was first introduced by Sprague and Tatum (1942), and later elaborated by Hallauer and Miranda (1988). GCA refers to the average performances of parents in cross combinations and SCA is the deviation of individual crosses from the average performance of the parents involved. The additive portion of genotypic variance is related to the general combining ability (GCA), determined by mean hybrid performance of a line. The non-additive portions such as dominance and epistasis relate to the specific combining ability (SCA), a measure for cases where some hybrid combinations are better, or worse, than expected based on mean performance of the lines involved. The diallel mating design is among the most widely used genetic designs appropriate to estimate the magnitude of the GCA effects of parents and the SCA effects of their crosses for yield and yield components (Griffing, 1956; Hallauer and Miranda, 1988; Hallauer et al., 2010). In this design, a set of lines are crossed pair-wise in all possible combinations, providing an assessment of their relative merits to guide selection and testing schemes for the trait under consideration.

Information on the combining ability of maize germplasm is of great value to maize breeders. GCA and SCA effects are important indicators of the potential value of inbred lines in hybrid combinations (Sprague and Tatum, 1942). Combining ability of inbred lines is the ultimate factor determining future usefulness of the lines for hybrid development (Hallauer and Miranda, 1988; Hallauer et al., 2010). Besides, combining ability studies allow classification of selected parental materials with respect to breeding behaviour (Hallauer and Miranda, 1988; Sleper and Poehlman, 2006). Using the concept of combining ability, genetic variance is partitioned into two

components: variance due to GCA and variance due to SCA (Hallauer and Miranda, 1988; Hallauer et al., 2010). The relative importance of additive versus non-additive effects in diallel crosses is an indication of the type of gene action (Baker, 1978; Hallauer et al., 2010). The greatest proportion of total genetic variance can be attributed to additive effects for most agronomic traits in maize (Hallauer et al., 2010).

1.5.3 Genotype-by-environment interaction (GEI)

The performance of a cultivar is a function of the genotype and the nature of the production environment (Cooper and Byth, 1996). Environmental factors have greater effect on quantitative traits than on qualitative traits, as a result of which performance evaluations of potential cultivars are conducted in multiple seasons/years and locations (Bernardo, 2002). In addition to genotype and environment main effects, performance of cultivars is largely influenced by the genotype-by-environment interaction (GEI). GEI is the differential response of cultivars to environmental changes (Hallauer and Miranda, 1988; Crossa et al., 1990; Vargas et al., 1999). Various biotic and abiotic stresses have been implicated as causes of GEI. Fluctuation in growing temperatures, seasonal rainfall amount and distribution, length of growing season, within-season drought, sub-soil pH and socio-economic factors that result in sub-optimal input use are often the causes of GEI in maize production in Africa (Banziger et al., 2006). Multi-environment trials (METs) are systematic approaches exploited to identify promising new cultivars with average yield stability in representative growing or test environments (Shakhatreh et al., 2001; Yan et al., 2007). GEI is complex and often it represents a significant impediment to genetic improvement in crop breeding programs (Basford and Cooper, 1998).

The relative magnitude of genotype x environment provides information concerning the likely area of adaptation of a given genotype. It is also useful in determining efficient methods of using time and resources in a breeding program (Ceccarelli, 1989; Yan and Kang, 2003). Consequently, improving a resistance or tolerance of a given genotype to different stresses to which it would likely be exposed may minimize GEI (Kang, 1998). Large GEI is expected when genotypes are grown under

a wide range of environments and outside their normal zone of adaptation (Beck et al., 1991). Selection of multi-environment sites to sample stresses adequately, where GE interaction are major sources of variation, is a critical step in a successful breeding program (Edmeades et al., 2006; Yan and Holland, 2010). The existence of GEI thus necessitates breeders to evaluate genotypes in more than one environment in order to obtain repeatable rankings of genotypes with average yield stability (Hallauer and Miranda, 1988; Yan and Kang, 2003). However, GEI becomes of practical significance only when crossover interactions occur (Crossa and Cornelius, 1997). Crossover interactions occur in evaluation trials when ranks of cultivars change across environments (Russel et al., 2003; Frashadfar et al., 2012; Nzuve et al., 2013). The Additive Main Effect and Multiplicative Interaction (AMMI) (Zobel et al., 1988) and genotype and genotype-by-environment interaction (GGE) biplot (Gabriel, 1971) are multivariate methods which are widely applied to analyze complex set of GE data obtained from METs (Gauch and Zobel, 1996; Yan et al., 2000).

1.6 Importance of participatory research in QPM breeding

In recent years, there has been an increasing interest towards participatory research in general, and participatory plant breeding (PPB), in particular. This interest is partly associated with the perception that the impact of agricultural research, including plant breeding, has been below expectations, particularly in developing countries and for marginal growing environments and poor farmers (Ceccarelli, 2011). The main reason for the limited impact of plant breeding in marginal environments is the existence of large interactions between the performance of breeding materials in research stations (the selection environment) and in the field of poor farmers of marginal areas (the target environment) (Ceccarelli, 2011). Scientists have become increasingly aware that end users' participation in technology development may substantially increase the probability of adoption of the technology (Rhoades and Booth, 1982; Ceccarelli, 2011). The main rationale for PPB in developing countries' agriculture is the existence of important cropping systems in marginal regions where the adoption of modern varieties is low (Waker, 2007). PPB has evolved mainly to address the difficulties of small-scale farmers in developing countries (Ceccarelli, 2011). PPB is, therefore, defined as "*that type of plant breeding in which farmers, as*

well as other partners, such as extension staff, seed producers, traders, and NGOs, participate and collaborate in the development of a new variety, is expected to produce varieties which are targeted (focused on various typologies of partners), relevant (responding to real needs, concerns, and preferences), and appropriate (able to produce results that can be adopted)" (Bellon, 2006).

1.7 Conclusions

QPM has the potential to combat malnutrition and being a component of food security crop in sub-Saharan Africa. Research has shown that the yield gap between QPM genotypes and normal maize counterparts is no longer a concern. However, more research is needed to develop high and stable yielding and farmers-preferred QPM varieties across the highland regions of east Africa. This chapter reviewed important aspects of breeding maize in general, and QPM, in particular. There is still research need to know the effect of conversion processes of normal maize inbred lines into QPM on the existing heterotic groups of inbred lines. The review also highlighted the purpose of studying genetic diversity in crops including maize using a combination of different methods. The combined use of molecular markers that allow direct comparison of the similarity or variability of inbred lines with morphological and combining ability studies facilitate the classification of inbred lines into distinct heterotic groups. The SSR markers are widely used to study genetic relationships for heterotic grouping because of their high genetic information content, high reproducibility, and simplicity to use. Separation of inbred lines into heterotic groups helps to make designed crosses and to improve selection gains.

It was also reviewed that conducting a combining ability study on F_1 hybrids can lead to the understanding of the underlying gene actions and thus which breeding strategy to follow. This is done through evaluation of crosses in multi-environment trials for precise estimation of the genotype and environmental main effects and their interaction. In this review, participatory research approach is also highlighted to enhance the potential for adoption of newly developed QPM varieties. In general, all the information included in this chapter may serve as background information for quality protein maize breeders towards developing improved cultivars with high yield, using the conventional approach and molecular markers.

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CHAPTER 2

Farmers' perceptions of maize production systems and breeding priorities, and their implications for the adoption of new varieties in selected areas of the Highland agro-ecology of Ethiopia

2.1 Abstract

Maize (*Zea mays* L.) plays a critical role in smallholder food security in Ethiopia. Its production is rapidly increasing to the Highlands of Ethiopia where it has been a minor crop in the past. This study aimed to assess the magnitude and production systems of Highland maize, farmers' production constraints, and their implications for the adoption of new maize cultivars in two zones of the Oromia Regional State, representing the Highland sub-humid agro-ecology of Ethiopia. A participatory rural appraisal (PRA) was conducted with eight peasant associations involving 160 experienced maize farmers from four districts during 2012. Primary data were collected through Focused Group Discussions (FGDs) and Semi-structured Interviews (SSI). Farmers' maize cultivar preferences showed that few adopted Highland cultivars are available. Instead a two-decade old cultivar, 'BH660', originally released for the mid-altitude agro-ecology, has been widely adopted in most Highland areas. As regards cultivars' trait preferences, non-significant variation ($P > 0.05$) was observed among farmers between the two study zones. Farmers (both men and women) in the study areas unanimously considered grain yield as the most important trait for maize cultivar selection. Major production constraints were also identified and listed by farmers, of which limited access to inputs (improved maize seeds and inorganic fertilizers), and late on-set and inadequate rainfall were the primary constraints across the study areas.

***Keywords*:** Ethiopia, Focused group discussion, Highland maize, Participatory rural appraisal, Maize traits.

2.2 Introduction

Agriculture is the mainstay of Ethiopia's economy. The majority of farmers operate mainly on small farms, in rain-fed cropping systems and livestock production. Small-scale farming predominately produces limited food to support the small-holder farmers and their family for consumption, leaving little to sell. Consequently, improving agricultural productivity is a national priority in Ethiopia. According to the Central Statistical Agency of Ethiopia (CSA, 2010), the low level of crop productivity in the country is attributed to inefficient farming methods that utilize outdated crop production and protection technologies, and fragmented pieces of land holdings . To bridge the gap between the demand and supply for food, and also to enhance the desired change in the livelihood of majority of the population, the government of Ethiopia launched a five year Growth and Transformation Plan (GTP). The plan includes transformation of the agricultural sector towards surplus-producing, market-oriented smallholder agriculture capable of achieving accelerated and sustainable economic development in the country (FDRE, 2011).

The need for food security and the diverse agro-climatic conditions in Ethiopia have prompted the majority of subsistence farmers to grow various crops. One major shift is that over the past 20 years, the area dedicated to maize cultivation in Ethiopia has expanded progressively. Of the major cereal crops, maize ranks second to tef [*Eragrostis tef* (Zucc.)] in area and first in production (Mosisa et al., 2002; Mosisa et al., 2012). Over the last 20 years, the area under maize has increased by about 50% and production by 66%, with the national average yield of maize increasing from 1.6 to 3.0 t ha^{-1} (CSA, 2011). The per capita consumption of maize is 60 kg $year^{-1}$ per annum in Ethiopia (Mosisa et al., 2012). Maize is therefore a crucial for Ethiopia in the short and medium term, and the GTP proposes a maize doubling of production by 2015 (FDRE, 2011).

Although a substantial quantity of maize is produced in the lowland areas, most maize is grown in the most productive agricultural lands in the mid and highland areas of the country. The crop is increasingly grown to the Highlands of Ethiopia where it has been a minor crop in the past. The high altitude, sub-humid maize agro-ecology (1800-2400 m.a.s.l.) in Ethiopia is estimated to cover 20% of the land

devoted to annual maize cultivation. More than 30% of small-scale farmers in this agro-ecology depend on maize production for their livelihoods (Twumasi-Afriyie et al., 2002). To meet the needs of increasing maize production in the Highlands of Ethiopia, the Ethiopian Highland Maize Breeding Program was established with the support of the International Maize and Wheat Improvement Center (CIMMYT), in 1998. The program is based at the Ambo Plant Protection Research Center of the Ethiopian Institute of Agricultural Research. It is aimed at developing and popularizing improved Highland maize cultivars, and enhancing their crop management technological packages.

From 1999 to 2011, the breeding program released five superior Highland maize hybrids including: AMB02SYN1-'*Hora*', AMH800-'*Arganne*', AMH850-'*Wenchi*', AMH851-'*Jibat*', and AMH760Q-'*Webi*', for large-scale production. AMH760Q was released as quality protein maize (QPM) hybrid, which was developed from the most popular, top-yielding non-QPM hybrid 'BH660'. Over 5.8 million hectares of potential suitable land was identified for the highland maize hybrids in the country (Demeke et al., 2012). However, the hybrids have not been aggressively popularized, and have not been adopted by farmers in the Highlands. Reasons for the slow rate of adoption include: (1) lack of awareness of the released cultivars in the Highland environment, and (2) the carryover effect of lack of awareness of the cultivars has resulted in untargeted seed distribution of the previously released mid-altitude maize cultivars in the Highlands. According to Dawit et al. (2010), one of the main reasons for seed leftovers in either public or private seed stocks during high demand has been associated with the limited efficiency of targeting seed production and distribution in Ethiopia. It is also believed that some superior cultivars that have been released might not have been adopted because of lack of sufficient considerations of farmers' preferences in their development process (Banziger and Cooper, 2001; Derera et al., 2006). Participatory rural appraisal (PRA) is a research tool to integrate farmers' perceptions, constraints and preferences into agricultural research so that improved technologies can be developed that become widely adopted (De Groote and Bellon, 2000).The objective of the current study was, therefore, to assess the magnitude of production of maize, the systems used and the farmers' production constraints, and their implications for the adoption of new maize cultivars in two zones of the Oromia Regional State representing the Highland, sub-humid agro-ecology of Ethiopia.

2.3 Methodology

2.3.1 Study area description

A participatory rural appraisal study was conducted in 2012 in two administrative zones, i.e., West Shoa and East Arsi of the Oromia Regional State of Ethiopia. The zones were selected based on their experience of growing Highland maize cultivars and local varieties. There are 18 and 24 districts in the West Shoa and East Arsi zones, respectively. The districts are situated near the Highland maize research centres because of the extension and popularization work done by the research centres in these districts. Previously released Highland maize cultivars widely grow in these districts. Further, the areas represent a High altitude, sub-humid agro-ecology with altitudinal ranges between 2000 and 2400 mean above sea level. The study zones are known for their agricultural potential particularly the rain-fed production of small grain cereals (wheat, tef, barley). In these zones, maize is largely grown as a rain-fed crop, although a few farmers in West Shoa also produce maize using local irrigation schemes during the off-season. In most years, both zones receive enough rainfall for crop production in the main season. The 'Meher' season, which encompasses June to October, is the main cropping and rainy season for both zones. Five major cereal crops, namely tef (*Eragrostis tef* Zucc.*)*, maize (*Zea mays* L.), sorghum (*Sorghum bicolour* L.), wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) are grown in the West Shoa and East Arsi zones. In the West Shoa zone, maize ranks second to tef in cultivation area, while it ranks fourth after wheat, barley, and tef in the East Arsi zone, which is a wheat belt of Ethiopia.

2.3.2 Sampling procedures

A multi-stage sampling was carried out whereby two districts (administrative units below the zonal level) in each administrative zone were selected. Ambo and Toke-Kutayei districts represented the West Shoa zone, and Tiyo and Hitosa, the East Arsi zone. From each district, two sub-districts or peasant associations (PAs) were further selected making the total number of eight PAs (Table 2.1). Furthermore, 20 farmers from each PA were interviewed, providing a total of 160 farmers for the study. The selections from the zonal through the districts, PAs and farmers levels were based

on the criterion of a long production background of the farmers in Highland maize production. Individual farmers were selected from each PA representing various socio-economic backgrounds (data not shown) and both genders. Thus the farmers selected for this micro-study were representative of the highland maize farmers in the two zones.

2.3.3 Data collection and analyses

All primary data were collected through the PRA process. The secondary data was obtained from the Central Statistical Agency of Ethiopia (www.csa.gov.et). Local extension staff and sub-district managers at the respective localities facilitated the PRA process by mobilizing farmers to participate on the Focused Group Discussions (FGDs). FGDs, accompanied by semi-structured interviews (SSI), were held in January- February 2012 at eight sites, one per sub-district. Each FGD and SSI

Table 2. 1 The selected study areas in West Shoa and East Arsi zones of the Oromia Regional State in Ethiopia

Zones	Districts	Sub-districts (PAs)	Altitude (m)	No. of farmers	
				Women	Men
West Shoa	Ambo	Gosu Kora	2350	3	17
		Boji Gebisa	2250	2	18
	Toke-Kutayei	Kolba-Lencha	2350	2	18
		Birbirsa-Dogoma	2200	3	17
East Arsi	Tiyo	Gora silingo	2300	4	16
		Oda dewata	2280	9	11
	Hitosa	Sheki Sherera	2250	-	20
		Oda Jila	2300	2	18

PA=peasant association

meeting was held outdoors and started with a minimum of 15 participants who varied in gender and age (data not shown). In the course of the process, the number increased to 20 participants. The farmers sat together and discussed maize farming issues that were common and general in nature. They were also interviewed individually to explore more specific issues and particularly to deal with issues that were traditionally sensitive to the farmers. Both the FGDs and individual farmers' interviews were held in local language, "*Afan Oromo*", using semi-structured

questionnaires designed to collect data, as well as to guide the discussions, yet to allow the participants' freedom to raise relevant issues of their own. The FGDs focused mainly on the following four themes: farm characteristics which highlighted the importance of maize for Highland farmers, farmers' preferences for named maize cultivars, traits of interest in maize cultivars and the perceived constraints affecting maize production.

The FGDs with farmers included questions involving the main cropping season, the major crops grown, the benefits of maze for Highland farmers, and ranking of the major crops based on estimated farm operations, which later involved matrix ranking following Ceccarelli's (2012) procedure. Further, farmers were asked to list their main criteria for maize cultivar selection, ranking the criteria, and to identify the preferred traits found in the maize varieties they had been growing. The scale used for the assessment of the selection criteria was: 1 = poor, 2 = fairly good, 3 = good, 4 = very good, 5 = best and 6 = excellent. An independent sample t-test was run to test equality of means of ranks obtained from the eight PAs of the two zones for each preferred trait. Using grain yield as the criterion set by farmers, the maize cultivars known to farmers and those commonly grown in the study areas were also listed and ranked one up to six depending on the number of cultivars and score interval at the specific locations- one being the worse, three for good, and six for excellent. The FGDs also helped to identify and prioritize production constraints for Highland maize.

Household interviews included questions such as the area of land cultivated by each farmer, and the proportion of land devoted to maize cultivation. Descriptive statistics were applied to the data, together with a Pearson correlation analysis to test for an association between the area of land cultivated and the proportion devoted to maize production. Farmers' awareness of quality protein maize (QPM) and their interest in QPM were emphasized during the FGD. Accordingly, attitudes towards QPM were determined from each respondent farmer using a 4-point scale where 1 = 'strong acceptance', 2 = 'acceptance', 3 = 'neutral', and 4 = 'negative'. These attitudinal data were subjected to a statistical test for independency in a 4 x 4 contingency table. SPSS (version 19) computer software (SPSS, 1989-2010) was used to perform statistical analyses for this study.

2.4 Results

2.4.1 Farm characteristics and maize production systems

2.4.1.1 Importance of growing maize

In all the study areas land has been used for growing crops, livestock grazing, human settlement and afforestation (data not shown). As expected, the '*Meher*' season was the only major maize growing season. During this season, famers grow other major cereal crops including wheat, tef, barley, sorghum as well as pulse and oil crops. During group discussions, farmers expressed their views that the experience of maize farming as a homestead crop in all the study areas dated back to more than half a century. However, it was only a few decades since maize became a major field crop in the Highland environment.

Farmers listed six primary reasons for increasing maize production in their area. Firstly, due to its high productivity relative to other cereal. Secondly, maize is versatile as a food. Thirdly, surplus maize is also a cash crop which can be sold to generate household income either as green cobs or as grain after satisfying the households' food requirements. Fourthly, maize is used for livestock feed using maize stover. Fifthly, it is used in crop rotation systems with small cereals such as wheat and barley. Sixthly, it provides an edible crop earlier than barley and wheat, filling a critical "hunger" period.

2.4.1.2 Area under maize production

In the study areas most farmers allocated a substantial portion of their lands for maize production (Figure 2.1). For instance, a relatively larger area (0.75 ha) was allocated to maize by several subsistence farmers in the three PAs in the West Shoa zone. Maximum values of up to 1.5 ha were also recorded for a few farmers in one of the PAs ('*Birbirsa-Dogoma'*) in the West Shoa zone, where 75% of the respondent farmers grow maize on >0.5 ha of land. On the other hand, a maximum land allocation of 0.5 ha was recorded for most of the PAs in the East Arsi zone, although one farmer allocated 0.75 ha to maize. Minimum areas under maize cultivation

ranged from 0.125 to 0.25 ha, and 0.1 ha were also recorded for the PAs in the West Shoa and East Arsi PRA zones, respectively.

An associational study between the area of land owned by farmers and the area allocated to maize suggested a highly significant and positive correlation (r = 0.41, P< 0.01) (Figure 2.2). In general, as the size of the total land holding (farm) increased, the size of land allocated for maize production also increased. However, there were incidences of exceptional farmers who allocated less land for maize production. Considering the 80 farmers in each PRA zone, the average total land owned by individual farmers (or mean farm size) was: 1.7 ha (*Oda Dewata*), 2.4 ha (*Gora Silingo*), 2.6 ha (*Sheki Sherera*), 3.4 ha (*Oda Jila*), 3.8 ha (*Kolba-Lencha*), 3.9 ha (*Gosu Kora*), 3.6 ha (*Boji-Gebisa*), and 2.5 ha (*Birbirsa-Dogoma*). Similarly,

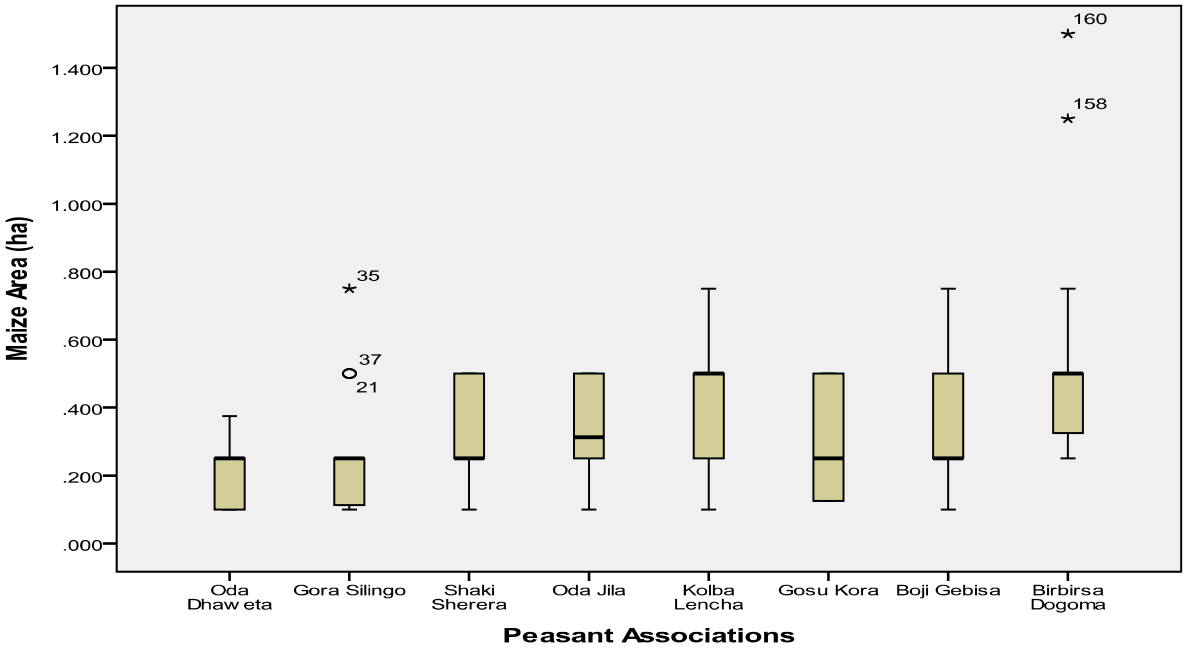

Figure 2. 1 Mean area (ha) allocated to maize production among eight peasant associations in two administrative Zones of the Oromia regional state of Ethiopia.

the corresponding mean maize production area on each farm was: 0.19 ha (*Oda Dewata*), 0.24 ha (*Gora Silingo*), 0.30 ha (*Sheki Sherera*), 0.34 ha (*Oda Jila*), 0.41 ha (*Kolba-Lencha*), 0.30 ha (*Gosu Kora*), 0.38 ha (*Boji-Gebisa*), and 0.53 ha (*Birbirsa-Dogoma*). Hence, more land was allocated to maize in the West Shoa zone than the East Arsi zone.

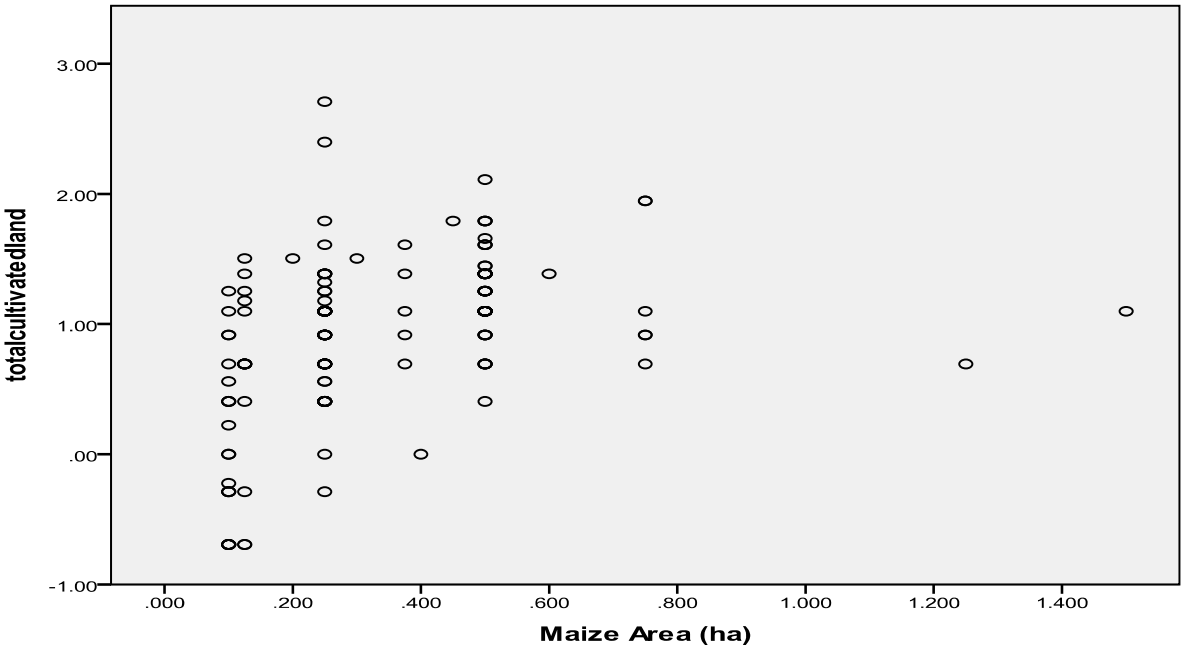

Figure 2. 2 Association between the total cultivated land and area allocated to maize production

2.4.1.3 Farming activities

Farmers in the study areas were also asked to rank the primary farm operations for the major cereal crops grown. This was done based on their estimation of costs involved per activity by crops (Table 2.2). A matrix ranking approach was used which showed that maize ranked on the top in terms of cost estimations for two out of the five farm operation, namely planting, and weeding and/or cultivation. Farmers explained that the cost of these operations was highest for “improved” varieties of maize. Seeds of improved maize varieties are manually planted in rows and then require 2-3 rounds of fertilizer application and cultivation for weed control. The farmers needed to hire labour, or to request assistance from neighbouring households, during critical periods of the cropping season. Because of this, some of the farmers explained that they usually prefer to plant their own local varieties which demand less crop management practices, and thus lower production costs and labour demands.

During group discussions, women farmers were requested to provide their crop preferences for use as food. All the groups in all areas unanimously chose maize. Women confirmed that maize could be consumed three times a day by the households’ family either as green cob or as a processed product mixed with other

cereal crops such as wheat and tef. However, the groups also noted that maize could not be consumed alone because of its low nutritive value and fast digestibility that result in hunger soon after feeding. The farmers' groups added two more reasons why they used maize as a staple crop: Firstly, because it matures early, maize plays a critical role in providing food to farmers and their families during a three month "hunger period" when the other cereal crops (wheat, tef and barley) are maturing and cannot be consumed. This is also the time when grain reserves from the previous season have started to run out; Secondly, the other cereal crops such as tef and wheat fetch better prices than maize in these areas, which persuades farmers keep the maize for home consumptions, and to sell the other cereals.

2.4.2 Maize cultivar preferences and farmers-preferred traits

2.4.2.1 Types of cultivars

Table 2.3 shows the maize cultivars and local varieties that were known and listed by farmers during the group discussions. A few cultivars were known to most farmers but were grown only in small quantities in almost all the study areas, with the exceptions of one cultivar 'BH600', which was widely grown in substantial quantities. Among the varieties grown on a small area of land (< 0.25 ha) by a limited number of farmers were the relatively recently released Highland maize cultivars such as *Arganne, Wenchi, Jibat, and Hora*, which were still being grown in demonstration plots. In contrast, local varieties such as 'Oromei' are widely grown by most homesteads. BH660 is a long season three-way hybrid developed for the mid-altitude and transitional highland agro-ecologies, with a wide adaptation spanning an altitudinal range of between 1600-2200 m.a.s.l. On the other hand, the four highland maize cultivars, *Arganne, Wenchi, Jibat, and Hora*, were released for transitional to true highland agro-ecologies. However, their adoption has been very limited. The first three are hybrids, while the fourth is an open pollinated variety (OPV). *Kuleni* and *Katumani* are OPVs with contrasting adaptations. The former was adapted to mid-altitude and transitional highland areas while the latter is an old variety initially introduced from Kenya for drought stressed areas in Ethiopia. From the group discussions, it was clear that *Katumani* was only grown to a small extent by some farmers in the East Arsi zone. Not only *Katumani* but also other OPVs including *Hora*

and *Kuleni* had better acceptance by farmers in the East Arsi compared to the West Shoa zone where hybrids were more preferred than the OPVs.

Table 2. 2 Ranking of the cost of farm operations for cereal crops

Farm operations	Crops	Individual rank based on cost estimations*					Rank index	Overall rank
		1	2	3	4	5		
Land preparation	Maize	35	20	70	5	30	455	3
	Wheat	15	5	20	80	40	605	2
	Tef	15	10	20	45	70	625	1
	Barley	40	75	20	10	15	365	4
	Sorghum	55	50	30	20	5	350	5
Purchase of seed	Maize	45	70	20	10	15	360	4
	Wheat	20	10	15	43	72	617	1
	Tef	10	5	25	82	38	613	2
	Barley	35	20	65	10	30	460	3
	Sorghum	50	55	35	15	5	350	5
Planting	Maize	10	7	15	50	78	659	1
	Wheat	35	20	70	5	30	455	3
	Tef	20	8	25	75	32	571	2
	Barley	40	70	20	15	15	375	4
	Sorghum	55	55	30	15	5	340	5
Weeding and cultivation	Maize	10	10	25	35	80	645	1
	Wheat	50	62	25	15	8	349	4
	Tef	15	5	20	90	30	595	2
	Barley	50	63	25	15	7	346	5
	Sorghum	35	20	65	5	35	465	3
Harvesting and transportation	Maize	47	61	30	13	9	356	4
	Wheat	6	9	25	86	34	613	2
	Tef	20	12	13	39	76	619	1
	Barley	37	18	63	10	32	462	3
	Sorghum	50	60	29	12	9	350	5

Notes: Rank index = Summations of rank × number (Ceccarelli, 2012); * Where 1 = low cost, 3 = intermediate, and 5 = high cost based on the farm operation under question

Table 2. 3 The maize cultivars listed and ranked by famers in the study zones based on grain yield during FGD

Zones	Peasant Associations	Cultivars and year of release							
		BH660 (1993)	Arganne (2005)	Wenchi (2007)	Jibat (2009	Hora (2005)	Kuleni (1995)	Katumani	Local*
East Arsi	Gora Silingo	6	4	-	-	5	3	2	1
	Oda Dewata	4	-	-	-	3	-	2	1
	Sheki Sherera	6	4	√	√	5	3	2	1
	Oda Jila	√	4	√	-	3	2	-	1
West Shoa	Gosu Kora	6	3	5	4	2	-	-	1
	Boji Gebisa	5	4	2	√	3	-	-	1
	Kolba-Lencha	4	2	3	√	-	-	-	1
	Birbirsa-Dogoma	5	2	4	-	3	-	-	1

* = different types of varieties; √ = the cultivar was known by a few farmers; 1 = worse, 2 = fairly good, 3 = good, 4 = very good, 5 = best, and 6 = excellent.

In all the study areas, farmers indicated that they had used several local varieties for more than half a century (Table 2.3). The source of these varieties was unknown but they had been grown in small areas around homesteads where the soil fertility was reasonably good and farmers do not need to apply inorganic fertilizers. Farmers do not apply inorganic fertilizers to local varieties because (1) these varieties are considered potentially low yielders and consequently farmers do not want to incur fertilizer costs; and (2) the farmers believed that their local varieties would start to "want" fertilizer like the improved varieties that were developed by research centres under conditions of high fertility.

2.4.2.2 Trait preferences

The mean rankings of farmers-preferred traits for maize cultivars are presented in Table 2.4. A non-significant difference ($P>0.05$) was observed between farmers in ranking their preferences of cultivars' trait in the study zones. High yield was the most important criterion used in selection of cultivars in the study areas. Most of farmers (98 %) in all PAs, except *Sheki Sherera* and *Gora Silingo,* identified the maturity period of cultivars as the second most important after yield, while plant height was ranked second to yield by 70% of the farmers in two PAs, *Sheki Sherera* and *Gora Silingo* (data not shown). Farmers of these two PAs strongly preferred intermediate plant height after yield and explained that short statured cultivars were

more prone to attacks by either wild or stray domestic or wild animals such as dogs and porcupines than an intermediate or a tall variety.

Table 2. 4 The most important traits of maize varieties, as ranked by farmers in both PRA zones

Best criterion	East Arsi Zone	West Shoa Zone	Overall	Sig.(2-tailed t-test)
Yield	5.75	6.00	5.88	0.356
Maturity	4.50	5.00	4.75	0.134
Plant height	4.50	4.00	4.25	0.134
Storability	1.50	1.25	1.38	0.537
Marketability	2.50	3.00	2.75	0.356
Feed	1.50	1.75	1.62	0.537

Ranking: 1 represented the least preferred and 6 the most preferred trait.

Conversely, tallness was not desirable because of the associated problem of lodging. On the other hand, most farmers who preferred early maturity as the second most important trait explained that they usually practice a relay cropping system whereby pulse crops, such as chickpea (*Cicer arietinum* L.) and grass pea (*Lathyrus sativus* L.), would be sown immediately after physiological maturity of maize and before the land dried out completely. Earliness is a relative term because the farmers preferred intermediate season cultivars to very short season cultivars. Almost all farmers in the study areas, however, do not want extra-early maturing cultivar because such a cultivar might be the first to mature in an area and therefore might suffer from exceptional animals' and bird damage.

Marketability ranked fourth among farmer-preferred traits. During group discussions, farmers explained that a cultivar whose grains have a glossy (flint-textured) characteristics and hard endosperm types command better acceptability in local markets than dent-textured and chalky types, when sold as both green and grain maize. Farmers' also considered local varieties to provide superior quality in the preparation of traditional beverages. But in terms of all other characters listed in Table 2.4, local varieties were considered inferior to the improved cultivars. In general, farmers in all PRA areas were not concerned much about storability and feed quality in maize and ranked them low. Farmers argued that they had not seen a maize cultivar with resistance to storage pests, nor with special qualities as feed for animals.

2.4.3 Major production constraints

The focused groups at each study area described important constraints to maize production. The groups also identified and listed the major constraints recognized and agreed on by most participants in the two study zones (Table 2.5). The constraints were listed in five categories, including pre-planting and planting of maize, vegetative growth, harvesting and consumption, and marketing. Farmers were then guided to prioritize the constraints under each category in each PA which, in turn, helped to establish the single most important constraint from each of the five categories so as to make pair-wise comparisons among the selected constraints independently for each PA. Overall, there were coincidences of similarity between PAs in their prioritization of constraints. PAs having similar priorities and rankings in their pair-wise comparisons were merged and presented in pairs in Table 2.6. The two most important production constraints were late and inadequate rainfall, and limited access to improved maize cultivars in all the study areas.

2.4.3.1 Inadequate rainfall

Farmers in all but two of the PAs (*Gora Silingo* and *Oda Jila*) explained that inadequate rainfall is a challenge to maize production in their area. In some cropping seasons farmers were forced not to plant maize and they panted the land prepared for maize with other small grain cereal crops. The consequence of not planting maize for a season usually led to hunger in their families especially in the months of July to September when no grain reserves remained.

2.4.3.2 Inefficient input distribution

All but two of the PAs (*Gosu Kora* and *Oda Dewata)* mentioned that poor seed distribution of improved cultivars, both temporal and spatial, by the extension system, cooperatives and unions was a major problem. There was also no alternative commercial seed available on the formal market. This situation was further exacerbated by the untargeted and limited capacity nationally for seed production and distribution that focused on few maize cultivars, both by the public and private seed sectors. Farmers further clarified that seed distributions through the

extension system have usually been effected very late in the cropping season after the rains had started and land preparations accomplished.

The same problem existed for fertilizer distribution. The price of inorganic fertilizers was a further issue and the key fertilizers were priced beyond their means, and their return from the maize they produced. On the other hand, when farmers responded to the question as to why should they use inorganic fertilizer if they could not afford the costs; they said that their farm lands have already been adapted to inorganic fertilizers and thus it would be unthinkable to grow improved cultivars without inorganic fertilizers. Although organic fertilizers such as compost and manure could, in theory, replace inorganic fertilizers, their reality was that the bulk required per unit area, and limited availability to farmers limited their large scale application, and dependency on inorganic fertilizers.

2.4.3.3 Low maize price

As summarized in Table 2.6, the low price of maize was the third most important constraint to farmers in *Birbirsa-Dogoma, Boji Gebisa*, and *Gosu Kora* PAs. The three PAs that ranked the low price of maize at the third level were the PAs in which the extent of maize production was relatively high in terms of area coverage. However, individual farmers of these PAs had decided not to increase the magnitude of their present production levels, given that the prevailing price of maize. This was unattractive for several reasons, including imbalance between supply and demand of maize, and lack of a year-round market. In seasons of bumper maize production, supply exceeded demand, which resulted in maize being left to rot both in the field and storage.

2.4.3.4 Biotic constraints

Post-harvest loss due to weevils was perceived as the third most important constraint to *Kolba-Lencha, Sheki Sherera, Oda Dewata, Gora Silingo*, and *Oda Jila* farmers. Out of these five PAs, the former (*Kolba-Lencha)* was from West Shoa and the latter four were from the East Arsi zone, suggesting the universal importance of this constraint in both zones. In contrast stalk borer is a localized problem. It was

Table 2. 5 Maize production constraints identified and listed under five categories by farmers during FGD in the study areas

Constraints	Pre-planting to planting	Seedling to harvesting	Post-harvest	Consumption	Marketing
Descriptions	- Late onset of rain - Shortage of rain - Limited access to improved seed & high cost of inorganic fertilizers - Untimely distribution of inputs - Weak extension services	- Stalk borer - Shortage of rain - Weak extension services - Wild and domestic animals	- Weevils - Termites - Rodents	- Poor nutritional value - Poor baking quality	- Low maize price - Instability of maize price

Table 2. 6 Pair-wise comparisons of five major constraints identified and prioritized by farmers in the eight PAs

PAs	Constraints	1	2	3	4	5	Total score	Rank
Gosu Kora	1. Late on-set and shortage of rain	*	1	1	1	1	4	1
	2. Stalk borer		*	3	2	5	1	4
	3. Weevils			*	3	5	2	3
	4. Poor baking quality				*	5	0	5
	5. Low price of maize					*	3	2
	Total						10	
Oda Dewata	1. Late on-set and shortage of rain	*	2	1	1		2	2
	2. Stalk borer		*	2	2		3	1
	3. Weevils			*	3		1	3
	4. Low price of maize				*		0	4
	Total						6	

Table (Cont'd)

PAs	Constraints	1	2	3	4	5	Total score	Rank
Kolba-Lencha and Sheki Sherera	1. Limited access to improved seeds	*	1	1	1	1	4	1
	2. Shortage of rain		*	2	2	2	3	2
	3. Weevils			*	3	3	2	3
	4. Poor nutritive value				*	5	0	5
	5. Low price of maize					*	1	4
	Total						10	
Gora Silingo and Oda Jila	1. Limited access to improved seeds	*	1	1	1	1	4	1
	2. Stalk borer		*	2	2	2	3	2
	3. Weevils			*	3	3	2	3
	4. Poor baking quality				*	4	1	4
	5. Low price of maize					*	0	5
	Total						10	
Birbirsa-Dogoma and Boji Gebisa	1. Shortage of rain	*	1	1	1	1	4	1
	2. Limited access to improved seeds		*	2	2	2	3	2
	3. Weevils			*	3	5	1	4
	4. Poor nutritive value				*	5	0	5
	5. Low price of maize					*	2	3
	Total						10	

Where, rank 1 stands for high priority and 5 for the least.

recorded as the most important problem ahead of all other constraints in *Oda Dewata,* and as the second most important constraint in *Gora Silingo* and *Oda Jila,* but it was not even mentioned as problem in the other PAs.

2.4.3.5 Poor nutrition and processing quality

Finally, the two quality constraints (poor nutritive value and baking quality) were ranked as the least important constraints. Farmers described the poor baking quality of maize in relation to the level of elasticity observed for one of the traditional and favourite food “Injera”, which is often made from maize flour. The “Injera” from maize is often crumbly after baking. To improve such character, farmers usually mix grains of maize with tef in varied proportions before milling. Tef is an indigenous and popular crop in Ethiopia well known for making good quality “Injera”.

With respect to the poor nutritive value of maize, farmers associated this with the rapid digestibility of maize, which would subsequently predispose the household’s family, especially of children, to hunger immediately after feeding on maize or maize products. Farmers further commented that there was no any weight gain by children being fed maize foods only. The farmers’ solution to this problem was to mix maize with other cereal grains such as wheat or tef in the preparation of staple food. However, when QPM (nutritionally enhanced maize) was put forward for discussion as a superior option against malnutrition the farmers were very eager to grow such varieties, provided that they would get comparable yields from the QPM varieties. Table 2.7 reveals farmers’ opinion towards QPM in the different study areas. The degree of acceptance of QPM was not the same across the four districts of the two PRA zones as determined by the significant chi-square test ($P < 0.01$, Pearson chi-square value = 30.022). Strong acceptance for QPM was exhibited by more district in West Shoa than in East Arsi, where neutral opinions were expressed. This suggested the need for an educational program to create awareness about QPM in the farm households at large, to reduce the number of households with either neutral or negative opinions on QPM.

Table 2. 7 Association between degree of acceptance to QPM and the four districts in two zones of the Oromia regional state of Ethiopia

Districts	Degree of acceptance*				Total
	strong acceptance	acceptance	neutral	negative	
Tiyo	6	11	15	8	40
Hetosa	7	14	17	2	40
Toke-Kutaye	21	12	5	2	40
Ambo	16	11	6	7	40
Total	50	48	43	19	160

* Degree of acceptance is statistically significant at the $P < 0.01$ and degrees of freedom (df) = 9

2.5 Discussion

In the past, small cereal crops such as tef, wheat and barley were the most widely grown food crops in the Highlands of Ethiopia, especially in the West Shoa and East Arsi zones, where maize used to be considered a homestead crop of minor importance. To date maize has become the most important cereal crop of the Highland farmers in Ethiopia. The major reasons have been described earlier. Furthermore, the price of maize and other food crops become high during the planting season of the small grains, and before their harvest (July-October) in Ethiopia. This is the time when farmers and their families suffer from a serious shortage of food. Consequently, the green maize is a vital crop that helps to supply early season food. In another PRA study conducted in the *Dendi* district of the West Shoa zone, it was also revealed that there has been a crop change over the last ten years, whereby maize had been replacing major crops such as wheat and barley (Land Use Planning and Resource Management Project in Oromia Region, Unpublished work 1999).

Although maize is being rapidly adopted in the Highland agro-ecology, the few highland cultivars that have been released have not been adopted by most highland farmers. Instead the mid-altitude hybrid cultivar ‘BH660’ has been widely adopted in most Highland areas. Even in case of seed shortages or the absence of this cultivar, farmers prefer to plant either the recycled seeds of the same hybrid, or their own local varieties. This has been a common phenomenon with many new crop varieties’ failure before meeting their targets that they are intended for. For example, McGuire (2008) reported that despite 25 years of sorghum breeding in Ethiopia, most of the

released cultivars had been poorly adopted by small-scale farmers. The failure of adoption occurs as a result of plant breeders try to identify the key problem and provide solutions without talking with the farming communities they serve (Girma et al., 2005). In addition, most research-extension-farmer linkages in Ethiopia followed a top-down approach in the past (Alene et al., 2000).

Many maize cultivars have been released in Ethiopia without including inputs from the farmers in the process of developing of the cultivars. This has been accomplished in such a way that agricultural research centres routinely assemble breeding nurseries and form experimental varieties to be tested under regional and national performance trials with the objective of generating desirable agronomic data to identify the best cross combinations or varieties for release (Mosisa et al., 2002). The problem of such highly controlled breeding and varietal selection processes is that they usually do not represent farmers' circumstances (such as low fertility, acid soil). Secondly, the limited number of traits considered by breeders as important traits in a cultivar may not represent farmers' preferences. A study by De Groote et al. (2002) was also in agreement with this idea in that poor correlations were observed between farmers' and breeders' evaluations of maize varieties using the Mother-Baby methodology of participatory breeding. Many studies, however, support the idea that including farmers in problem identification and solution search may facilitate success in adoption of new technologies.

Although farmers' trait preferences are diverse and complex (Witcombe and Virk, 1997), the farmers in this study described and prioritized the most important traits they use for maize cultivar selection. It was observed that there was no statistically significant difference regarding farmers' trait preferences between the two PRA zones, suggesting that these two zones could be included under same recommendation domain when new maize cultivar development and deployment are sought. It was interesting to note that farmers in all the study areas were in agreement that grain yield of maize is the most important trait compared to all other traits, including enhanced nutrition in maize (e.g. QPM). This is the reason that a two-decades-old and yet top yielding maize hybrid 'BH660', despite its lateness in maturity and tall plant height, dominates the seed sale in major maize producing areas of Ethiopia to date. Girma et al. (2005) found that farmers in moisture stress

areas were willing to adopt drought tolerant maize varieties since they were confident of their high yield and earliness. A similar PRA conducted in Zimbabwe also found that high yield of maize was the most important criterion in variety selection by farmers (Derera et al., 2006).

Yield potential of maize can be enhanced through agronomic interventions such as improved seeds, chemical fertilizers, integrated pest management, crop rotation, irrigation, and improved technical efficiency. However, the maize production system in the two PRA zones has been challenged by a number of constraints across the value chain that limit the productivity and income of smallholder farmers. According to the International Food Policy Research Institute (IFPRI, 2010), in Ethiopia, maize producers are mostly subsistence farmers, both in terms of numbers and in terms of total product volume. They are characterized by ownership of small fields (usually less than 2 hectares, as witnessed in this study) and low utilization of yield enhancing technologies such as hybrid seeds and inorganic fertilizers. Moreover, maize farmers sell the majority of their produce immediately after harvest when the price is lowest, because of urgent cash needs (including loan repayments) and fear of losses to storage pests due to unimproved storage structures. Hence, a balance between farmers-preferred traits and solutions to production constraints should be the breeders' goal in order to enhance cultivar dissemination and uptake by farmers (Hussein and Laing, 2012). This can be achieved through the implementation of participatory plant breeding approaches that involve a wide number of relevant stakeholders in the system.

2.6 Conclusion and recommendations

From the trend in maize production over the past five years, as reflected in this study, it is possible to forecast increasing demand for maize in the Highland agro-ecology of Ethiopia. Maize will grow as a component of household food security. Therefore, in the context of developing the maize production system toward improving the food, nutrition and income security of subsistence farmers in Ethiopia in general, and in the two PRA zones in particular, it is important to envision a comprehensive intervention approach that embraces the entire value chain. This will involve key stakeholders during the processes of improvements in variety

development, adoption, production, aggregation and marketing. Being able to create such an effective chain will help to fast track holistic improvements in supply, demand and market mechanisms.

Regarding improvements in varietal development, the overall issues that need to be addressed by the Highland maize breeders include developing a suitable maize cultivar which incorporate the trait preferences of farmers such as high yield, intermediate plant statures and maturity periods, flint textured grains, field and storage pest resistance/tolerance, and enhanced nutritional and baking quality. While incorporating all or some of these traits into new cultivars, breeders should make sure that there is no yield penalty in these cultivars since this is an essential trait for farmers in the study areas.

As to the non-breeding interventions, many farmers in the study areas reported that improved seeds and fertilizer were either not available, or that their delivery was too late. Where they were available, they were unaffordable. Therefore, institutional directions to facilitate the adoption of new maize cultivars should focus on reducing input costs by making sustainable improvements in infrastructure, transportation, credit availability, and markets. The results of this study also identified the need for extension services in awareness creation among farmers about new Highland maize cultivars and how to manage them best.

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CHAPTER 3

Phenotypic variation among quality protein and normal maize inbred lines adapted to tropical-highlands

3.1 Abstract

A well-characterized germplasm would enhance the development of productive and better adapted cultivars. Morphological and molecular markers and pedigree analyses are widely used in germplasm characterization, to establish genetic diversity and relationship in crop plants. The objective of this study was to determine genetic variation among converted quality protein maize (QPM) and normal inbred lines using phenotypic traits for breeding and cultivar development. Thirty-six maize inbred lines (30 QPM and six normal) adapted to tropical highlands were phenotyped under alpha lattice design (0, 1) with two replications using 18 traits at two locations in Ethiopia. Significant phenotypic variation was observed among inbred lines for all measured traits. Grain yield had positive and highly significant correlations with ear height, ear length, and number of kernels per row. It also showed moderate and high genotypic coefficients of variation and phenotypic coefficients of variation, respectively. Traits with heritability value less than 0.50 included grain yield, anthesis date, anthesis-silking interval and ear diameter. Ear height, tassel size, and thousand kernels weight had high heritability (≥ 0.70) and high genetic advance as percent over mean at the 5% selection intensity. Principal component and unweighted paired group method using arithmetic averages (UPGMA) cluster analyses revealed the presence of three distinct cluster groups. Seven inbred lines [KIT32Q, 142-1eQ, SRSYN20Q, FS67(BC_2), FS170Q, FS60, and F7215] with complementary phenotypic traits and relatively better yield performance were selected for making crosses for further genetic analysis and breeding. Overall, the phenotypic traits were found useful for primary characterization of the maize inbred lines.

***Keywords*:** Genotype, Phenotype, Phenotypic traits, Quality Protein Maize, Tropical-highland, Zea mays.

3.2 Introduction

Maize (*Zea mays* L.) is the principal crop in the world and ranks first in total production followed by wheat and rice. It is an important food security crop in rural areas of the developing world, especially in sub-Saharan Africa (SSA) and Latin America (Shiferaw et al., 2011). The highland maize mega-environments majorly include Tropical Highlands (2,000-3,600 metres above sea level [masl]), Tropical Highland Transition zones (1,500-2,000 masl), and Temperate Highlands (1,000-2,500 masl) (Bjarnason, 1994). In the Tropical Highlands, farmers commonly grow unimproved local varieties which are low yielders, long maturing, tall plant height and susceptible to lodging. In addition, these varieties show narrow genetic bases and variability for economic traits (Bjarnason, 1994). In the past, limited research is conducted on Tropical-highland maize when compared to lowland tropical, subtropical, mid-altitude or temperate maize. Recently some public and private breeding programs are developing highland maize varieties due to socio-economic importance of the crop in the highland agro-ecologies. Consequently, maize production is considerably expanding in the tropical highland agro-ecologies in sub-Saharan Africa.

In Ethiopia, the highland agro-ecologies were previously known for their highland crops production including barley, wheat, broad beans, and potatoes (Demissew et al., 2013). The highland agro-ecologies are densely populated and limited lands are available for crop production. Deployment of nutritionally enhanced and high yielding maize cultivars in these environments would enhance food and nutritional security. Besides, introduction of improved highland maize genotypes may diversify the cropping system and significantly improve the livelihoods of poor households. In eastern and central Africa (ECA), maize is also the priority crop among food staples. Hence, maize has accorded research priority by the Staple Crops Program of the Association for Strengthening Agricultural Research in East and Central Africa (ASARECA) in pursuit of food security in the region (www.asareca.org).

In developing countries, maize remains one of the main staple food and sometimes the only source of protein for humans who have limited access to other protein food sources such as animal products (Prasanna et al., 2001; Sofi et al., 2009). Normal

maize is deficient in two essential amino acids (lysine and tryptophan) leading to malnutrition and protein deficiency. Therefore, conversion of normal maize into quality protein maize significantly improves its nutritional value for humans and animals (Prasanna et al., 2001; Sofi et al., 2009). Quality protein maize genotypes (QPM), originally developed in the late 1990s at CIMMYT-Mexico, possesses improved protein quality due to enhanced lysine and tryptophan contents regulated by the *opaque-2* gene and associated modifiers (Gupta et al., 2009). QPM contains 90% the protein quality of casein in milk compared to 40% present in the conventional maize (Atlin et al., 2011).

In 1998, the International Wheat and Maize Improvement Center (CIMMYT) together with the Ethiopian Institute of Agricultural Research and National Agricultural Research Systems (NARs) in ECA initiated a highland maize improvement project in Ethiopia. The project aim was to develop a pool of both highland normal and quality protein maize (QPM) improved germplasm for the highland zones of the region (Twumasi-Afriyie et al., 2012). Consequently, a large number of early generation normal maize and QPM lines were introduced and developed with general adaptation to highland agro-ecologies in the region (Krivanek et al., 2007). The highland normal maize lines were converted to QPM through backcrossing the non-QPM local lines with QPM donor parents introduced from CIMMYT (Twumasi-Afriyie et al., 2012).

The QPM inbred lines developed should be systematically characterized for effective hybrid breeding to exploit heterosis. A well-characterized and evaluated germplasm would contribute in cultivar development with enhanced productivity and better adaptation at target growing environments (Yunbi et al., 2009; Prasanna, 2012). The availability and maintenance of genetic variability is crucial to widen the genetic base for effective breeding (Hallauer, 1980). Morphological and molecular markers and pedigree analyses are widely used in germplasm characterization and to establish genetic diversity and relationship in crop plants (Smith and Chin, 1997; Gomez et al., 2000; Bernardo and Kahler, 2001; Liu et al., 2003; Warburton et al., 2005). Morphological traits are among the earliest markers widely used in germplasm characterization and management (Goodman and Bird, 1977; Smith and Smith, 1992; Karanja et al., 2009). Abu-Alrub et al. (2006) used kernel characteristics as the

best descriptors followed by ear traits for classifying Peruvian highland maize germplasm. Beyene et al. (2006) reported genetic diversity analysis of Ethiopian highland maize accessions based on 15 morphological traits and AFLP markers. Ranatunga et al. (2009) studied the extent of genetic diversity available in maize inbreds using qualitative and quantitative traits and simple sequence repeat markers. The objective of this study was therefore to determine genetic variation among quality protein (QPM) and normal maize inbred lines using phenotypic traits for breeding and cultivar development.

3.3 Materials and methods

3.3.1 Germplasm

Thirty-six white maize inbred lines comprised of 31 highland adapted lines from the highland maize breeding program, two lines from mid-altitude breeding programs in Ethiopia, and three CIMMYT maize lines (CMLs) were used for this study. Their protein quality (tryptophan) levels were reasonably good (Table 3.1). Twenty seven of the 36 inbred lines are converted elite QPMs developed through backcross breeding procedure as described by Vivek et al. (2008). Briefly, 27 highland adapted non-QPM inbred lines from three heterotic groups (8 Ecuador, 4 Pool 9A, and 9 Kitale) and six unassigned inbreds were crossed to two CIMMYT QPM lines (CML144 and CML176). The conventional (non-QPM) and QPM lines were used as recurrent and donor parents, respectively in the successive backcrosses. CML144 (a tropically adapted inbred line) was used as a QPM donor parent for most lines from the Kitale heterotic group, while CML176 (a sub-tropical adapted inbred line) was used as a donor parent for the majority of the lines from Ecuador and Pool 9A heterotic groups. The BC1F1 and BC2F1 generations were selfed for 5 generations to develop the QPM inbred lines following rigorous field and light box selection in the laboratory. This facilitated selection for grain modification, good agronomic characters and resistance to common rust caused by Puccinia sorghi and northern corn leaf blight (Exserohilum turcicum). The non-QPM versions of the two QPM inbred lines (F7215Q and 142-1-eQ) are well-adapted tester lines used for maize breeding to the mid-altitude and highland transition agro-ecologies in Ethiopia

Table 3. 1 Pedigree and protein profile of the 36 QPM and non-QPM inbred lines used in the study

S/N	Name	Pedigree	Origin	*Protein %	Tryptophan %	Quality Index (%)	Heterotic group†	Remark
1	142-1-eQ	Unknown (derived from Ecuador-573)	ETHIOPIA	9.44	0.092	0.98	Ecuador	
2	CML144	Pob62c5HC182-2-1-2-B-B-3-1-#-#	CIMMYT	10.82	0.081	0.75	Ecuador	
3	CML176	(P63-12-2-1/P67-5-1-1)-1-2-B-B	CIMMYT	10.48	0.065	0.62	Unknown	
4	CML491	(6207QB/6207QA)-1-4-#-2-2-B-B	CIMMYT	10.09	0.083	0.82	A	
5	F7215Q	Unknown (derived from Kitale Syn. II)	ETHIOPIA	10.83	0.055	0.51	Kitale	
6	FS111	[POOL9Ac7-SR(BC2)]FS111-6-1-1-2-1-#/CML176BC1F1-8-1-2-1-1-#-#-#	CIMMYT	10.33	0.078	0.76	Ecuador	
7	FS112	[POOL9Ac7-SR(BC2)]FS112-4-2-1-1-2-#/CML144(BC2)-25-8-2-1-3-1-#-#	CIMMYT	10.71	0.060	0.56	Unknown	
8	FS151-3SR	[POOL9Ac7-SR(BC2)]FS151-3SR-1-2-1-1-#/CML176BC1F1-2-3-1-#-#	CIMMYT	11.44	0.071	0.62	Pool 9A	
9	FS170N	[POOL9Ac7-SR(BC2)]FS170-2-1-3-2-2-1-#-#-#	CIMMYT	–	–	–	Unknown	Non-QPM
10	FS170Q	[POOL9Ac7-SR(BC2)]FS170-2-1-3-1-#/CML176(BC2)-5-2-1-3-1-#-#	CIMMYT	12.81	0.075	0.59	Unknown	
11	FS211-1SR	[POOL9Ac7-SR(BC2)]FS211-1SR-1-1-1-#/CML144(BC2)-14-21-1-3-2-1-#-#-#	CIMMYT	8.72	0.068	0.78	Kitale	
12	FS232N	[POOL9Ac7-SR(BC2)]FS232-4-1-3-1-2-1-3-##-#-#-#-#	CIMMYT	–	–	–	Pool 9A	Non-QPM
13	FS232Q	[POOL9Ac7-SR(BC2)]FS232-4-1-3-1-#/CML176(BC2)-17-1-1-1-#-#	CIMMYT	10.17	0.068	0.67	Pool 9A	
14	FS2-3SR	[POOL9Ac7-SR(BC2)]FS2-3SR-2-1-2-#/CML176BC1F1-18-2-2-1-1-#-#-#	CIMMYT	9.32	0.064	0.69	Unknown	
15	FS4-3SR	[POOL9Ac7-SR(BC2)]FS4-3SR-1-1-1-#/CML176(BC2)-8-2-1-1-1-#-#-#	CIMMYT	11.30	0.085	0.75	Unknown	
16	FS45	[POOL9Ac7-SR(BC2)]FS45-3-2-2-1-#/CML144(BC2)-8-14-2-1-4-1-#-#-#	CIMMYT	11.51	0.091	0.79	Ecuador	
17	FS48	[POOL9Ac7-SR(BC2)]FS48-1-1-1-1-1-#/CML144(BC2)-6-25-5-2-1-4-#	CIMMYT	9.24	0.080	0.87	Kitale	
18	FS48-1SR	[POOL9Ac7-SR(BC2)]FS48-1SR-2-1-2-1-#/CML144(BC2)-7-4-1-3-2-1-#	CIMMYT	10.85	0.085	0.78	Kitale	
19	FS59-2	[POOL9Ac7-SR(BC2)]FS59-2-2-1-1-#/CML144(BC2)-9-9-3-2-2-1-#	CIMMYT	11.26	0.067	0.59	Kitale	
20	FS59-4N	[POOL9Ac7-SR(BC2)]FS59-4-1-2-1-1-#-B-B-B-B-B	CIMMYT	10.03	0.047	0.47	Ecuador	Non-QPM check
21	FS59-4Q	[POOL9Ac7-SR(BC2)]FS59-4-1-2-1-1-#/CML176BC1F1-3-2-3-#-#	CIMMYT	10.78	0.066	0.61	Ecuador	
22	FS60	[POOL9Ac7-SR(BC2)]FS60-2-1-1-1-#/CML176BC1F1-5-3-1-2-1-#	CIMMYT	11.70	0.065	0.56	Pool 9A	
23	FS67(BC1)	[POOL9Ac7-SR(BC2)]FS67-1-2-1-1-1-#/CML144(BC1)F1-11-1-2-2-2-#	CIMMYT	9.72	0.078	0.81	Kitale	
24	FS67(BC2)	[POOL9Ac7-SR(BC2)]FS67-1-2-3-1-#/CML144(BC2)-10-11-2-4-1-2-#	CIMMYT	10.73	0.065	0.61	Kitale	
25	FS67-N	[POOL9Ac7-SR(BC2)]FS67-1-2-3-1-#-B-B-B-B-B	CIMMYT	10.14	0.048	0.47	Kitale	Non-QPM check
26	FS68(BC1)	[POOL9Ac7-SR(BC2)]FS68-1-1-2-1-1/CML144(BC1)F1-1-1-2-1-1-#-#	CIMMYT	11.63	0.067	0.57	Kitale	
27	FS68(BC2)	[POOL9Ac7-SR(BC2)]FS68-1-1-2-1-1/CML144(BC2)-33-1-1-1-#-#	CIMMYT	11.65	0.056	0.48	Kitale	
28	KIT12	[KIT/SNSYN[N3/TUX]]c1F1-##(GLS=1)-12-2-1-#/CML176(BC2)-6-2-3-3-1-#-#-#	CIMMYT	10.17	0.072	0.70	Ecuador	
29	KIT29	[KIT/SNSYN[N3/TUX]]c1F1-##(GLS=2)-29-35-2-3/CML144(BC2)-29-24-1-1-2-1-#-#-#	CIMMYT	7.28	0.070	0.96	Unknown	
30	KIT31	[KIT/SNSYN[N3/TUX]]c1F1-##(GLS=1.5)-31-17-1-1/CML144(BC2)-31-14-1-1-1-2-#-#-#	CIMMYT	8.95	0.059	0.66	Unknown	
31	KIT32N	[KIT/SNSYN[N3/TUX]]c1F1-##(GLS=2.5)-32-1-1-1-#-#-#-#-#-#-#-#	CIMMYT	–	–	–	Ecuador	Non-QPM
32	KIT32Q	[KIT/SNSYN[N3/TUX]]c1F1-##(GLS=2.5)-32-1-1-#/CML176BC1F1-12-1-3-1-1-#-#-#	CIMMYT	9.71	0.075	0.77	Ecuador	
33	KIT34	[KIT/SNSYN[N3/TUX]]c1F1-##(GLS=2.5)-34-2-1-#/CML176BC1F1-6-1-1-1-1-#	CIMMYT	10.39	0.102	0.99	Ecuador	
34	SRSYN20N	SRSYN95[ECU//SC/ETO]F1-##(GLS=3.5)-20-2-1-1-#-#-#-#-#-#	CIMMYT	–	–	–	Pool 9A	Non-QPM
35	SRSYN20Q	SRSYN95[ECU//SC/ETO]F1-##(GLS=3.5)-20-2-1-#/CML176(BC2)-4-2-2-3-2-#-#-#	CIMMYT	12.06	0.069	0.57	Pool 9A	
36	SRSYN48	SRSYN95[ECU//SC/ETO]F1-##(GLS=3.5)-48-1-1-#/CML176(BC2)-11-2-1-1-1-#-#	CIMMYT	10.12	0.077	0.76	Ecuador	

† Putative heterotic grouping based on phenotypic data of the non-QPM counterparts before conversion to QPM

*Protein quantity and quality of the inbred lines were determined in 2011 at the CIMMYT Maize Nutrition Quality and Plant Tissue Analysis Laboratory following procedures described by Nurit et al. (2009). Whole grain samples were used to determine concentrations of nitrogen and tryptophan. Percent tryptophan (% Trp) was determined by the colorimetric method based on glyoxilic acid while percent nitrogen (% N) was determined by Micro-Kjeldahl methodology. Protein content was determined based on the formula for maize: Protein = %N*6.25. Quality Index (QI) was calculated as QI = (%Trp /%Protein)*100.

3.3.2 Experimental sites

Two experiments were conducted under rain fed conditions during the main rainy season (June-September) of 2012 at two locations, Ambo and Kulumsa in Ethiopia. Ambo is situated at 8^{o}57' N latitude, 38^{o}07' E longitude with an altitude of 2225 m above sea level (masl) and Kulumsa at 8^{o}13' N latitude, 39^{o}13' E longitude and 2180 masl. Both locations represent the highland sub-humid maize growing agro-ecology in Ethiopia. The soil type at Ambo is clay (heavy vertisol) with a pH of 7.8 for the most top soil (0–30 cm). The long-term total annual rainfall of this site is 1115 mm, and average minimum and maximum temperatures are 11.7^{o}C and 25.4^{o}C, respectively. Kulumsa has clay soil (eutric vertisol) with a pH of 6.8 at 0-30 cm of soil depth. The long term total annual rainfall at Kulumsa is 824 mm, with average minimum and maximum temperatures of 10^{o}C and 23^{o}C, respectively.

3.3.3 Experimental design and field procedures

The inbred lines were planted in an alpha (0, 1) lattice design (Patterson and Williams, 1976) with two replications at each location. In both locations, planting was done during the first week of June. All entries were sown and thinned to one plant per hill after 35 days of emergence giving a population density of 53,333 plants ha^{-1}. Each entry was planted in two rows, each 5.25 m long, with a spacing of 0.25 m within and 0.75 m between rows. The experiments at both locations received 69 kg ha^{-1} of phosphate (P_2O_5) in the form of di-ammonium phosphate fertilizer (DAP) and one-third of 119 kg nitrogen (N) ha^{-1} in the form of Urea as basal dressing during planting. Second and third doses of N (each one-third of 119 kg) were side-dressed 37 days after the crop emergence and before tasseling, respectively. Weeds were controlled manually by hand.

3.3.4 Data collection and analyses

Observations were made on eighteen phenotypic traits according to maize descriptors of IBPGR (International Board for Plant Genetic Resource) and CIMMYT (IBPGR and CIMMYT, 1991). The list of traits used in the study with their abbreviations and description are summarised in Table 3.2.

Table 3. 2 List of phenotypic traits, abbreviations used and their description

No	Abbreviation	Trait	Units	Trait description
1	GY	Grain yield	Tonnes per hectare ($t\ ha^{-1}$)	The total grain yield from all the ears of each experimental unit; moisture level was adjusted to 12.5% and 80% shelling percentage to estimate grain yield per hectare
2	AD	Days to anthesis	Days (D)	The number of days from planting to 50% of the plants in a plot shed pollen
3	ASI	Anthesis-silking interval	D	Number of days between anthesis and silking dates
4	PH	Plant height	Centimeters (cm)	The height from the soil surface to the base of the tassel branching; the measurement was made two weeks after pollen shedding has ceased
5	EH	Ear height	(cm)	The height from the ground level to the upper most ear bearing node, it was also measured two weeks after pollen shedding ceased
6	EA	Ear aspect	1-5	Overall phenotypic appearance of the ear; where 1= excellent and 5= poor
7	PA	Plant aspect	1-5	Overall phenotypic appearance of the plant; where 1= excellent and 5= poor
8	ED	Ear diameter	cm	Measured at the mid-way along ear length, as the average diameter of 10 randomly taken ears
9	EL	Ear length	cm	Length of the ear from the base to tip; it was measured as the average length of ten randomly sampled ears from each experimental unit
10	TKW	Thousand kernel weight	Grams (g)	After shelling, random kernels from the bulk of each experimental unit was counted and weighed in grams after the moisture was adjusted to 12.5%
11	RPE	Number of rows per ear	Number (No.)	This was recorded as the average number of kernel rows per ear from the 10 randomly sampled ears
12	KPR	Number of kernels per row	No.	Recorded as the average number of kernels per row from the 10 randomly taken ears
13	LL	Leaf length	cm	Length of the leaf from ligule to apex; the measurement was taken after flowering from the leaf that subtends the uppermost ear
14	LW	Leaf width	cm	Measurement was taken from the same leaf as leaf length at mid-way along its length
15	LA	Leaf area	Square cm (cm^2)	Area of the upper most ear leaf computed as maximum width x length x 0.75
16	TS	Tassel size	Tassel size 3, 5 or 7	Recorded after milk stage as 3 (small), 5 (medium) and 7 (large)
17	LN	Number of leaves above the ear	No.	Counted after flowering; observation was made on 10 randomly selected plants in a plot
18	FR	Foliage rating	Foliage rating 3, 5, or 7	Rating of total leaf surface after milk stage as 3 (small), 5 (intermediate) and 7 (large); and the observation was made on 10 randomly selected plants/plot

Analysis of variance (ANOVA) for each experimental site was done separately using REML (Patterson and Thompson, 1971) analysis in GenStat® Release 14 statistical software (Payne et al., 2007). Inbred lines were assigned as fixed factor, and replicates and incomplete blocks within replicates were random factors. Data for both locations were then pooled after testing homogeneity of variances using Hartley's F_{max}-test (Hartley, 1950). Combined ANOVA was conducted with inbred lines and locations being considered as fixed factors, and replicates within locations and incomplete blocks within replicates as random factors.

Variance components and corresponding standard errors were further estimated to identify genetic variability among lines and determine genetic and environmental effects on various traits. REML analysis was used to estimate variance components as genotypic, environmental (locations) and their interactions, assuming both the lines and environments as random factors in the model. Additionally, phenotypic variances (σ^2_p) were calculated as the sum total of genotypic variances (σ^2_g) and their interaction with environments (σ^2_{gxe}) plus error variances (σ^2_e) (Hallauer et al., 2010). Phenotypic and genotypic coefficients of variations (Burton, 1952), heritability in the broad sense, and genetic advance expressed as percent of the mean (Johnson et al., 1955) were estimated using the components of variances. Pearson phenotypic correlation coefficients were also calculated among all traits to determine the linear relationships between the traits.

Principal components analysis (PCA) was conducted using the correlation matrix in order to assess the importance of different traits in explaining multivariate variation. Since principal components of a set of traits depend critically on the scales used to measure the traits, standardization of the measurements was made to ensure that all have equal weight in the analysis (Manly, 2005). Standardization of the data was thus carried out by subtracting from each observation the mean value of the trait and subsequently dividing by its respective standard deviation. Finally, Cluster analysis using Euclidean dissimilarity matrices was carried out to observe phenotypic similarities among the lines. The sequential agglomerative hierarchical nested cluster analysis (SAHN) method (Sneath and Sokal, 1973) of the NTSYS-pc 2.10 software (Rohlf, 2000) was used to compute Euclidean distance matrix, and cluster the

distance matrix in the form of dendrogram using the Unweighted Pair Group Method using Arithmetic Averages (UPGMA) with average linkage (Sneath and Sokal, 1973). Cophenetic correlation coefficient' was estimated using COPH and MXCOMP options of the NTSYS-pc 2.10 to test the goodness of fit of the cluster analysis to the dissimilarity matrix on which it was based (Mantel, 1967).

3.4 Results

3.4.1 Analyses of variance

Significant differences were detected among inbred lines for all 18 phenotypic traits studied at the two locations. The mean with its standard error (SE), the range and the F-test for each of the 18 phenotypic traits are presented in Table 3.3. The average performance of each line for most of the traits over the two locations is nearly similar although considerable variability was observed among individual lines (Table 3.3 and Figure 3.1). However, considering some traits such as grain yield and days to anthesis, a large proportion of the inbred lines had relatively higher grain yields and delayed anthesis at Ambo than Kulumsa site (Figure 3.2).

Combined analysis of variance across the two locations showed significant effects of lines and their interaction with locations for most of the traits. Whereas the effect of environment (location) was significant only for a few traits *viz*., leaf length, number of leaves, ear length, and number of kernels per row (Table 3.4). Data were not pooled to undertake combined analysis for traits such as anthesis date, anthesis silking interval, plant height, and ear diameter because of heterogeneity of error variance observed from Hartley's F_{max}-test. Table 3.5 shows the mean performance of individual lines over the two locations for 14 traits.

3.4.2 Agro-morphological traits variation among inbred lines

Considerable variability existed among the lines for all traits (Table 3.5). The highest grain yield records were obtained for a normal maize check, FS67-N (4.5 t ha^{-1}), and three other QPM lines, namely, 142-1-eQ (4.4 t ha^{-1}), KIT32Q (4.1 t ha^{-1}), and FS2-

3SR (4.0 t ha^{-1}). All the four lines but 142-1-eQ had also better plant and ear aspect records. On the other hand, 142-1-eQ is superior in terms of ear length which, in turn

Figure 3. 1 Partial view of inbred lines studied for phenotypic variability at Ambo (2012)

was a contribution for having larger number of kernels per row of this line compared to all other lines. Out of the six normal maize lines, and their QPM counterparts evaluated, three (FS67-N, FS59-4N, and FS232N) had better grain yields than their QPM versions (Table 5). Whereas, the two normal maize lines (KIT32N and SRSYN20N) were inferior grain yielders while the remaining one (FS170N) had equivalent yield compared to the QPM counterparts. However, all the six normal maize lines reached flowering relatively earlier than their QPM versions (Data not shown). Line FS60, with maximum leaf width (13.1 cm) and ear length (14.4 cm), had a moderate grain yield record of 3.4 t ha^{-1}. On the other hand, FS48 had the minimum leaf width of all the lines although big ear length (14.5 cm) and moderate grain yield (3.6 t ha^{-1}) values were recorded for this line. CML144, CML491 and FS60 had big tassel size, relatively wide leaf area, and maximum foliage rating scores.

Table 3. 3 Mean, standard error of the mean [SE(m)], range, F-test and coefficient of variation (CV) of 18 phenotypic traits of maize inbred lines evaluated at Ambo and Kulumsa, 2012

Trait[§]	Ambo				Kulumsa			
	Mean ± SE(m)	Range	F test	CV (%)	Mean ± SE(m)	Range	F test	CV (%)
GY	3.2 ± 0.04	1.4 - 4.8	**	13.3	2.9 ± 0.13	2.0-4.3	**	15.6
AD	107.4 ± 0.22	96.6 - 118.7	**	1.4	99.1 ± 0.26	92.0 - 109.5	**	2.2
ASI	2.4 ± 1.02	-4.6 - 8.1	**	47.0	3.1 ± 0.03	1.0 - 5.5	**	16.8
EA	2.8 ± 0.02	1.7 - 4.5	**	10.1	2.8 ± 0.07	1.7 - 4.0	**	9.1
PA	2.7 ± 0.03	1.5 - 4.5	**	17.2	2.7 ± 0.02	1.3 - 4.9	**	13.8
EH	69.1 ± 0.36	44.7 - 92.1	**	6.4	70.1 ± 0.29	49.3 - 96.6	**	5.9
PH	152.6 ± 0.89	117.9 - 196.1	**	4.6	146.9 ± 0.26	112.7 - 180.0	**	2.9
LL	67.9 ± 0.39	53.6 - 82.4	**	4.9	66.5 ± 0.59	53.5 - 79.0	**	6.3
LW	9.7 ± 0.08	6.7 - 12.5	**	5.3	9.7 ± 0.06	6.3 - 13.7	**	5.7
LA	493.6 ± 5.87	354.6 - 619.6	**	10.1	482.6 ± 12.17	307.0 - 664.8	**	12.2
TS	5.3 ± 0.22	3.0 - 7.1	**	13.1	5.1 ± 0.08	3.0 - 7.0	**	13.9
LN	5.4 ± 0.02	4.3 - 7.8	**	4.8	5.3 ± 0.02	4.2 - 6.8	**	6.1
FR	5.2 ± 0.11	2.9 - 7.1	**	10.3	5 ± 0.34	3.0 - 7.0	*	9.6
EL	12.7 ± 0.06	8.3 - 16.0	**	5.2	12 ± 0.11	8.5 - 14.9	**	4.6
ED	3.9 ± 0.02	3.3 - 4.4	**	2.7	3.7 ± 0.02	2.9 - 4.2	*	8.9
RPE	12.4 ± 0.09	10.0 - 15.0	**	6.2	12.1 ± 0.18	8.9 - 15.6	**	4.6
KPR	25.7 ± 0.37	14.1 - 36.5	**	8.4	22.2 ± 0.34	13.4 - 30.0	**	9.1
TKW	262.3 ± 2.59	167.9 - 379.4	**	3.9	269.5 ± 1.58	136.4 - 415.5	**	2.9

[§]See Table 3.2 for abbreviation; * $P \leq 0.05$; ** $P \leq 0.01$

Table 3. 4 Mean squares from combined analysis of variance for 14 phenotypic traits

Trait	Location (df=1)	Line (df=35)	Location x Line (df=35)	Error (df=62)
Grain yield (t ha^{-1})	4.0	1.5**	0.6**	0.195
Ear aspect (1-5)	0.0	1.4**	0.4**	0.078
Plant aspect (1-5)	0.02	1.9**	0.5**	0.178
Ear height (cm)	36.4	617.1**	42.8**	18.85
Leaf length (cm)	67.5*	178.2**	12.6	14.78
Leaf width (cm)	0.06	6.6**	0.80	0.462
Leaf area (cm^2)	4311.8	29479.1**	3058.0	3058.0
Tassel_size_(3, 5 or 7)	1.3	6.8**	0.7	0.515
Number of Leaves (No.)	3.5*	1.8**	0.2*	0.087
Foliage rating (3, 5 or 7)	1.8	3.2**	0.6**	0.262
Ear length (cm)	19.3**	9.3**	1.2**	0.366
Number of rows/ear (No.)	4.3	6.5**	0.8*	0.471
Number of kernels per row (No.)	400.8**	54.3**	10.0**	3.38
Thousand kernel weight (g)	88.1	12309.8**	1491.7**	587.3

* Significant at $P \leq 0.05$ level; ** Significant at $P \leq 0.01$ level; df= Degrees of freedom

Figure 3. 2 The response of 36 QPM and non-QPM maize inbred lines for days to anthesis and grain yield at Ambo and Kulumsa, 2012

Table 3. 5 Mean performances from combined analysis of 14 phenotypic traits for the 36 QPM and non-QPM maize inbred lines evaluated at Ambo and Kulumsa

No.	Lines	Traits[§]													
		GY	EA	PA	EH	LL	LW	LA	TS	LN	FR	EL	RPE	KPR	TKW
1	KIT31	1.9	3.8	3.0	53.3	67.0	10.3	514.2	4.1	5.1	6.0	9.7	13.0	20.3	212.1
2	KIT29	2.7	2.2	2.3	74.0	72.4	9.8	535.3	5.0	5.3	5.0	12.5	12.3	23.5	387.2
3	FS112	2.1	4.1	3.8	51.3	72.5	9.2	500.6	6.5	5.3	6.0	12.4	15.0	22.6	199.1
4	FS211-1SR	2.9	3.0	3.9	53.5	55.4	8.3	343.2	3.6	4.9	3.0	12.3	11.2	24.4	218.0
5	FS45	2.3	2.9	3.9	73.3	70.7	9.9	523.2	5.9	4.7	5.5	9.2	12.2	17.1	335.9
6	KIT32Q	4.1	2.0	2.0	61.0	70.2	10.1	529.1	4.9	5.1	5.5	12.1	11.8	21.8	260.0
7	FS111	3.0	2.5	2.4	53.8	72.7	10.5	571.6	5.0	6.7	5.5	12.5	12.4	22.1	224.5
8	FS2-3SR	4.0	1.9	2.1	76.3	66.8	9.1	458.8	2.9	4.5	4.9	13.6	11.2	30.2	241.0
9	KIT12	2.9	3.2	2.6	81.5	78.1	9.4	550.1	4.9	5.6	5.0	11.9	11.8	21.9	237.7
10	FS4-3SR	2.9	3.3	3.5	54.3	54.9	9.9	408.9	5.1	5.3	3.5	12.4	10.8	28.1	200.2
11	SRSYN20Q	3.1	2.6	1.9	88.3	77.7	11.0	644.2	4.5	6.2	5.0	11.4	13.5	22.3	287.0
12	SRSYN48	2.4	3.0	2.0	59.3	57.7	9.4	406.5	5.0	5.1	5.0	14.1	12.0	27.6	197.3
13	KIT34	2.6	3.1	2.1	52.8	68.8	9.6	497.9	5.6	6.0	6.0	12.9	13.4	23.3	215.8
14	FS48	3.6	2.2	3.8	69.0	68.0	6.5	328.9	6.9	4.4	3.0	14.5	12.3	28.1	238.3
15	FS67(BC2)	3.6	3.1	2.6	83.3	60.5	8.7	394.8	3.0	4.6	4.5	12.2	12.2	24.9	253.9
16	FS59-2	3.0	3.6	2.3	55.5	60.7	9.9	451.5	2.9	4.9	5.0	13.5	9.8	22.3	276.1
17	FS68(BC1)	2.2	3.5	3.3	48.8	60.6	9.7	445.5	4.4	5.5	5.0	9.8	10.7	20.5	243.5
18	FS48-1SR	3.3	2.3	2.5	72.0	66.9	8.6	429.3	4.9	4.9	5.0	13.3	13.8	25.4	245.5
19	FS59-4Q	2.9	2.5	3.1	79.3	62.0	7.4	342.8	6.5	4.6	5.0	12.4	13.3	23.7	229.5
20	FS60	3.4	2.3	2.4	65.8	58.5	13.1	574.5	7.0	4.9	7.0	14.4	13.9	29.0	239.0
21	FS151-3SR	3.1	2.9	2.5	76.0	75.5	10.0	567.5	6.6	5.6	6.1	13.8	13.0	22.7	237.6
22	FS170Q	3.1	3.5	3.0	70.8	66.6	11.2	556.3	4.6	5.4	5.0	12.0	12.8	24.3	212.3
23	FS232Q	2.8	3.4	2.9	57.3	70.2	7.7	407.7	5.5	5.6	4.5	13.3	10.4	23.5	239.7
24	FS68(BC2)	2.8	2.4	1.9	88.0	60.4	9.7	438.6	5.4	5.6	5.0	10.4	9.8	18.0	375.3
25	FS67(BC1)	3.5	3.3	3.5	59.5	62.7	9.2	432.1	3.1	6.0	5.0	12.4	10.0	23.5	298.8
26	CML491	3.0	2.1	2.5	75.0	75.5	9.8	562.6	7.0	7.3	7.0	12.2	13.3	24.2	227.6
27	F7215Q	3.3	3.3	2.5	83.5	81.0	9.0	546.1	4.9	4.7	5.0	12.7	13.1	24.5	277.3
28	142-1-eQ	4.4	2.5	2.8	86.0	71.3	11.2	593.5	6.5	5.2	5.0	15.5	12.2	33.3	257.6
29	CML144	2.8	3.0	2.6	68.5	76.2	10.5	606.7	6.6	6.3	7.0	11.4	14.7	23.9	154.1
30	CML176	2.1	3.3	4.6	55.8	69.6	8.7	449.8	5.0	6.2	4.5	9.6	13.0	23.5	171.8
31	FS67-N	4.5	1.8	1.4	77.8	67.6	12.2	620.6	6.1	4.7	5.5	14.1	10.3	28.4	373.6
32	FS59-4N	3.9	2.0	2.1	87.3	57.4	8.4	361.0	4.6	5.2	4.5	13.4	12.0	23.8	348.9
33	FS232N	3.3	2.5	2.9	73.5	62.6	10.7	506.8	4.6	5.5	5.0	12.2	11.9	22.0	316.7
34	KIT32N	3.4	2.3	1.8	80.3	71.1	10.1	540.3	7.0	5.2	5.0	13.3	12.4	29.9	243.2
35	SRSYN20N	2.4	2.9	2.8	86.8	62.4	8.9	417.3	4.4	5.9	4.5	8.4	12.6	13.7	320.7
36	FS170N	3.2	2.5	2.6	73.8	67.8	10.1	514.3	6.0	4.9	5.0	12.4	12.3	26.9	218.6
	Mean	3.1	2.8	2.7	69.6	67.2	9.7	488.1	5.2	5.4	5.1	12.3	12.2	24.0	256.0
	Min	1.9	1.8	1.4	48.8	54.9	6.5	328.9	2.9	4.4	3.0	8.4	9.8	13.7	154.1
	Max	4.5	4.1	4.6	88.3	81.0	13.1	644.2	7.0	7.3	7.0	15.5	15.0	33.3	387.2
	$LSD_{0.05}$	0.63	0.40	0.60	6.12	5.50	1.01	77.97	1.02	0.47	0.74	0.88	0.99	2.70	35.71
	CV (%)	14.2	10.0	15.6	6.2	5.7	7.0	11.3	13.9	5.5	10.0	4.9	5.6	7.7	9.5

[§] See table 3.2 for abbreviations of traits; CV(%)= Coefficient of variation; LSD=Least Significant Difference; Min= minimum; Max= maximum

3.4.3 Correlation analysis

Correlation coefficients of 14 traits are presented in Table 3.6. Grain yield had positive and highly significant correlations with ear height, ear length, and number of kernels per row, while it had a negative and highly significant correlation with ear aspect. Low score values for ear aspect had also negatively and significantly correlated with ear height, ear length, number of kernels per, and thousand kernel weight. Foliage rating had positive and significant association with foliar traits such as leaf length, leaf width, leaf area, number of leaves above the ear, number of rows per ear, and tassel size. Similarly, leaf area had positive and significant correlations with leaf length, leaf width, number of leaves above the ear, and number of rows per ear. Leaf length had positive and significant associations with number of leaves, number of rows per ear, and tassel size. Ear height showed positive and highly significant correlations with thousand kernel weight and grain yield, but associated negatively and significantly with plant aspect. Ear length exhibited high positive correlation with number of kernels per row in addition to grain yield. Number of rows per ear had positive and highly significant correlation with tassel size.

Table 3. 6 Pair-wise phenotypic correlation coefficients among 14 traits of 36 QPM and non-QPM maize inbred lines evaluated at Ambo and Kulumsa, 2012

Traits[§]	EA	EH	EL	FR	GY	KPR	LA	LL	LN	LW	PA	RPE	TKW
EH	-0.52**												
EL	-0.35*	0.06											
FR	-0.03	-0.02	-0.02										
GY	-0.68**	0.46**	0.64**	-0.08									
KPR	-0.33*	0.04	0.81**	-0.08	0.58**								
LA	-0.12	0.19	0.05	0.68**	0.16	0.09							
LL	-0.02	0.23	-0.01	0.41*	0.01	-0.04	0.67**						
LN	0.09	-0.12	-0.32	0.38*	-0.29	-0.33	0.35*	0.34*					
LW	-0.13	0.05	0.08	0.59**	0.20	0.16	0.80**	0.11	0.17				
PA	0.51	-0.43**	-0.36	-0.35	-0.46	-0.17	-0.40	-0.13	-0.01	-0.41			
RPE	0.06	0.07	-0.04	0.48**	-0.23	0.02	0.33*	0.45**	0.18	0.08	0.10		
TKW	-0.42*	0.52**	-0.07	-0.12	0.30	-0.28	0.04	-0.09	-0.16	0.11	-0.37	-0.44	
TS	-0.27	0.15	0.24	0.38*	0.02	0.25	0.33	0.37*	0.10	0.16	-0.04	0.47**	-0.15

[§]Abbreviations for traits as explained in Table 3.2

3.4.4 Variance components and heritability

Table 3.7 shows estimates of variance components of 18 traits of 36 QPM and non-QPM maize inbred lines tested at two locations. Of the total phenotypic variances

(σ^2_p), the proportion of genotypic variances (σ^2_g) was higher than the error or environmental (σ^2_e) and genotype-environment interaction (σ^2_{gxe}) variances for most traits except anthesis data, anthesis silking interval, and ear diameter. In most cases, variance components were greater than the respective standard errors for majority of the traits (Table 3.7).

The estimates of genotypic coefficients of variations (GCV), phenotypic coefficients of variations (PCV), heritability (H^2) in broad sense, and genetic advance as percent of the mean are presented in Table 3.8. The highest estimate of GCV was observed for plant aspect (22.8%) followed by thousand kernel weight (22.0%), and tassel size (21.9%). Ear aspect (17.9%), ear height (17.2%), grain yield (16.1%), leaf area (16.1%), and foliage rating (16.0%) showed moderate GCV. However, low GCV values were expressed by some traits such as anthesis date (3.2%), anthesis silking interval (3.3%), ear diameter (5.9%) and rows per ear (9.9%). Similarly, the highest PCV estimates were recorded for anthesis silking interval (56.3%), plant aspect (31.1%), tassel size (26.3%), grain yield (25.6%), ear aspect (25.5%), thousand kernel weight (24.7%), and foliage rating (20.6%). In contrast, low PCV were observed for traits such as anthesis date (4.9%) and ear diameter (8.8) while moderate values were recorded for leaf area (19.7%), ear height (19.0%), and kernels per row (18.0%).

Broad sense heritability (H^2) estimates ranged from 0.004 for anthesis silking interval to 0.82 for ear height, while genetic advance as percent over mean was found to vary between 0.41% (anthesis siliking interval) and 40.29% (thousand kernel weight) (Table 3.8). Most traits had heritability values greater than 0.50. Traits with heritability value less than 0.50 included grain yield, anthesis date, anthesis silking interval, ear diameter. Anthesis date, anthesis silking interval, and ear diameter traits were also low in terms of genetic advance. However, ear height, tassel size, and thousand kernels weight had high heritability (≥ 0.70) expressing high genetic advance as percent over mean expected from selecting the top 5% of the lines. Whereas high heritability and moderate genetic advance was exhibited by plant height, leaf width, leaf number, and ear length. Besides, leaf length and kernel rows per cob had relatively high heritability values although they showed low genetic advance.

Table 3. 7 Estimates of components of variances and their standard errors from pooled data for 18 phenotypic traits of the 36 QPM and non-QPM maize inbreds evaluated at Ambo and Kulumsa, 2012

Trait[§]	$\sigma^2_g \pm$ SE	$\sigma^2_e \pm$ SE	$\sigma^2_{gxe} \pm$ SE	$\sigma^2_p \pm$ SE
Grain yield (t ha^{-1})	0.25 ± 0.10	0.20 ± 0.03	0.18 ± 0.07	0.63± 0.20
Anthesis date (Days)	10.90 ± 4.46	3.55 ± 0.61	11.26 ± 3.14	25.71 ± 8.21
Anthesis Silking Interval (Days)	0.01 ± 0.52	0.60 ± 0.10	2.78 ± 0.73	3.39 ± 1.35
Ear aspect (1-5)	0.25 ± 0.09	0.08 ± 0.01	0.18 ± 0.05	0.51 ± 0.15
Plant aspect (1-5)	0.38 ± 0.12	0.18 ± 0.03	0.15 ± 0.06	0.71 ± 0.21
Ear height (cm)	143.75 ± 37.01	18.42 ± 3.11	12.14 ± 5.33	174.31 ± 45.48
Plant height (cm)	284.86 ± 77.62	32.91 ± 5.55	59.93 ± 18.3	377.70 ± 101.47
Leaf length (cm)	43.30 ± 11.15	15.22 ± 2.54	0.00 ± 2.00	58.52 ± 15.69
Leaf width (cm)	1.48 ± 0.39	0.28 ± 0.05	0.24 ± 0.09	2.00 ± 0.53
Leaf area (cm^2)	6154.0 ± 1654.0	3055.0 ± 521.0	0.00 ± 433	9209.0 ± 2608.0
Tassel size (3, 5 or 7)	1.29 ± 0.35	0.51 ± 0.09	0.05 ± 0.09	1.85 ± 0.53
Number of leaves (No.)	0.38 ± 0.10	0.08 ± 0.01	0.03 ± 0.02	0.49 ± 0.13
Foliage rating (3, 5 or 7)	0.67 ± 0.20	0.25 ± 0.04	0.19 ± 0.08	1.11 ± 0.32
Ear length (cm)	2.12 ± 0.58	0.39 ± 0.07	0.40 ± 0.15	2.91 ± 0.80
Ear diameter (cm)	0.05 ± 0.02	0.06 ± 0.01	0.00 ± 0.01	0.11 ± 0.04
Number of rows/ear (No.)	1.46 ± 0.40	0.50 ± 0.09	0.16 ± 0.11	2.12 ± 0.60
Number of kernels per row (No.)	10.92 ± 3.32	4.70 ± 0.78	3.05 ± 1.35	18.67 ± 5.45
Thousand kernel weight (g)	3165.75 ± 857.8	86.1 ± 14.85	756.62 ± 191.6	4008.47 ± 1064.25

[§] See table 3.2 for abbreviations of traits; σ^2_e = error variance; σ^2_g = genotypic variance; σ^2_p = phenotypic variance; σ^2_{gxe} = genotype x environment variance; SE= standard error

Table 3. 8 Estimates of genotypic (GCV) and phenotypic (PCV) coefficients of variation, broad sense heritability (H^2) and genetic advance (GA) based on pooled data for 18 phenotypic traits of the 36 QPM and non-QPM maize inbreds evaluated at Ambo and Kulumsa, 2012

Trait	GCV (%)	PCV (%)	H^2	GA	GA (% of Mean)
Grain yield (t ha^{-1})	16.1	25.6	0.40	0.65	20.96
Anthesis date (Days)	3.2	4.9	0.42	4.43	4.26
Anthesis Silking Interval (Days)	3.3	56.3	0.004	0.01	0.41
Ear aspect (1-5)	17.9	25.5	0.49	0.72	25.79
Plant aspect (1-5)	22.8	31.1	0.54	0.93	34.35
Ear height (cm)	17.2	19.0	0.82	22.46	32.28
Plant height (cm)	11.3	13.0	0.75	30.24	20.20
Leaf length (cm)	10.0	11.4	0.74	11.68	17.37
Leaf width (cm)	12.6	14.6	0.74	2.16	22.35
Leaf area (cm^2)	16.1	19.7	0.67	132.30	27.10
Tassel size (3, 5 or 7)	21.9	26.3	0.70	1.96	37.76
Number of leaves (No.)	11.5	13.1	0.78	1.12	20.89
Foliage rating (3, 5 or 7)	16.0	20.6	0.60	1.31	25.67
Ear length (cm)	11.8	13.8	0.73	2.56	20.78
Ear diameter (cm)	5.9	8.8	0.45	0.31	8.21
Number of rows/ear (No.)	9.9	11.9	0.69	2.07	16.90
Number of kernels per row (No.)	13.8	18.0	0.58	5.21	21.71
Thousand kernel weight (g)	22.0	24.7	0.79	103.15	40.29

3.4.5 Principal component analysis

The principal component analysis computed using 12 correlated traits found four principal components (PC) accounting 81% of the total variation existing among the lines. Principal components (eigenvectors) as well as the latent roots (eigenvalues) with their contribution to total variation are summarised in Table 3.9. The first PC which explained 32.2% of the variation among the genotypes was mainly associated with increased grain yield and ear height, and decreased ear and plant aspect scores. Foliar characters including leaf length, leaf width, leaf area, and foliage rating, with a relatively highest negative weights of -0.345, -0.381, -0.486, and -0.497, respectively, were important in PC2 that accounted to the 21.2% variation. The third PC, accounting for 18.0% of the total variation, was dominated by traits such as ear height, plant height, and thousand kernel weight with negative weights, and ear length and number of kernels per row with positive weights. Plant height and leaf length, with positive signs, and leaf width and thousand kernel weights, with negative signs, were important delineating traits associated with the fourth PC that explained 9.3% of the total variation. Based on principal component scores of the two most important principal components, PC1 and PC2, a plot of Y_1 and Y_2 was made that provided a recognizable cluster of the genotypes (Figure 3.3). From the bi-plot, two major groups (the Ecuador group represented by the symbol 'EC' and Kitale by 'KT') are recognised for representing half of the inbred lines while the remaining 50% hardly showed clear group patterns (Figure 3.3).

3.4.6 Cluster analysis

Euclidean genetic distance estimates based on 18 phenotypic data for all 630 pairwise comparisons among the 36 maize inbred lines ranged from 2.80 (FS59-4Q vs FS48-1SR) to 9.84 (FS67-N vs FS112 and FS67-N vs CML176) with mean of 5.84 (Table 3.10). The low morphological distances were observed between inbred lines FS59-4Q and FS48-1SR (2.80), FS232-N and Kit29 (2.84), FS151-3SR and Kit34 (3.00), FS170-N and FS232-N (3.03), Kit34 and FS111 (3.08), and SRSYN20Q and Kit12 (3.09). In contrast, high morphological distance estimates were observed between FS67-N and FS112 (9.84), FS67-N and CML176 (9.84), CML144 and FS211-1SR (9.40), SRSYN20-N and FS67-N (9.23), CML144 and FS48 (9.14),

Table 3. 9 Principal components (PCs) for 12 phenotypic traits of the 36 QPM and non-QPM maize inbreds evaluated at Ambo and Kulumsa, 2012

Variables	Eigenvectors/Principal components			
	PC1	PC2	PC3	PC4
Grain yield (t ha^{-1})	0.383	0.255	0.188	-0.046
Ear aspect (1-5)	-0.368	-0.214	0.010	0.145
Plant aspect (1-5)	-0.399	0.043	0.046	0.201
Ear height (cm)	0.348	0.138	-0.353	0.222
Plant height (cm)	0.277	0.112	-0.392	0.316
Leaf length (cm)	0.161	-0.345	-0.068	0.640
Leaf width (cm)	0.251	-0.381	0.141	-0.421
Leaf area (cm^2)	0.288	-0.486	0.051	0.066
Foliage rating (3, 5 or 7)	0.159	-0.497	0.076	-0.106
Ear length (cm)	0.256	0.220	0.457	0.116
Number of kernels per row (No.)	0.213	0.195	0.514	0.192
Thousand kernel weight (g)	0.229	0.149	-0.422	-0.368
Eigen value	3.859	2.548	2.160	1.119
Individual variation explained (%)	32.2	21.2	18.0	9.3
Cumulative variation explained (%)	32.2	53.4	71.4	80.7

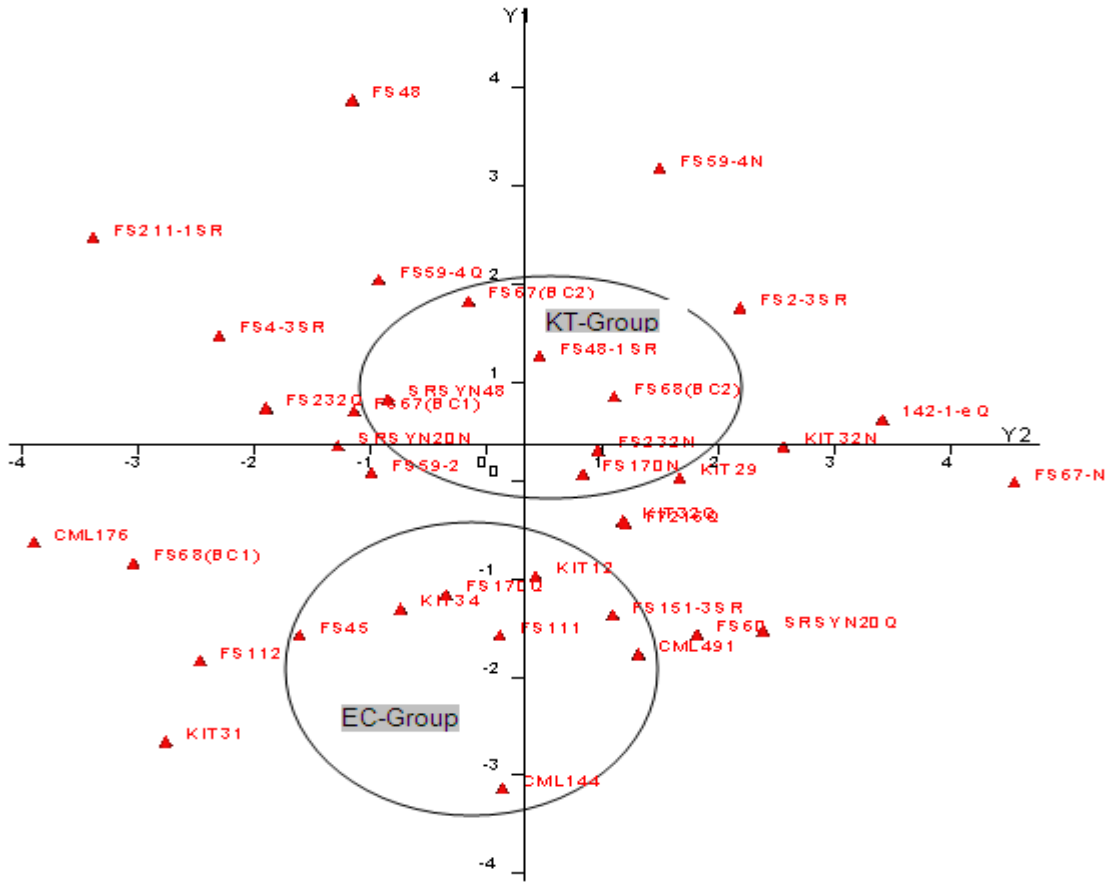

Figure 3. 3 Scatter plot of PC1 (32.2%) and PC2 (21.2%) of the 36 QPM and non-QPM inbred lines based on 12 correlated phenotypic traits combined over the two locations

FS67-N and Kit31 (9.07), and FS48 and SRSYN20Q (9.02). Of these seven pairs of inbreds with high morphological distances, three consisted of CIMMYT tropical (CML144) and subtropical lines (CML176) which were distinct from the highland adapted genotypes. Moreover, FS67-N (a non-QPM line) also contributed for the high morphological distances observed in most pairwise comparisons than any other line in the present study.

The dendrogram constructed using UPGMA cluster analysis on the basis of combined phenotypic data from the two locations classified the test genotypes into three clusters and seven outlier inbreds (Figure 3.4). The estimated cophenetic correlation value was in the range of poor fit (r_{cop}= 0.63). Outlier inbreds were identified for having either highest or lowest data values for one or more traits. FS67-N, one of the outlier inbreds, is a non-QPM line characterized with the highest grain yield, and best ear and plant aspect scores. Outliers FS60 and FS48 inbreds had the highest and the lowest leaf width, respectively. Out of the two CMLs, CML491 had the maximum number of leaves above the ear, and CML144 had the lowest thousand kernel weight which made them outliers. Similarly, the smallest ear length and number of kernel per row were recorded for an outlier inbred SRSYN20N while the maximum plant height was recorded for another outlier inbred FS68(BC2).

Among the three clusters, cluster I is dominated by three inbred lines from Kitale heterotic group although one from Ecuador, one from pool9A heterotic group, and one uncategorized line were included in this cluster. The lines in cluster I had short ear heights with average values of grain yields, number of leaves above the ear, ear diameter, and ear aspect. Whereas cluster II contained 50% of the lines which are mainly of lines from Ecuador heterotic group having high grain yield, good ear and plant aspects, and good ear length and diameter. This cluster also consisted of lines from Pool9A and Kitale group, and uncategorized lines. Cluster III, on the other hand, involved five lines (one from Kitale, one from Ecuador, and three unrecognized lines) characterized by low grain yield, increased anthesis silking interval, and short ear height and ear length.

Table 3. 10 Euclidean genetic distances for pair-wise comparison of the 36 maize inbred lines based on 18 phenotypic traits evaluated at Ambo and Kulumsa

Line[§]	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
1	0.00																																			
2	6.63	0.00																																		
3	4.11	6.98	0.00																																	
4	6.57	7.20	7.89	0.00																																
5	4.43	5.03	5.58	6.96	0.00																															
6	6.08	5.04	7.00	7.09	5.60	0.00																														
7	4.88	5.14	5.90	6.65	5.94	4.93	0.00																													
8	7.70	4.85	8.32	6.45	7.55	4.75	6.36	0.00																												
9	5.64	5.14	5.75	7.62	5.92	5.12	5.13	5.65	0.00																											
10	6.04	7.06	7.08	3.43	7.24	6.89	5.71	6.29	6.30	0.00																										
11	6.79	5.43	7.21	9.17	7.04	5.86	5.30	6.55	3.09	7.67	0.00																									
12	5.46	6.03	6.20	5.18	7.19	5.98	4.98	5.22	5.34	3.44	6.54	0.00																								
13	3.87	5.26	4.16	6.57	5.55	4.29	3.08	6.14	4.49	5.62	5.47	3.94	0.00																							
14	8.20	6.66	7.49	5.17	7.17	6.68	7.41	5.96	7.35	5.93	9.02	6.15	6.83	0.00																						
15	7.04	5.77	7.93	5.39	7.14	5.63	6.94	4.01	5.05	5.31	6.40	4.90	6.09	6.42	0.00																					
16	5.17	6.03	7.19	4.84	6.60	6.02	5.11	5.64	5.87	4.15	7.13	3.81	5.15	7.20	5.28	0.00																				
17	3.53	6.42	5.86	5.00	5.08	6.87	4.76	7.45	6.45	4.55	7.71	5.05	5.07	7.42	7.03	3.94	0.00																			
18	6.00	4.19	5.87	5.82	5.76	3.72	5.26	3.60	4.47	5.72	5.64	4.31	3.91	4.79	3.74	5.76	6.57	0.00																		
19	6.36	5.43	5.82	5.86	5.81	5.55	6.49	5.21	4.97	5.58	6.70	4.51	5.00	4.38	4.35	6.38	6.45	2.80	0.00																	
20	6.91	6.55	7.01	8.55	7.05	5.17	6.05	6.78	7.39	7.53	7.47	6.28	5.16	8.13	7.64	7.48	8.01	5.53	6.87	0.00																
21	5.25	4.30	4.71	7.50	5.14	4.32	3.63	5.67	3.73	6.49	4.59	5.03	3.00	6.50	6.16	5.88	6.12	3.86	4.84	4.87	0.00															
22	4.23	4.69	4.97	5.81	4.81	4.34	4.14	5.19	4.24	5.11	5.30	4.77	3.53	6.65	4.84	4.95	5.11	3.85	5.15	5.03	3.43	0.00														
23	5.39	5.57	5.82	4.55	5.85	5.14	4.82	5.66	4.51	4.12	6.74	3.95	3.93	5.04	4.89	4.20	4.85	4.26	4.40	7.38	4.67	4.50	0.00													
24	7.24	4.51	8.32	7.84	6.07	6.25	6.54	6.19	5.09	6.99	5.62	6.22	6.52	8.08	5.82	6.00	6.35	5.88	5.65	8.34	5.99	6.22	6.05	0.00												
25	5.48	5.58	6.53	5.29	5.68	4.68	5.32	5.37	5.05	5.13	6.70	5.39	4.86	6.59	4.96	4.27	5.05	4.84	5.65	7.27	5.55	4.28	3.69	5.73	0.00											
26	6.62	5.80	6.25	9.00	6.57	5.25	4.14	7.20	5.12	7.93	5.14	6.66	4.00	8.20	7.69	7.92	7.50	5.38	6.29	5.85	3.50	5.34	6.14	6.92	6.52	0.00										
27	6.01	3.74	5.78	7.18	5.55	5.50	5.25	4.79	3.80	6.67	4.86	5.62	5.02	6.21	5.20	5.75	6.40	4.05	4.96	7.00	3.40	4.04	5.11	5.86	5.92	6.06	0.00									
28	8.29	5.41	7.71	8.11	7.72	5.80	6.03	4.82	6.08	6.90	6.34	6.27	6.25	6.41	6.63	7.08	8.24	5.15	6.41	5.23	4.42	5.05	6.61	7.43	6.77	6.21	4.75	0.00								
29	6.34	7.21	5.81	9.40	7.18	5.71	5.81	7.85	4.96	8.19	5.42	6.98	4.40	9.14	7.30	8.50	8.36	5.63	6.57	6.04	4.53	5.11	6.64	8.26	7.23	3.83	6.53	7.30	0.00							
30	4.25	6.93	4.62	5.42	5.07	6.61	5.31	7.73	5.81	5.42	7.43	6.14	4.77	6.62	6.84	6.69	4.66	5.70	5.66	7.95	5.83	4.60	4.66	7.84	5.15	6.25	6.48	8.15	6.28	0.00						
31	9.07	5.27	9.84	8.84	8.06	5.36	6.73	5.27	6.99	7.82	6.70	7.06	7.20	8.27	6.79	6.95	8.81	6.19	7.65	5.82	5.94	6.41	7.40	6.54	7.32	7.28	6.26	4.50	8.31	9.84	0.00					
32	7.84	4.22	8.28	6.36	6.57	5.46	6.55	4.23	6.64	6.78	7.27	5.90	6.36	5.41	5.18	6.04	6.96	4.16	4.76	7.19	5.82	5.84	5.90	4.88	5.48	7.20	5.39	5.80	8.90	7.80	6.10	0.00				
33	6.25	2.83	7.01	5.88	5.29	4.93	4.69	4.41	4.99	5.51	5.49	5.17	5.09	6.59	4.44	5.00	5.56	4.01	5.01	6.13	4.43	3.63	5.08	4.24	4.68	5.90	4.21	5.14	6.90	6.27	5.12	4.08	0.00			
34	7.22	4.14	6.60	8.10	6.56	5.16	5.55	4.72	5.46	7.18	5.96	5.79	5.19	6.14	6.72	7.05	7.31	4.32	5.31	5.47	3.72	4.75	6.12	6.12	6.59	5.38	4.13	3.49	6.77	7.37	5.51	4.89	4.80	0.00		
35	5.96	5.57	6.86	7.77	4.61	6.41	6.92	7.57	5.63	7.95	6.47	7.49	6.30	8.16	6.62	7.32	6.18	5.92	5.72	8.87	6.38	5.80	6.52	4.57	5.67	7.01	6.35	8.87	7.77	6.05	9.23	5.87	5.55	7.02	0.00	
36	5.86	3.90	6.08	5.86	5.65	4.43	4.50	3.80	4.12	4.80	5.16	3.91	4.16	5.45	4.24	5.26	5.83	2.90	3.79	5.18	3.30	3.16	4.32	5.36	5.33	5.22	3.44	3.99	5.60	5.75	5.05	4.91	3.03	3.53	6.54	0.00

[§] Number 1 to 36 represents inbred lines as listed down in Table 5 in that order

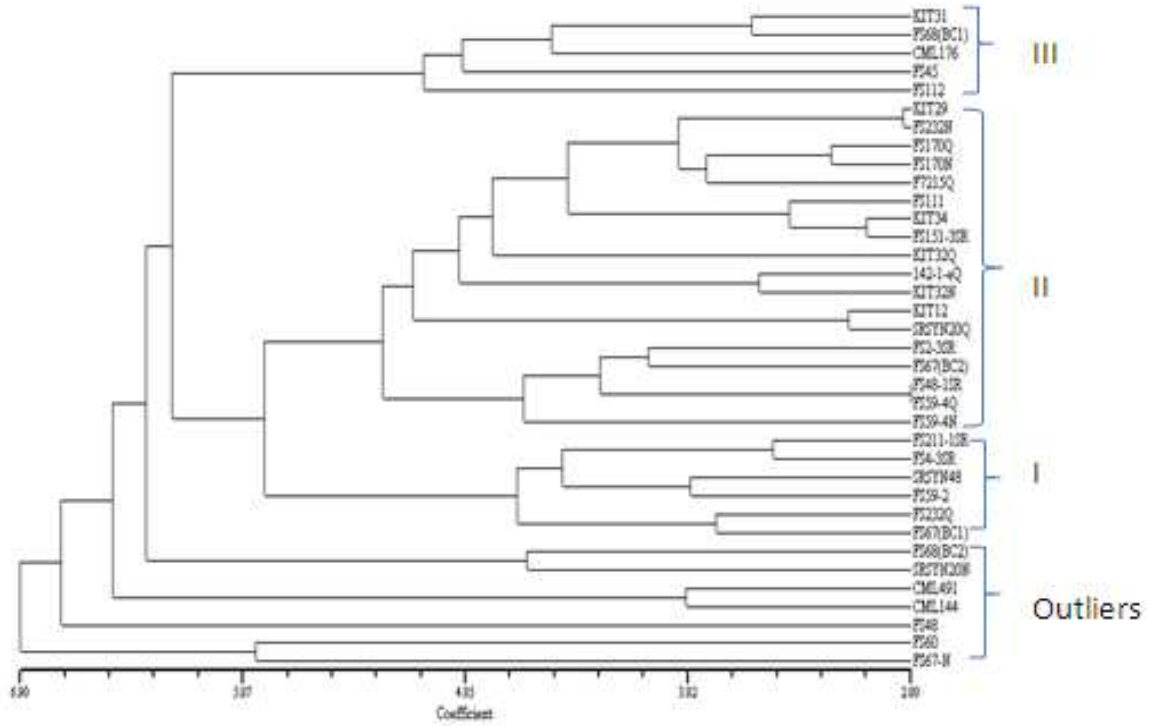

Figure 3. 4 Dendrogram of the 36 QPM and non-QPM maize inbred lines revealed by UPGMA cluster analysis based on 18 phenotypic traits when evaluated at two locations

3.5 Discussion

In the present study, observations on 18 phenotypic traits across the 36 maize inbred lines revealed useful information of the existing diversity in the Tropical-highland QPM breeding lines. The range of variability observed among the inbred lines for all traits was significant, indicating the presence of high level of phenotypic diversity in the inbred lines. Similarly, Beyene et al. (2006) observed high genetic diversity among the traditional Ethiopian highland maize accessions using both morpho-agronomic traits and molecular markers. Legesse et al. (2009) detected the availability of significant genetic variability in the study of combining ability and heterotic grouping among highland maize inbred lines using agronomic traits. Understanding the genetic diversity and relationships that may exist in maize germplasm is an important and a priority task for breeders. It assists in decision making for selection of parents to be involved in crosses, and also helpful to enhance the genetic base of breeding programs. To this end, it is necessary to make use of the available genetic variability to explore desirable traits including grain yield and other yield attributes, quality traits and their association (Mohammadi and

Prasanna, 2003; Has et al., 2009). According to FAO's prediction, an additional 60 million tonnes of maize grain will be needed in the tropics from the annual global harvest by 2030 (Paliwal et al., 2000). To meet such requirements, conservation and utilization of useful genetic diversity is crucial. Because it is fundamental for sustainable genetic gain of economically desirable traits, and can help prevent losses due to biotic and abiotic stress (Pollak and Scott, 2005; Osorno and Carena, 2008; Has et al., 2010).

The broad range of phenotypic variation detected in the current study implied great potential for the development of improved open-pollinating varieties, inbred lines and hybrids of QPM adapted to the highland agro-ecologies. The ranges in days to anthesis at Ambo (96.6–118.7) and Kulumsa (92.0–109.5), for example, suggests the possibility to develop cultivars with different maturity groups for the diverse highland environments. The combined analysis of variance showed highly significant location x line interaction for most of the traits indicating rank differences in performance of the lines across the two locations. Luxurious vegetative growth and better yield performances of the lines were observed at Ambo than Kulumsa site which could be attributed to inadequate rainfall during the growing season at Kulumsa. It was interesting to note that out of the six non-QPM inbreds used in this study three had better grain yield, one provided equivalent and the rest two had lower grain yield than their QPM converted counterparts. The result conforms to previous reports by Prasanna et al. (2001), Sofi et al. (2009), and Atlin et al. (2011) that it could be possible to develop a QPM genotype which is comparable or even better in agronomic performances than the normal maize genotypes. The implication is that QPM adoption, especially in Africa, could be facilitated if the QPM cultivar is agronomically better than the non-QPM for farmers' acceptance and marketing (Krivanek et al., 2007).

Understanding of the relationships among traits is important in designing effective selection programs for crop improvement. Genetic correlations are of interest to determine degree of association between traits and how they may enhance selection (Hallauer et al., 2010). In this study, grain yield was highly correlated with ear length, number of kernels per row, ear aspect, and ear height. All these traits are ear traits, except ear height, which dominated the correlation with grain yield. According to

Hallauer et al. (2010), average genetic correlations with yield observed from several experimental results were larger for ear traits than for plant and ear height. The positive correlation of grain yield with its components such as ear diameter and length, thousand kernel weight, and number of kernel per row was also reported by other workers (Bolaños and Edmeades, 1996; Edmeades et al., 1997). Unlike the reports of Dagne (2008) and Tollenaar et al. (2004), weak correlations of grain yield with foliar traits such as leaf length and width, leaf number and area, and foliar rating were observed in the present study. In general, the traits with greater heritability value and strong association with yield can be considered as secondary traits during indirect selection for grain yield. Indirect selection is the selection for a secondary trait with the purpose to obtain a positive response in the desirable or primary trait (Hallauer et al., 2010).

Variance components, coefficients of variability, heritability and genetic advance parameters provide estimates of genetic variation of quantitative traits. The variance component derived from further partitioning of genotypic differences into phenotypic, genotypic, and environmental coefficient of variation and heritability is a good index of transmission of characters from parents to their offsprings (Falconer, 1960). The proportions of genotypic variances (σ^2_g) were higher than both error variances (σ^2_e) and genotype x environment interactions (σ^2_{gxe}) for most of the traits. It means that the proportion of the heritable component of the variances is overwhelming, indicating potential of the lines that can be exploited through selection. However, the proportion of σ^2_{gxe} component of the phenotypic variance for anthesis date and anthesis silking interval traits is greater than the σ^2_g. This, in turn, contributed to the small genotypic coefficients of variations and genetic advance values of these two traits. The result is also in agreement with the report of Assefa et al. (1999) that genetic coefficients of variation together with heritability estimates would give the best picture of genetic advance to be expected from selection.

On the other hand, traits such as thousand kernel weight, ear height, and tassel size exhibited relatively high estimates of genotypic coefficient of variation coupled with high heritability and genetic advance as percent of the mean. High heritability estimates along with expected genetic advance are more useful in predicting the response to selection. Johnson et al. (1955) also suggested that the estimate of

heritability and genetic advance should always be considered simultaneously. The genetic variance for the three traits could be attributed to their high additive gene effects (Johnson et al., 1955) and thus there is better scope for improvement of these traits through direct selection. For example, based on the result of this study selection of the top 5% with higher thousand kernel weight may lead to expected increase of thousand kernel weight by 40.3% after one cycle of selection. Dagne (2008) reported that QPM line development should focus on thousand kernel weight as selection criteria to increase grain yield because this trait had also positive correlation with grain yield. However, heritability and selection response expressed as a percentage of the mean were very low for anthesis date, anthesis silking interval, and ear diameter. This implied the predominant role of non-additive gene action and environmental effect in governing these traits. Shanthi et al. (2011) also reported low values of heritability in broad sense and genetic advance as percent of the mean for days to 50% tasseling and silking, protein and oil contents. The authors further pointed out the major role of non-additive gene action for these traits and thus improvement of the traits may be possible through hybrid breeding.

The presence of broad phenotypic diversity among the highland maize inbred lines was further substantiated by principal component analysis, which indicated that the total variation was fairly distributed across all the 12 morpho-agronomic traits. It was also quite conceivable that these traits were adequately represented by four principal components to measure underlying ‘dimensions’ in the data. Accordingly, the first principal component is just a weighted average of standardized measurements of four traits with equal positive (for grain yield and ear height) and negative (for plant and ear aspects) signs, and showing weights that are more or less similar. Furthermore, these four traits contributed more than the other traits for the 32.2% variation accounted for by the first principal component (PC1). The variations in PC2 and PC3 were contributed more by foliar and ear traits, respectively, while PC4 was dominated by a mixture of traits that contributed more variation in PC2 and PC3. The cumulative variation explained by the four PCs (80.7%) in the current study is greater than the finding’s of Beyene et al. (2006). The authors reported 71.8% of the total variation in 62 traditional Ethiopian highland maize accessions was represented by the first four PCs. Similarly, Dagne (2008) found a comparable result of 78% total variation explained by the first six PCs among CIMMYT QPM inbred lines. The work

of Alika et al. (1993) also supports the major role of morphological traits in phenotypic variation observed in this study.

Genetic distance estimates and cluster analysis based on the phenotypic data further revealed the existence of considerable variability among the inbred lines. The dendrogram presented the resolution power of the phenotypic traits for grouping the inbred lines following more or less similar patterns of previous classifications using combining ability study. In agreement to this finding, Beyene et al. (2006) classified 62 traditional highland maize accessions into three groups using 15 morphological traits. Lucchin et al. (2003) clustered 20 Italian flint maize landraces using 34 morphological and agronomic traits. Wietholter et al. (2008), on the other hand, emphasized the contribution of traits *viz*., plant height, ear insertion, female flowering, male flowering and kernel row number per ear in the classification of Brazilian corn landraces. Besides, Abu-Alrub et al. (2006) reported that tassel traits were found to be less reliable descriptors unlike kernel and ear traits for classifying Peruvian highland maize germplasm.

3.6 Conclusion

Maize breeding relies on the extent of genetic variability available among genotypes. The high genetic variability observed among the inbred lines used in this study for various traits implied great potential for the development of improved QPM open-pollinating varieties, inbred lines and hybrids for the highland agro-ecologies. Besides, it was shown that QPM lines performed comparably the same with their non-QPM counterparts and thus witnessed the possibility of developing competent QPM cultivars. This could help to further alleviate the doubt in the farming community that QPM genotypes are agronomically inferior in performance to the non-QPM or normal genotypes, and also facilitates the adoption of new QPM cultivars. Both principal component and cluster analyses of the present study showed important contributions of the phenotypic traits for the total variation existing among the inbred lines. The analyses also enabled to partially categorize the lines according to previously made classifications based on combining ability study. The cluster analysis allowed distinguishing three groups of the maize inbred lines with distinctive morpho-agronomic traits. This will be useful to set breeding and

conservation strategies for QPM germplasm adapted to the highland environments. The dendrogram clearly displayed stratification of the inbred lines under three clusters and an outlier group depending on their potentials in terms of agronomic performances. Accordingly, cluster I consisted of lines with intermediate potential in agronomic performance, while cluster II and III comprised of lines that are agronomically high and low performing ones, respectively. Based on the cluster groups, seven inbred lines [KIT32Q, 142-1eQ, SRSYN20Q, FS67(BC_2), FS170Q, FS60, and F7215] with complementary phenotypic traits and relatively better yield performance were selected to make crosses for further genetic analysis and breeding. The phenotypic traits were found useful for primary characterization of the maize inbred lines, although correlating variation at the molecular level to phenotypic diversity is an important prerequisite for future breeding and conservation plans.

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CHAPTER 4

The genetic purity and patterns of relationships among highland adapted quality protein and normal maize inbred lines using microsatellite markers

4.1 Abstract

Conversion of normal maize (*Zea mays* L.) into quality protein maize (QPM) significantly improves its nutritional value for humans and animals. Highland adapted normal maize inbred lines were backcrossed with selected QPM donor lines to incorporate the *opaque-2* gene. The objectives of this study were to verify the genetic purity, determine the effect of conversions of normal maize lines to QPM on the original heterotic system and understand patterns of relationships among 36 white maize inbred lines (30 QPM and 6 non-QPM) using 25 simple sequence repeat (SSR) markers. The proportion of observed heterogeneity within an inbred line varied from 4 to 16.7% and the average was 7.9%. Twenty of the 36 inbred lines (55.6%) showed higher than the expected 6.25% mean residual heterogeneity for inbred lines developed after 5 generations of selfing. The genetic distances between pairwise comparisons of the 36 inbred lines ranged from 0.077 to 0.780 and the average was 0.52. Nearly 98% of the pairwise comparisons had a distance between 0.30 and 0.78, which indicates large genetic differences among most lines. The model-based population structure, principal coordinate and neighbor-joining cluster analyses revealed the presence of 3 groups, which is generally consistent with pedigree information and partly with heterotic grouping. Analysis of molecular variance indicated that differences among heterotic groups explained 8.6 to 15.4% of the total SSR variance, indicating the presence of moderate to great genetic differentiation among heterotic groups.

Key words: Genetic diversity, Heterotic group, Highland maize, Microsatellite marker, Quality Protein Maize.

4.2 Introduction

Maize (*Zea mays* L., 2n = 2x = 20) is an important source of food and nutritional security for millions of people in the developing world, especially in sub-Saharan Africa (SSA) and Latin America (Shiferaw et al., 2011). It is a staple food in many of the SSA countries and is commonly grown by resource poor, small-scale farmers in rural areas. Farmers in the highland zone with an elevation of >1,800 meters above sea level (Hassan et al., 2001) generally grow local varieties that are characterized by long maturity, vulnerable to frost damage, tall in plant stature and susceptible to lodging, which together contribute to low yield potential (Twumasi-Afriyie et al., 2002). Due to various socio-economic importance of maize in the highland zone, a CIMMYT-led maize project was initiated in Ethiopia to develop varieties adapted to the highland zones of east and central African (ECA) countries. The early non-QPM inbred lines were extracted from three populations: Kitale Synthetic II x N3-types, Ecuador-573 x/SC-type, and Pool9A x/IITA's Mid-altitude streak resistant (Legesse et al., 2007). Most of the lines were then top crossed with three population testers representing three different heterotic groups (Kitale Syn II, Ecuador 573 and Pool 9A). Combining ability analyses were conducted and most inbred lines were putatively assigned into one of the three heterotic groups although some lines had a mixed heterotic pattern (Twumasi-Afriyie et al., 2002).

Normal maize has poor protein quality due to a deficiency in levels of the amino acids lysine and tryptophan limiting its nutritional value for humans and animals. The discovery of high protein quality maize caused by a recessive mutation, *opaque-2* (*o2*), during the 1960s was an important milestone in quality protein maize (QPM) breeding (Mertz et al., 1964). QPM, originally developed in the late 1990s at CIMMYT-Mexico, possesses improved protein quality due to enhancement in lysine and tryptophan that are known to be regulated by *opaque-2* gene and associated modifiers (Gupta et al., 2009). QPM lines were also developed for the highlands of east and central African countries by backcrossing the non-QPM lines with QPM donor lines in collaboration with CIMMYT (Krivanek et al., 2007). Results of grain sample analysis at CIMMYT-Mexico showed that the converted QPM inbreds were phenotypically stable with protein levels of 8-14 g per 100 g protein and tryptophan levels of 0.65-0.90 g per 100 g protein (Twumasi-Afriyie et al., 2012). Maintenance of

the genetic purity and interrelationships among the QPM and non-QPM inbred lines is thus an important quality control function in maize breeding programs (Semagn et al., 2012a).

Maize breeders are constantly developing genetically complementary inbred lines to develop new hybrids with increased hybrid vigor (Duvick, 2001). Maize inbred lines are primarily developed by crossing elite lines within heterotic groups followed by inbreeding and selection, while hybrids are produced by crossing parents that belong to different heterotic groups. A heterotic group is a collection of genetically closely related inbred lines which tend to result in hybrid vigor when crossed with lines from unrelated heterotic groups (Lee, 1995). Depending on the objectives of the breeding program, breeders use different conventional breeding methods in selecting the best parents for making crosses and for assigning lines to a particular heterotic group, including (a) pedigree relationships, (b) phenotypic performance for specific traits, (c) adaptability and yield stability, (d) top crosses, and (e) diallel crosses. In addition, genetic distance estimates using molecular markers are reportedly helpful to identify the best parent combinations for new pedigree starts and to assign lines into heterotic groups (Melchinger et al., 1990; Benchimol et al., 2000; Reif et al., 2003a; Reif et al., 2003b; Bertan et al., 2007; Flint-Garcia et al., 2009; Lu et al., 2009).

Genetic distance can be estimated from various types of molecular markers, including restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), simple sequence repeats (SSRs) and single nucleotide polymorphisms (SNPs). Advances in marker technology have shifted toward SNP markers, particularly for model organisms with substantial genomic resources. Hamblin et al. (2007) used 89 SSRs and 847 SNPs markers to characterize 259 maize inbred lines, and compared these markers in elucidating the population structure and the genetic relationships among individuals. The SSRs performed better at clustering germplasm into populations and provided more resolution in measuring genetic distance than SNPs. For germplasm characterization, SSR markers were reported to provide much better information than SNPs (Hamblin et al., 2007; van Inghelandt et al., 2010). The objectives of the present study were to verify the genetic purity within each inbred line, determine the effect of conversions of normal maize lines to QPM on the original heterotic system and understand patterns

of relationships among 36 white maize inbred lines (30 QPM and 6 normal) adapted to highland agro-ecologies using SSR markers.

4.3 Materials and methods

4.3.1 Germplasm

The 36 inbred lines used for phenotypic characterization in Chapter 3 were also used for genotyping experiment in this chapter. Detailed descriptions of the inbred lines used for this experiment are presented under the materials and methods section of Chapter 3.

4.3.2 DNA extraction and genotyping

Seedlings of all genotypes were grown in plastic seed trays for 3 weeks in a screen house at the Biosciences for east and central Africa (BecA) hub in Nairobi, Kenya. Leaf tissue from each line was cut into pieces with scissors, and transferred into 1.2 ml strip tubes that contained two 4 mm stainless steel grinding balls. The tissue was freeze-dried (lyophilized) for 3 days using a Labconco freeze dryer (http://www.labconco.com). The lyophilized leaf samples were ground into fine powder using GenoGrinder-2000 at 500 strokes per minute for 6 minutes. Genomic DNA was extracted using a modified version of the mini-prep Cetyl Trimethyl Ammonium Bromide (CTAB) method of CIMMYT protocol **(**http://www.generationcp.org/capcorner/chile_wksp_2005/manuals/manual_01.pdf**).** The quality of the isolated DNA was checked after running aliquots of DNA samples on a 0.8% agarose gel that contained 0.3 μg/ml GelRed (Biotium). DNA concentration was measured using NanoDrop ND-800 Spectrophotometer (Thermo Scientific).

Thirty SSR markers selected from the list of markers that had previously been used for genetic characterization of CIMMYT maize germplasm (Warburton et al., 2002) were used for this study. The SSRs were chosen based on prior information, including chromosomal distribution, minor allele frequency (MAF), polymorphic information content (PIC), and repeat length. Polymerase chain reaction (PCR) was

performed in 96-wells plates in a total reaction volume of 10 µl that consisted of 50 ng template DNA, 1× magnesium-free PCR buffer, 2.3 mM $MgCl_2$, 0.20 µM of the forward primer labeled either with 6-FAM, PET, VIC or NED fluorescent dyes (PE-Applied Biosystems), 0.20 µM of the reverse primers, 0.20 mM each dNTP, and 0.375 unit Taq DNA polymerase. PCR amplifications were performed for each primer pair separately using Gene-Amp PCR System 9600 (PE-Applied Biosystems) according to the following protocol: 3 min initial denaturation at 94 ^{o}C, followed by 35 cycles of 94 ^{o}C for 30 sec, 1 min annealing between 52 and 60 ^{o}C (depending on the recommended annealing temperature for each primer; Table 2) and 72 ^{o}C for 2 min, and a final extension of 10 min at 72 ^{o}C. For high throughput and low cost genotyping, PCR products were separated by pooling 1.2 µl of PCR products from each of the 6-FAM, VIC, PET and NED-labelled markers and 9 µL of a mix of an injection solution (HiDi) and GS-500LIZ size standard (1 mL HiDi and 12 µl GS500 LIZ for a 96-well plate). DNA fragments were denatured for 3 minutes at 94 ^{o}C and size-fractioned using ABI 3730 Capillary DNA Sequencer with GeneScan software (PE-Applied Biosystems). Allele sizes were called using GeneMapper version 4 software. Both DNA extraction and SSR genotyping were done at the BecA hub.

4.3.3 Data analysis

Stuttering and plus-A amplification often cause ambiguity in automated SSR allele binning (Idury and Cardon, 1997). We used AlleloBin **(**http://www.icrisat.org/bt-software-downloads.htmhttp://www.icrisat.org/bt-software-d-allelobin.htm**)** for adjusting inconsistencies in allele calls obtained from GeneMapper software. Different types of multivariate analyses were performed on the adjusted SSR data of the 25 markers (Table 2). The data of the remaining 5 markers were excluded because they either showed ambiguous allele calls with high proportion of missing data points or were monomorphic. For each SSR marker, number of alleles per marker, number of genotypes, minor allele frequency (MAF), observed heterogeneity (the number of markers that were not homozygous due to mixture of two homozygous genotypes or heterozygosity due to pollen contamination during multiplication), and polymorphic information content (PIC) were computed using PowerMarker version 3.25 (Liu and Muse, 2005). An admixture model-based clustering method was used to infer population structure using the software package

STRUCTURE version 2.3.3 (Pritchard et al., 2000). STRUCTURE was run as described elsewhere (Semagn et al., 2012b) by varying the number of clusters (K) from 1 to 6, with each K repeated trice. Individuals with probability of membership > 60% were assigned to the same group while those with <60% probability memberships in any single group were assigned to a ‘‘mixed’’ group (Yang et al., 2011; Semagn et al., 2012b). Roger’s genetic distance (Rogers, 1972) was calculated between each pair of genotypes using PowerMarker. A phylogenetic tree was constructed from the genetic distance matrix using the neighbour joining method implemented in DARwin version 5.0.158 (http://darwin.cirad.fr/Home.php). Principal coordinates analysis (PCoA) was performed on the genetic distance matrix using DARwin software. The first two principal components were plotted for visual examination of the clustering pattern of the lines. Analysis of molecular variance (AMOVA) was used to partition the SSR variation among and within population components (Excoffier et al., 1992) using the ARLEQUIN version 3.5 (http://cmpg.unibe.ch/software/arlequin3). The inbred lines were assigned into populations on the basis of their putative heterotic grouping assigned by breeders based on combining ability (phenotype data) from field experiments.

4.4 Results

4.4.1 Marker Characterization

A summary of the 25 SSR markers used in the present study is given in Table 4.1. There were two to four pairs of markers for each chromosome except chromosome 9 that had only a single marker. The number of alleles scored for each marker varied from 2 in phi084 and umc2250 to 8 in umc1161. The 25 markers amplified a total of 98 alleles, with an average of 3.9 alleles per marker. Minor allele frequency was the lowest (0.194) in umc1367 and highest (0.681) in phi299852, and the overall average was 0.434. The polymorphism information content ranged from 0.303 (umc1367) to 0.735 (phi299852) and the overall average was 0.491. Minor allele frequency showed highly positive correlation ($r = 0.863$; $p < 0.001$) with the polymorphism information content.

Table 4. 1 Summary of marker characterization for the 25 SSRs used in the study

Marker	Chromosome	Bin number	Repeat length	Repeat motif	Annealing temperature (°C)	Minor allele frequency	Number of genotypes	Number of alleles	Observed heterozygosity	PIC†
nc130	5	5.0	3	AGC	54	0.333	3	3	0.000	0.404
nc133	2	2.1	5	GTGTC	54	0.343	3	3	0.000	0.454
phi029	3	3.0	4	AGCG	56	0.443	4	3	0.029	0.410
phi046	3	3.1	4	ACGC	60	0.472	3	3	0.000	0.412
phi056	1	1.0	3	CCG	56	0.561	5	4	0.030	0.633
phi065	9	9.0	5	CACTT	54	0.611	6	4	0.056	0.604
phi072	4	4.0	4	AAAC	56	0.306	5	4	0.056	0.401
phi075	6	6.0	2	CT	54	0.236	4	3	0.028	0.354
phi076	4	4.1	6	GAGCGG	60	0.600	9	6	0.143	0.663
phi079	4	4.1	5	AGATG	60	0.625	6	5	0.028	0.690
phi084	10	10.0	3	GAA	54	0.333	3	2	0.056	0.346
phi102228	3	3.1	4	AAGC	54	0.222	3	3	0.000	0.337
phi114	7	7.0	4	GCCT	60	0.515	4	4	0.000	0.524
phi123	6	6.1	4	AAAG	54	0.417	3	3	0.000	0.505
phi299852	6	6.1	3	AGC	58	0.681	8	7	0.028	0.735
phi308707	1	1.0	3	AGC	56	0.528	3	3	0.000	0.541
phi331888	5	5.0	3	AAG	58	0.458	5	4	0.028	0.512
phi374118	3	3.0	3	ACC	54	0.417	4	4	0.000	0.542
phi96100	2	2.1	4	ACCT	56	0.597	7	4	0.083	0.659
umc1161	8	8.1	6	GCTGGG	56	0.409	10	8	0.091	0.577
umc1304	8	8.0	4	TCGA	54	0.386	4	3	0.143	0.380
umc1367	10	10.0	3	CGA	62	0.194	4	4	0.000	0.303
umc1545	7	7.0	4	AAGA	54	0.314	5	5	0.000	0.423
umc1917	1	1.0	3	CTG	52	0.357	5	4	0.029	0.497
umc2250	2	2.0	3	ACG	58	0.500	1	2	1.000	0.375
Mean						0.434	4.68	3.9	0.073	0.491

† PIC, polymorphism information content

4.4.2 Genetic purity and population structure

The proportion of observed heterogeneity within an inbred line varied from 4% in CML144 to 16.7% in FS232Q and the average was 7.9%. Twenty of the 36 inbred lines (55.6%) showed heterogeneity higher than the 6.25% expected value for inbred lines derived after 5 generations of selfing (Figure 4.1). The estimated log probability of the data ((LnP(D)) increased between K=1 and K=3, and reached to a plateau between K=4 and K=6 (Figure 4.2). The ad hoc statistic ΔK showed a higher likelihood values at K=2, followed by K=3 with a sharp decrease when K increased from 3 to 6, suggesting the presence of 2 or 3 possible groups. A summary of the population structure with group membership between K=2 and K=6 showed that Group 1 consisted of 2 non-QPM and 9 QPM lines, with the majority of them belonging to the Ecuador heterotic group. Group 2 consisted of 4 non-QPM and 9

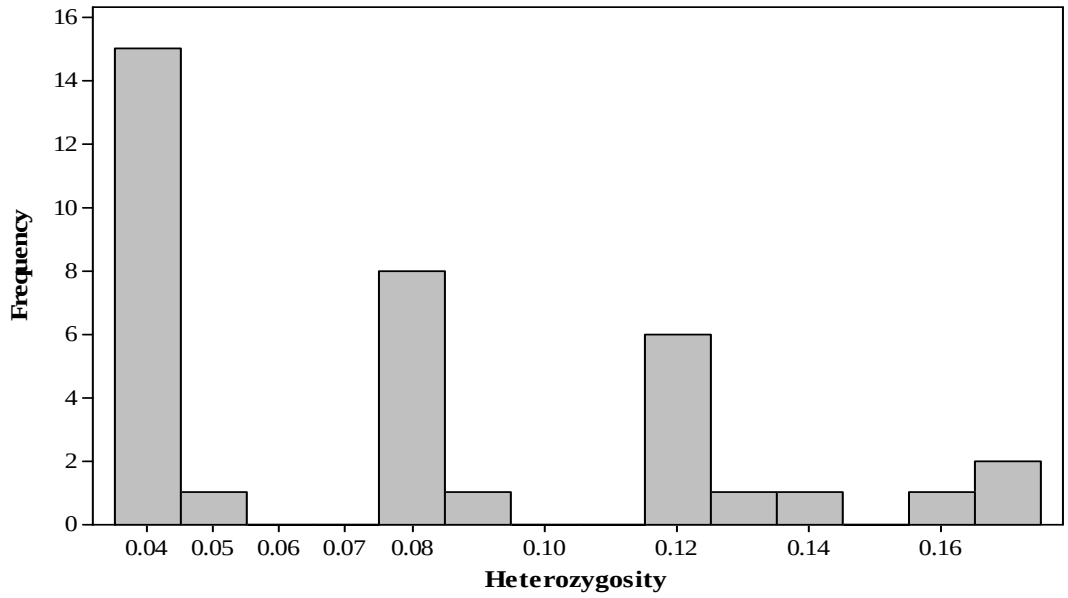

Figure 4. 1 Frequency distribution of the observed heterozygosity for the 36 inbred lines genotyped with 25 SSR markers

QPM lines that belong to all 3 heterotic groups plus to an unassigned ones. Group 3 consisted of 10 QPM lines, half of which belong to the Kitale heterotic group (data not given).

4.4.3 Genetic distance and relationship

Roger's genetic distances between pairwise comparisons of the 36 inbred lines ranged from 0.077 between CML144 and CML491 to 0.780 between FS111 and FS211-SR, and the overall mean genetic distance was 0.52. About 98% of the pairwise comparisons had a distance between 0.30 and 0.78 (Figure 4.3). The neighbor-joining tree generated from Roger's genetic distance matrix grouped the 36 lines into 3 groups (Figure 4.4). Group 1 consisted of a total of 9 inbred lines, of which 6 lines belong to Kitale, 1 to Ecuador, and the remaining two to Pool 9A. Group 2 comprised of 13 inbred lines that belongs to Ecuador (5 lines), Pool 9A (3 lines) and an unassigned (5 lines). Group 3 consisted of 14 inbred lines, including Ecuador (5 lines), Kitale (4 lines), Pool 9A (1 line) and an unassigned (4 lines). There was low concordance between the neighbor-joining clustering and model-based population partition in assigning lines into the different groups. The first five principal components (PCs) from principal coordinate analysis explained 48.8% of the total

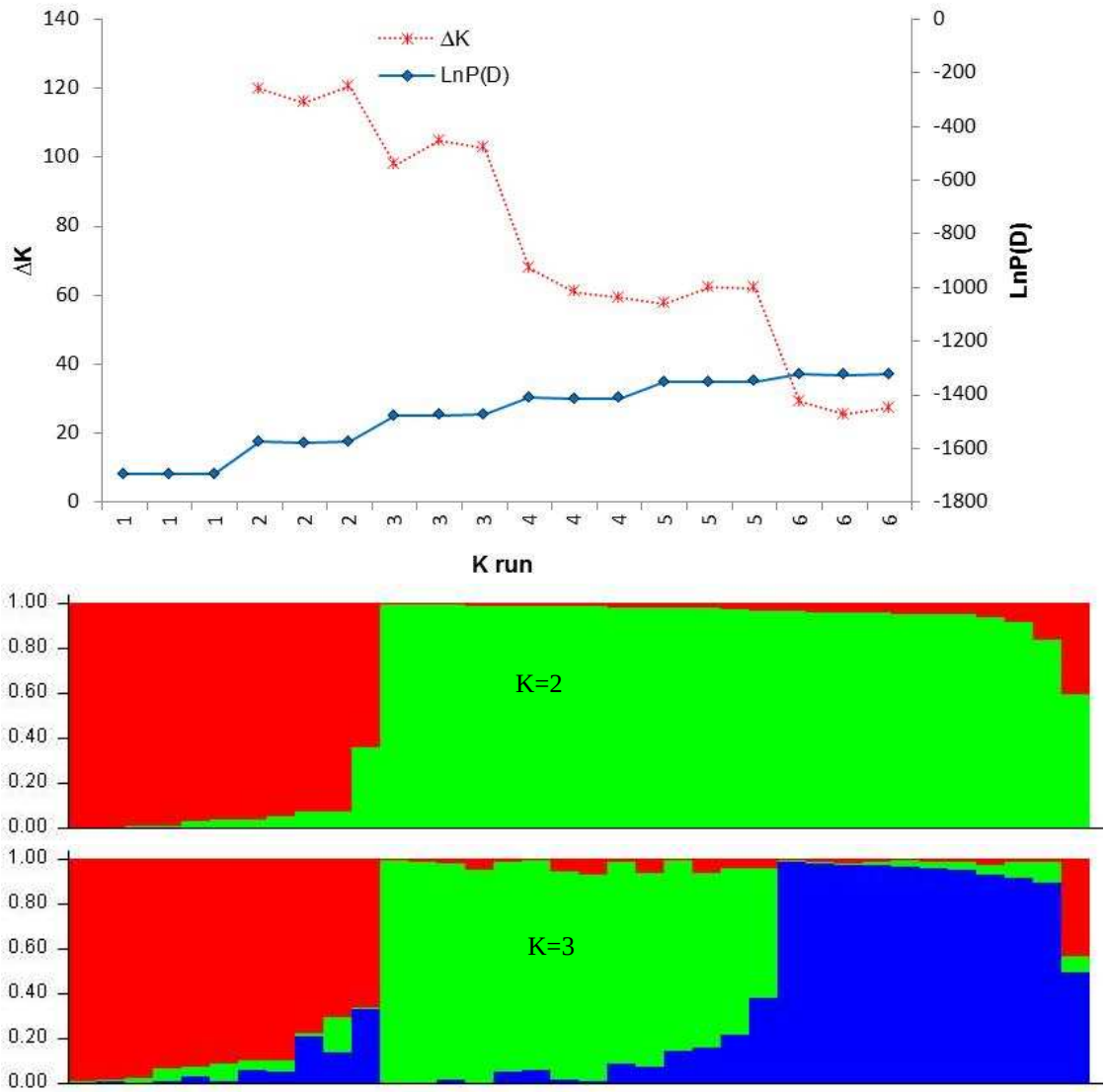

Figure 4. 2 Population structure of 36 maize inbred lines genotyped with 25 SSR markers: (a) plot of LnP(D) and an ad hoc statistic ΔK calculated for K ranging from 1 to 6, with each K repeated trice; (b) population structure of the 36 lines at K=2 and K=3. Each individual is represented by a single vertical line that is partitioned into K colored segments (K=2 and K=3) in the x-axis, with lengths proportional to the estimated probability membership (y-axis) to each of the K inferred clusters.

genetic variations among the inbred lines. A plot of PC1 (13.0%) and PC2 (10.5%) revealed 3 major groups (Figure 4.5). The principal coordinate analysis showed two major groups corresponding to the majority of lines that belong to the Ecuador (group 1) and Kitale (group 2) heterotic groups, with the unassigned and Pool 9A lines showing no clear patterns of grouping.

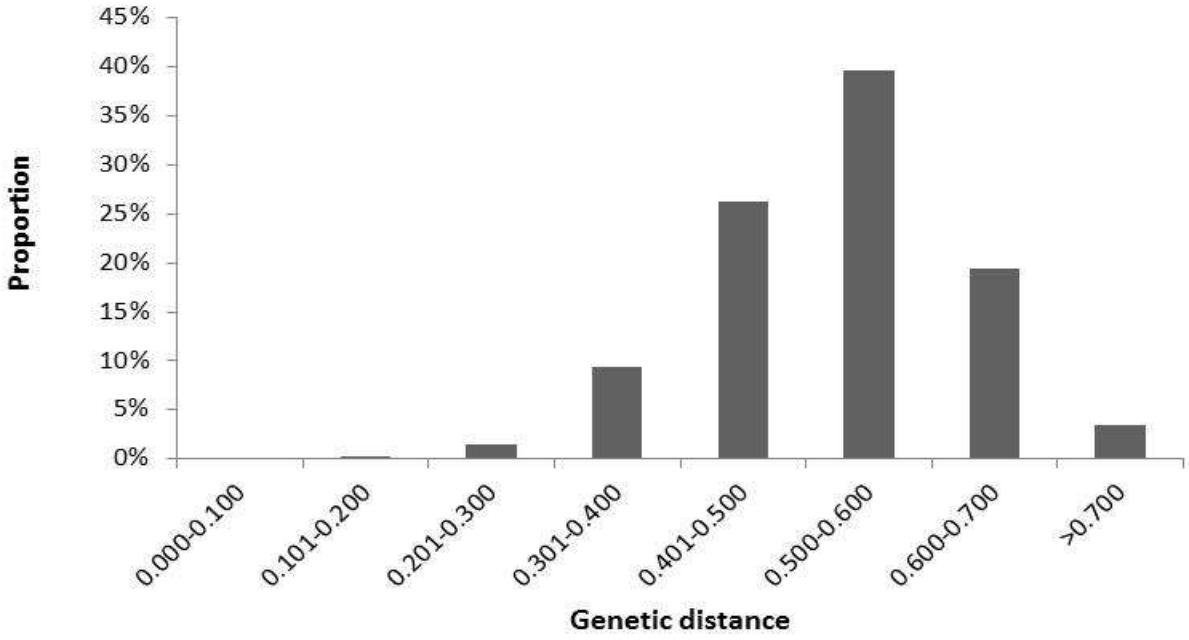

Figure 4. 3 Frequency distribution of pairwise Roger's genetic distance among 36 maize inbred lines genotyped with 25 SSR

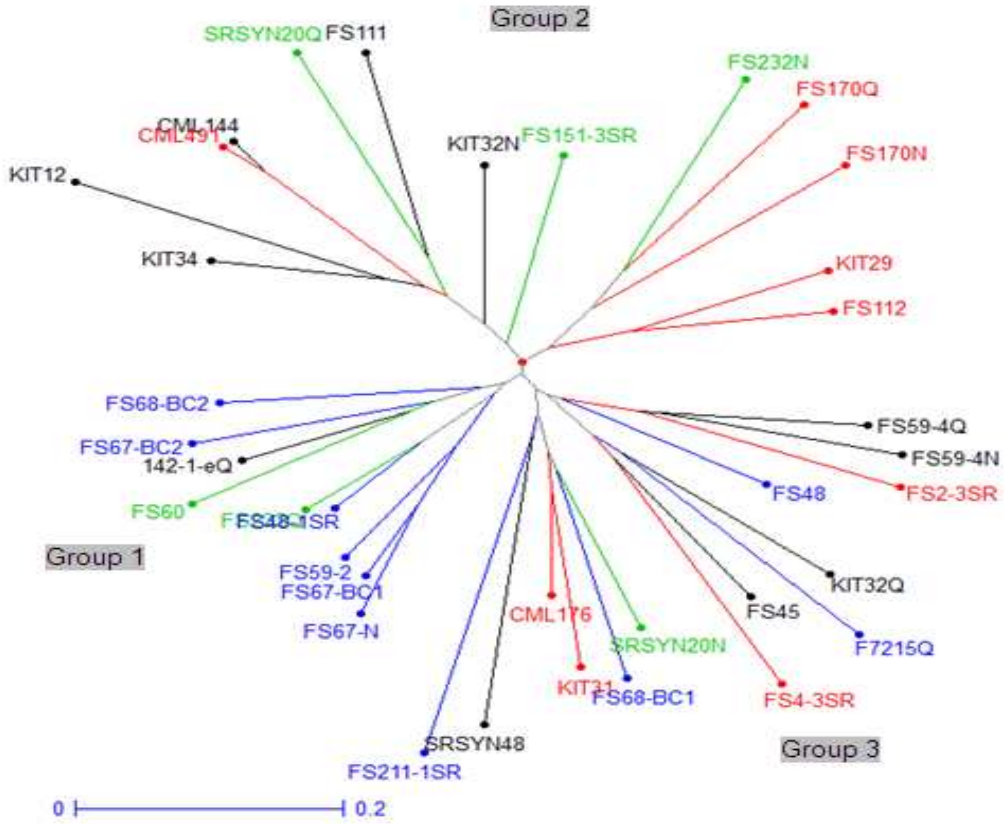

Figure 4. 4 Neighbor-joining tree for 36 inbred lines based on Roger's genetic distance calculated from 25 SSR markers. Lines that belong to the same heterotic group are indicated with the same color (Eucador=black; Pool9A = green; Kitale=blue and unknown=red).

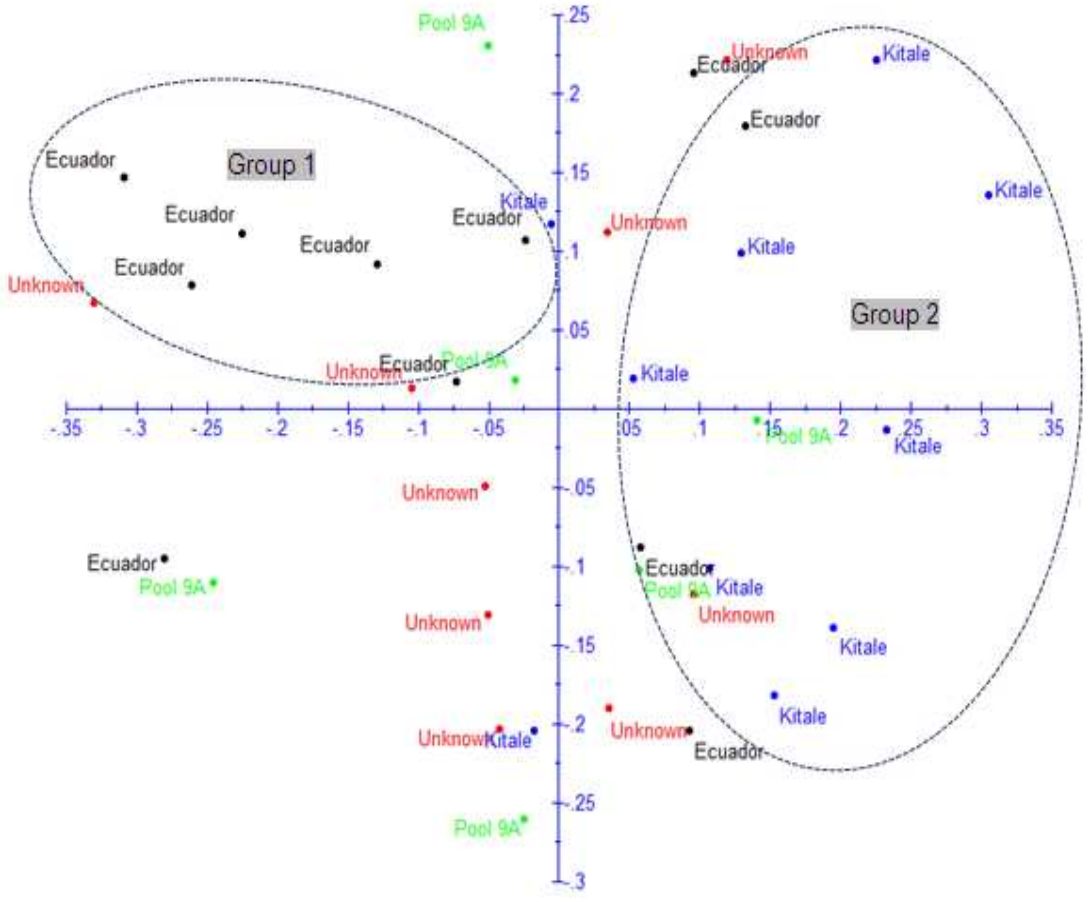

Figure 4. 5 Plot of PC1 (13.0%) and PC2 (10.5%) from principal coordinate analysis of 36 inbred lines. Lines that belong to the same heterotic group are indicated with the same colour (Eucador=black; Pool9A = green; Kitale=blue and unknown=red)

Analysis of molecular variance (AMOVA) was conducted to assess whether the SSR-based data agreed with the expected genetic differentiation between heterotic groups based on prior combining ability tests. This was conducted by assigning inbred lines that belong to Ecuador (11 lines), Kitale (10 lines), Pool9A (6 lines) and unkown/CIMMYT (9 lines). Partitioning of the overall molecular variance into hierarchical levels using AMOVA (Table 4.2) showed that differences among and within heterotic groups accounted for 8.6 to 15.4% and 84.6 to 91.4%, respectively. The genetic differentiation parameter, F_{ST} (Wright 1978), varied between the heterotic groups from 0.086 to 0.154. A random permutation test indicated that the proportion of variances attributable at all groups were highly significant ($p < 0.0001$).

Table 4. 2 Analysis of molecular variance (AMOVA) for the extraction of SSR variation among and within heterotic groups (populations)

Grouping	Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Heterotic groups (Eucador, Kitale, Pool9A and unassigned)	Between heterotic groups	3	68.56	0.98	15.38
	Within heterotic groups	68	367.92	5.41	84.62
	Total	71	436.49	6.39	100.00
Heterotic groups (Eucador, Kitale and Pool9A)	Between heterotic groups	2	29.48	0.52	8.56
	Within heterotic groups	49	285.15	5.59	91.44
	Total	51	314.63	6.11	100.00

4.5 Discussion

In the present study, a total of 98 alleles, with a mean of 3.9 alleles per marker, were detected across the 36 inbred lines. The number of alleles found in this study is in agreement with other studies (Beyene et al., 2006; Dhliwayo et al., 2009; Legesse et al., 2007; Makumbi et al., 2011). Beyene et al. (2006) genotyped 62 traditional Ethiopian highland maize accessions with 20 SSRs and reported a total of 98 alleles and a mean of 4.9 alleles per marker. Legesse et al. (2007) reported an average of 3.9 alleles per marker by genotyping 56 highland and mid-altitude non-QPM inbred lines using 27 SSRs. Krishna et al. (2012) reported a mean of 4.1 alleles using 48 SSR loci and 63 QPM inbred lines. The mean number of allele in the present study is, however, lower than the 6.4 and 5.4 alleles previously reported by Yao et al. (2008) and Wu et al. (2004), respectively, and higher than the 3.3 alleles reported by Bantte and Prasanna (2003), and the 2.4 to 3.4 alleles reported by Babu et al. (2009, 2012). The differences in mean numbers of alleles among different studies could be attributed to the type of germplasm, sample size and repeat length of the SSRs used (Rajab et al., 2006)**.**

Polymorphic information content provides an estimate of how informative is a particular marker by considering both the number of alleles that are expressed, and the relative frequencies of those alleles (Smith et al., 1997). In the present study, PIC

values ranged from 0.303 (less discriminative marker - umc1367) to 0.735 (highly discriminative marker - phi299852) with a mean of 0.491. According to Botstein et al. (1980) PIC guideline, 14 markers were reasonably informative (0.30 < PIC < 0.50) and the remaining 11 markers were highly informative (PIC > 0.50; Table 4.1). These values are in a general agreement with previous reports by Dhliwayo et al. (2009) and Mahar et al. (2009) but lower than that of Krishna et al. (2012). The relatively smaller PIC values in the present study may be due to the presence of only a single di-nucleotide repeat SSR as opposed to more di-nucleotides used in other studies (Babu et al., 2009; Babu et al., 2012; Bantte and Prasanna, 2003; Smith et al., 1997) or lower genetic variability among the germplasm used for the study.

The present study found 4.0 to 16.7% heterozygosity with the majority of the inbred lines showing higher than the expected values after 5 generations of selfing. In another study, a total of 88 maize inbred lines widely used by breeders at the Ethiopian Institute of Agricultural Research have been genotyped using a subset of 191 SNPs identified for a routine quality control analysis (Semagn et al., 2012a). Nearly 78% of the inbred lines showed very high proportion of heterogeneity (B. Tadesse, unpublished). High level of heterogeneity similar to the present findings have been observed across several CIMMYT derived maize inbred lines primarily attributed to human errors (e.g. seed admixture, pollen contamination, mislabelling of seed sources and mixing of different seed stocks for planting) (K. Semagn, unpublished). Small changes in allele frequencies may occur during seed regeneration, bulking during maintenance breeding, and possible contamination with seeds or pollen of other samples (Heckenberger et al., 2002; Warburton et al., 2010). However, large proportions of heterogeneity can significantly change the uniformity and performance of hybrids, and in the worst case may result in the distribution of wrong hybrids. Consequently, additional generations of purification for all lines with higher proportion of heterogeneity are essential. The levels should thus be monitored frequently, as *opaque 2* is a recessive gene which is liable to contamination. For new pedigree starts, such problems could be minimized by implementing a routine quality control genotyping using a subset of informative markers at different stages in a breeding program (Semagn et al., 2012a).

The study also investigated the extent of genetic differentiation, population structure, and patterns of relationship among 36 maize inbred lines using the model-based population structure analysis, NJ-cluster analysis, principal coordinate analysis, and discriminant analysis. All these different multivariate methods revealed the presence of 3 primary groups (Figures. 4.2, 4.4 and 4.5), which was in general agreement with pedigree information and partly with the putative heterotic groups. Maize breeders generally develop new parental inbred lines by selecting the progeny of intercrossed lines from within the same heterotic group. As heterotic group assignment is made based on combining ability from combining ability experiments, several authors suggested the use of molecular markers in heterotic grouping (Benchimol et al., 2000; Flint-Garcia et al., 2009; Lu et al., 2009; Melchinger et al., 1990; Reif et al., 2003a; Reif et al., 2003b).

In the present study, the model-based population structure analysis assigned about half of the inbred lines into their putative heterotic group defined by breeders. The failure of the SSR markers to assign the remaining 50% of the inbred lines into their heterotic groups may be attributed to lack of association between SCA estimates of phenotypic traits in identifying heterotic pools by breeders during the initial development of inbred lines. Therefore, future effort to identify heterotic pools could be based on genome wide marker information or through the use of SCA for certain economic traits. The present findings are in agreement with other reports that showed either partial or no clear heterotic patterns in subtropical and tropical CIMMYT maize inbred lines (Lu et al., 2009; Semagn et al., 2012b; Warburton et al., 2005; Wen et al., 2011; Xia et al., 2005). It is also clear from Figure 4.4 that the conversion to QPM was not done systematically hence the original heterotic system has been disrupted. The Kitale and Ecuador lines are spread throughout the three genetic clusters. The conversions had also been done using phenotypic selections without monitoring the genetic backgrounds. Consequently, recombinants were selected and very small portion of the genome of the recurrent parents was recovered. This suggests the use of marker assisted backcross or marker assisted selection (MAS) in the future. The marker assisted breeding and/or MAS will be used to facilitate background selection and avoid disruption of the newly established heterotic groups. Furthermore, the old CIMMYT maize breeding method used may have also contributed in the failure of molecular markers for heterotic grouping.

CIMMYT breeders initially used broad based pools and populations to develop open pollinated varieties (OPV). To exploit hybrid technologies, assignment of CIMMYT populations and inbred lines into heterotic groups via crossing to various representative testers has been intensified since the early 1990s. It is challenging to divide lines into heterotic groups when many lines were developed from the same original pool without regard to racial origin or heterotic pattern (Warburton et al., 2005). Therefore, many generations of reciprocal recurrent selection may be necessary before the lines from each heterotic group begin to be significantly diverged (Xia et al., 2005).

According to Wright's (Wright, 1978) guidelines for the interpretation of F_{ST}, the range 0-0.005, indicates little, 0.05-0.15 moderate, 0.15-0.25 great, and above 0.25 very great genetic differentiations. The F_{ST} value in the present study (0.086 to 0.154) indicated moderate to great genetic differentiation (Hamrick and Godt, 1997) among heterotic groups. This result supports the observed partial population structure based on heterotic grouping and proper assignment of nearly half of the inbred lines with *prior* information. The breeding system is one of the main factors that determine the genetic structure of plant populations. Several studies have shown that mixed-mating and out crossing species have less than 25% of their genetic variation among populations or groups and the remaining within populations or groups (Hamrick and Godt 1997; Huff et al. 1993; Nesbitt et al. 1995). The 8.6-15.4% genetic differences observed in this study is in good agreement with the above values and also other studies reported in maize (Semagn et al., 2012). The existence of greater variation within than between heterotic groups may also be attributed, in part, to the mix up of germplasm during the conversion process which, therefore, necessitates the establishment of new heterotic groups.

4.6 Conclusions

The inbred lines used in the present study were expected to be genetically pure with <6.25% heterogeneity but 20 of the 36 lines showed between 8.0 to 16.7% heterogeneity, which requires additional generations of purification. The overall mean genetic distance among the inbred lines was 0.52, with about 98% of the pairwise comparisons ranging from 0.300 to 0.780, which indicates large genetic

differences among most lines. The model-based population structure analysis, principal coordinate analysis and neighbor-joining cluster analysis revealed the presence of 3 groups, which is generally consistent with pedigree information and partly with the putative heterotic groups. AMOVA indicated that difference among heterotic groups explained 8.6 to 15.4% of the total SSR variance, which indicates the presence of moderate to great genetic differentiating among heterotic groups. The results from this study may enhance the development of hybrids of quality protein maize for the highland agro-ecological zones.

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CHAPTER 5

Combining ability and heterosis analyses of quality protein maize hybrids adapted to tropical-highlands

5.1 Abstract

Breeding for quality protein maize (QPM) is economical and sustainable approach to alleviate protein deficiency in developing countries where maize is a major staple crop. However, QPM cultivars with high grain yield and wide adaptation to the tropical-highland sub-humid maize agro-ecologies are yet to be developed for adoption by smallholder or commercial farmers. The objective of this study was, therefore, to determine the levels of heterosis and combining ability of elite QPM inbred lines and their hybrids for cultivar development. A 12-parent diallel cross was tested for grain yield and related agronomic traits under rain-fed conditions. The 66 experimental hybrids and two commercial hybrid checks were evaluated using an alpha lattice (0, 1) design at three sites representing highland sub-humid agro-ecology in Ethiopia. The ratio of dominance to additive genetic variance was relatively larger for grain yield compared to other traits, suggesting the genetic worthiness of QPM hybrid development. Inbred lines KIT32, FS60 and 142-1-EQ were found good general combiners for grain yield; while FS60 exhibited good general combining ability for days to anthesis, plant and ear height. Inbred line FS60 is genetically complementary to KIT32 and 142-1-EQ for QPM hybrid cultivar development. In general, the new QPM single-cross hybrids performed better than the checks. The best crosses identified were KIT32 x 142-1-EQ and SRSYN20 x FS60 that yielded 9.6 t ha^{-1} and 8.8 t ha^{-1}, respectively. These hybrids can be used as potential single cross testers for the development of three-way cross QPM hybrids for the highland ecologies.

***Key words*:**- Diallel**,** General combining ability, Hybrid vigour, QPM, Highland maize, Specific combining ability.

5.2 Introduction

Food insecurity is a major challenge facing sub-Saharan Africa countries (Badu-Apraku et al., 2013). In this region, the greatest contribution to food security is expected to come from highly productive and well-managed crops such as maize. It is estimated that maize supplies at least one fifth of total daily calories and accounts for 17 to 60% of the total daily protein supply of individuals in eastern and southern African countries (Krivanek et al., 2007). However, normal maize protein is deficient in two essential amino acids, lysine and tryptophan, limiting its biological nutritional value at 40% compared to that of milk (Bressani, 1991). Hence, normal maize diets need to be supplemented with other expensive protein sources such as legumes or animal products in order to meet daily protein requirements of poor households who depend on maize as a staple food.

Breeding for quality protein maize (QPM) is the most economical and sustainable approach to improve the nutritional value of normal maize for rural communities where high protein animal products are not readily available or affordable for consumption (Hoisington, 2002). Further, QPM cultivars could enhance food and nutritional security especially in the tropical highland farming-systems where farm lands are increasingly dwindling due to high population density. Of the total protein in whole grain maize flour, the lysine levels vary across genetic backgrounds from 1.6 to 2.6% in normal maize and 2.7 to 4.5% in their QPM counterparts (Moro et al., 1996).

Breeding and dissemination of QPM is steadily progressing in Africa (Krivanek et al., 2007). In the past years, some commercial cultivars were released in several African countries. The success rate of QPM variety adoption by smallholder farmers primarily depend on its competitive yield advantage compared to that of normal maize and the presence of an effective improved seed production and delivery mechanism. In Ethiopia, there are only few QPM hybrids so far released for production in the major maize growing agro-ecologies. In the high potential maize growing areas of the country, the released QPM varieties were less appealing to growers due to their comparatively low grain yield level and susceptibility to the major maize diseases such as *Puccinia sorghi* and *Exerohilum turcicum* when compared to the existing normal maize cultivars. In light of these, it is imperative to develop and deploy novel

QPM cultivars with high grain yield and stability and disease resistance adapted to the major maize growing agro-ecologies.

The Ethiopian Highland Maize Research Project based at Ambo develops QPM germplasm in collaboration with the International Maize and Wheat Improvement Center (CIMMYT), national agricultural research systems (NARS) of east and central African (ECA) countries, and the Association for Strengthening Agricultural Research in Eastern and Central Africa (ASARECA). The project, through its conversion program of normal maize genotypes to QPM, developed a pool of stable QPM inbred lines with general adaptation to highland agro-ecologies of the ECA region (Twumasi-Afriyie et al., 2012). The source germplasm used in the conversion program were highland maize inbred lines developed in the CIMMYT-NARS Highland Maize Breeding Project for eastern and central African countries (Twumasi-Afriyie et al., 2004). The inbred lines are locally known in the development of existing farmers-preferred normal hybrid maize varieties. The approach of using locally known inbred lines may facilitate the uptake of the new QPM varieties by smallholder or commercial famers similar to that of the normal maize hybrids (Krivanek et al., 2007). The converted inbred lines were also systematically characterized using agro-morphological and microsatellite markers that indicated their stability and genetic dissimilarity useful in the development of hybrids or synthetic varieties (unpublished data).

The *per se* performance of maize inbred lines does not predict the performance of hybrids for grain yield and yield related traits (Hallauer et al., 2010). Therefore, predictors of single cross hybrid value or heterosis between parental inbred lines could increase the efficiency of hybrid breeding programs (Betran et al., 2003). To accelerate the development of both hybrids and synthetic varieties, it is essential to determine the genetic potential of available QPM inbred lines in hybrid combinations through combining ability and heterosis studies. The objective of this study was, therefore, to determine the levels of heterosis and combining ability of elite QPM inbred lines and their hybrids for cultivar development.

5.3 Materials and Methods

5.3.1 Germplasm

The QPM inbred lines used for the study were selected from a large pool of QPM converted inbred lines developed in the CIMMYT-NARS Highland Maize Breeding Project for the ECA. The lines were selected through rigorous phenotypic evaluations of testcrosses and light table selections for endosperm modifications, and subsequent biochemical analyses of Tryptophan levels. In 2011, grain samples of 30 lines including the inbred lines selected for this study with endosperm modification scores of 2 (75% modified) and 3 (50% modified) were sent to CIMMYT-Mexico Quality and Plant Tissue Analysis Laboratory for Tryptophan analysis following the procedure outlined by Nurit et al. (2009). Based on Vivek et al. (2008), the result of analysis showed that the converted QPM lines had acceptable protein and tryptophan levels with protein levels >8.0 g 100 g^{-1} protein and tryptophan >0.06 g 100 g^{-1} protein (Table 5.1). Further, a series of testcrosses using the converted lines and two CIMMYT QPM inbred lines (CML144 and CML159 used as testers) were conducted for performance evaluation in the highland environments. The 12 inbred lines selected for this study were crossed in a diallel fashion in 2011.

5.3.2 Study sites, experimental design and field management

The 66 crosses together with one QPM and one normal maize three-way cross commercial hybrid checks were evaluated under rain-fed conditions during the main rainy season (June-August) of 2012 at three locations in Ethiopia. The study sites were: Ambo Plant Protection Research Center (8^{o}57' N, 38^{o}07' E, 2225 m above sea level [masl]), Kulumsa Agricultural Research Center (8^{o}13' N, 39^{o}13' E, 2180 masl), and Holetta Agricultural Research Center (9^{o}00' N, 34^{o}48' E, 2400 masl). The locations represent the highland sub-humid maize growing agro-ecology in Ethiopia. Entries were established using a 4×17 alpha-lattice design (Patterson and Williams, 1976) with two replications at each location. Concurrent trails involving the 12 parents were conducted at each location. The experimental unit for both trials was two rows of 5.25 m spaced at 75 cm. Two seeds were planted per hole spaced at 25 cm and later thinned to one plant giving a final population density of 53,333 plants ha^{-1}. The trials

received the recommended rate of 69 kg ha^{-1} of phosphate (P_2O_5) in the form of di-ammonium phosphate fertilizer (DAP) and one-third of 119 kg nitrogen (N) ha^{-1} in the form of Urea as basal dressing during planting at all locations. Second and third doses of N (each one-third of 119 kg) were side-dressed 37 days after the crop emergence and before tasseling, respectively. Pre-emergence herbicide, Primagram® Gold 660 SC (5 L ha^{-1}) was applied at planting followed by hand weeding to control weeds.

Table 5. 1 Pedigree and protein quality profiles of 12 QPM inbred parents used for the diallel crosses

No.	Name	Pedigree	*Putative heterotic groups	% Prot‡	% Trp‡	% QI§
P1	KIT32	[KIT/SNSYN[N3/TUX]]c1F1-##(GLS=2.5)-32-1-1-#/CML176BC1F1-12-1-3-1-1-#-#	Ecuador	9.71	0.08	0.82
P2	SRSYN20	SRSYN95[ECU//SC/ETO]F1-##(GLS=3.5)-20-2-1-#/CML176(BC2)-4-2-2-3-2-#-#	Pool 9A	12.06	0.07	0.58
P3	SRSYN48	SRSYN95[ECU//SC/ETO]F1-##(GLS=3.5)-48-1-1-#/CML176(BC2)-11-2-1-1-1-#-#	Ecuador	10.12	0.08	0.79
P4	FS67	[POOL9Ac7-SR(BC2)]FS67-1-2-3-1-#/CML144(BC2)-10-11-2-4-1-2-#	Kitale	10.73	0.07	0.65
P5	FS68	[POOL9Ac7-SR(BC2)]FS68-1-1-2-1-1/CML144(BC1)F1-1-1-2-1-1-#	Kitale	11.63	0.07	0.60
P6	FS59-4	[POOL9Ac7-SR(BC2)]FS59-4-1-2-1-1-#/CML176BC1F1-3-2-3-#	Ecuador	10.78	0.07	0.65
P7	FS170	[POOL9Ac7-SR(BC2)]FS170-2-1-3-1-#/CML176(BC2)-5-2-1-3-1-#	-	12.81	0.08	0.62
P8	FS60	[POOL9Ac7-SR(BC2)]FS60-2-1-1-1-#/CML176BC1F1-5-3-1-2-1-#	Pool 9A	11.70	0.07	0.60
P9	FS232	[POOL9Ac7-SR(BC2)]FS232-4-1-3-1-#/CML176(BC2)-17-1-1-1-#	Pool 9A	10.17	0.07	0.69
P10	F7215Q	Not available	Kitale	10.83	0.06	0.55
P11	142-1-eQ	Not available	Ecuador	9.44	0.09	0.95
P12	$CML144	Pob62c5HC182-2-1-2-B-B-3-1-#-#	A	10.82	0.08	0.74

*= Putative heterotic grouping based on line x tester analysis of the non-QPM counterparts before conversion into QPM; $= CIMMYT QPM line; ‡= Protein and Tryptophan contents (%) in whole grain; §QI=Protein Quality Index (the ratio of % Trp to % Prot concentration in grain expressed as a %)

5.3.3 Data collection

Data were recorded on plot basis on days from planting to 50% anthesis (AD) and silking (SD), anthesis–silking interval (ASI) = SD – AD, plant height (PH) measured

from the base of a plant to the insertion of the first tassel branch of the same plant, ear height (EH) measured from the base of a plant to the insertion of the top ear of the same plant, and ears per plant (EPP) (number of ears with at least one fully developed grain divided by the number of harvested plants). Ten random ears were selected from all harvested ears of each plot to measure ear diameter (ED) and length (EL), number of rows per ear (RPE) and kernels per row (KPR). EL was measured as the length of the ear from the base to tip, ED measured at the mid-way along ear length, RPE (the average number of kernel rows per ear), KPR (the average number of kernels per row). Unshelled grain weight was used to estimate grain yield (GY) (adjusted to 80% shelling percentage and 12.5% grain moisture content and expressed in t ha^{-1}), while thousand kernels were randomly sampled from the bulk of shelled cobs and weighed and expressed in grams after the moisture was adjusted to 12.5% to determine thousand kernel weight (TKW). A random 100-kernel sample was taken from the bulk grains of each plot and endosperm modification (MOD) scores were assessed under light box using a 1– 5 scale; where 1 = completely modified (normal phenotype); 2 = 75% modified; 3 = 50% modified; 4 = 25% modified; and 5 = completely opaque (Vivek et al., 2008). The endosperm modification score for a plot was the weighted mean score for the 100-kernel sample.

5.3.4 Data analyses

Analyses of variance (ANOVA) per individual location and across locations were done using restricted maximum likelihood (REML) (Patterson and Thompson, 1971) analysis in GenStat® Release 14 statistical software (Payne et al., 2007). Genotypes were considered as fixed effects, and replications and incomplete blocks within replications as random effects. When significant differences were detected among entries at each location, combined ANOVA was conducted with genotypes and locations being considered as fixed factors, and replicates within locations and incomplete blocks within replicates as random factors. Entry means adjusted for block effects and generated from significant individual location analysis of traits were used to perform individual and across locations combining ability analyses using the DIALLEL-SAS05 program (Zhang et al., 2005). General combining ability (GCA) effects of the parents and specific combining ability (SCA) effects of the crosses were

estimated following Griffing's Method IV of diallel crosses and experiments conducted at several environments (Ferreira et al., 1993). Analyses were performed based on the following model:

$$Y_{ii'k} = m + l_k + g_i + g_{i'} + s_{ii'} + (lg)_{ik} + (lg)_{i'k} + (ls)_{ii'k} + e_{ii'k}$$

Where $Y_{ii'k}$ is the mean over replications and blocks within replications of the single cross (i x i') in the k^{th} environment; m is the overall mean; l_k is the k^{th} environment effect; g_i, $g_{i'}$, and $s_{ii'}$ are general and specific combining ability effects (Griffing, 1956); $(lg)_{ik}$ and $(lg)_{i'k}$ are GCA effects of i and i' parents and their interaction with environment respectively; $(ls)_{ii'k}$ is SCA interaction with environment; $e_{ii'k}$ is the error term. The hybrid checks were excluded from the diallel analysis. Simple linear phenotypic correlations between grain yield and agronomic traits were computed using the means from both individual and combined analyses. Mid-parent heterosis (MPH) was calculated using means from combined analysis as MPH = $\frac{(F1-MPV)}{MPV} x\ 100$; where *F1* is the mean performance of the cross and *MPV* is mean of the two inbred parents.

In the combined analysis, the mean squares for hybrids and environments were tested against the mean squares for hybrid x environment as error term while hybrid x environment interactions mean squares were tested against pooled error. Similarly, the significance of GCA and SCA sources of variation was determined using the corresponding interactions with the environment as error terms. Error mean squares used to test the significance of GCA and SCA interactions with environment were obtained by dividing the pooled error mean squares from the ANOVA by the number of replications, because the combining ability mean squares were calculated based on entry means (Griffing, 1956; Dabholkar, 1992; Ferreira et al., 1993). The significance levels of GCA and SCA effects were determined by the t-test, using standard errors of GCA and SCA effects, respectively. For GCA effects of the inbred lines and SCA effects of the crosses, the restriction $\sum g_i = 0$ and $\sum s_{ij} = 0$ were imposed, respectively (Griffing, 1956). GCA (σ^2_{gca}) and SCA (σ^2_{sca}) equivalent variance components with the standard errors were estimated from the corresponding combining ability effects of the three locations using REML. The σ^2_{gca} and σ^2_{sca} were thus used to estimate the additive (σ^2_A) and dominance (σ^2_D) variances as $\sigma^2_A = 2\sigma^2_{gca}$ and $\sigma^2_D = \sigma^2_{sca}$ assuming inbreeding coefficient of zero ($F = 0$) (Hallauer et al., 2010). The additive and dominance variances were further used to compute genotypic and phenotypic

variances which, in turn, were used to estimate heritability in narrow sense (h^2) as $h^2 = \sigma^2_A / \sigma^2_P$, where σ^2_P represents phenotypic variance.

5.4 Results

5.4.1 Analyses of variance and performance of hybrids

The single cross hybrids exhibited highly significant differences ($P < 0.01$) in all measured traits except anthesis-silking interval at the three locations (Table 5.2). Mean squares due to hybrids and hybrid by location interaction showed significant differences ($P < 0.05$) for all traits during combined analysis. Whereas non-significant differences were detected for ear length, number of rows per ear and endosperm modification (Table 5.2).

Across locations analyses showed mean grain yield record of hybrids including the two commercial checks at 7.0 t ha^{-1} with maximum and minimum yields of 9.6 t ha^{-1} and 4.8 t ha^{-1}, respectively. A maximum GY of 11.3 t ha^{-1} was recorded at Kulumsa while a minimum (2.5 t ha^{-1}) was noted at Holetta (Table 5.2). About 11% of the 66 hybrids at Ambo, 6% at Holetta and 3% at Kulumsa had higher GY than the non-QPM commercial hybrid check. Also, in the combined analysis, 6% of the 66 hybrids had better GY than the non-QPM hybrid check (Table 5.2). Whereas about 38% of the 66 hybrids had higher grain yield than the QPM hybrid check in the combined analyses (data not presented). Days to anthesis of the hybrids varied between 97.1 and 108.3 with a mean of 101.5 at Ambo, 112.5 to 131.9 with a mean of 120.0 at Holetta, and between 96.4 and 115.3 with a mean of 106.7 days at Kulumsa. Across locations analyses revealed that the hybrids including checks took a maximum, minimum and mean of 117.3, 103.2 and 109.4 days to anthesis, respectively (Table 5.2). A higher plant high at 244.8 cm surpassing both checks was recorded for the experimental hybrids in the combined analyses. However, there was no significant difference in hybrid x location interaction for EL, RPE and MOD. Of the three locations, the hybrids performed better for number of ears per plant at Kulumsa than the other two locations. Among the traits considered, only grain yield had mean mid-parent heterosis above 100% (Table 5.2).

Table 5. 2 Selected statistics for grain yield and related agronomic traits of QPM hybrids evaluated at three study sites in Ethiopia (2012)

Locations	Statistics	GY	AD	ASI	PH	EH	EPP	EL	ED	RPE	KPR	TKW	MOD
Ambo	Grand mean	7.3	101.5	1.2	238.5	117.2	1.2	18.0	4.5	13.1	38.6	333.8	2.2
	Minimum	4.7	97.1	-2.4	200.2	90.5	0.9	15.2	4.0	10.7	32.0	254.7	1.2
	Maximum	10.1	108.3	4.0	271.9	148.0	1.6	20.7	5.0	15.5	46.7	436.7	4.0
	Mean of QPM check	7.1	103.8	1.4	250.6	152.0	1.2	18.4	4.7	13.7	45.0	350.8	2.5
	Mean of normal check	8.8	96.1	1.1	219.2	116.5	1.2	21.6	4.7	14.7	41.9	388.5	1.0
	F test	**	**	**	**	**	**	**	**	**	**	**	**
	SE(m)	0.2	0.2	0.1	1.2	0.5	0.0	0.1	0.0	0.1	0.2	0.9	0.1
	CV (%)	9.8	1.3	89.1	2.4	5.3	12.8	4.8	2.9	5.2	5.1	3.6	19.9
	% high yielding hybrids‡	10.6											
Holetta	Grand mean	5.8	120.0	2.9	219.1	130.5	1.0	16.3	4.5	12.8	32.2	357.2	1.7
	Minimum	2.5	112.5	-3.0	173.6	93.7	0.5	12.5	3.8	10.4	25.9	262.3	1.0
	Maximum	8.8	131.9	6.5	248.0	172.3	1.3	19.5	4.9	15.1	39.2	449.6	3.2
	Mean of QPM check	6.1	121.8	2.5	223.4	157.3	0.9	16.9	4.8	13.7	35.0	397.6	2.3
	Mean of normal check	7.5	111.3	3.0	223.5	119.1	1.0	19.1	4.5	13.4	38.2	374.5	1.0
	F test	**	**	**	**	**	**	**	**	**	**	**	**
	SE(m)	0.3	0.6	0.2	2.1	0.5	0.0	0.1	0.1	0.1	0.4	3.0	0.1
	CV (%)	11.6	1.3	48.3	2.5	4.3	11.6	5.6	2.8	4.7	5.5	3.4	22.9
	% high yielding hybrids	6.1											
Kulumsa	Grand mean	7.7	106.7	4.3	194.0	78.2	1.4	17.6	4.5	12.8	37.2	348.4	1.9
	Minimum	5.6	96.4	2.0	147.0	53.9	1.0	14.4	4.1	10.7	30.3	238.5	1.0
	Maximum	11.3	115.3	6.6	233.5	109.4	1.8	20.9	4.9	15.0	45.7	439.2	3.8
	Mean of QPM check	8.5	115.2	2.5	204.1	101.6	1.4	18.8	4.7	12.8	38.8	410.9	2.9
	Mean of normal check	9.5	100.8	4.4	184.5	68.4	1.4	21.3	4.6	13.2	40.2	413.4	1.0
	F test	**	**	**	**	**	**	**	**	**	**	**	**
	SE(m)	0.1	1.4	0.1	1.5	1.0	0.02	0.1	0.0	0.1	0.2	1.7	0.03
	CV (%)	9.5	1.6	22.2	3.0	7.5	12.6	5.0	3.7	6.0	4.8	2.5	18.9
	% high yielding hybrids	3.0											
Across Locations	Grand mean	7.0	109.4	2.8	217.2	108.9	1.2	17.4	4.5	12.9	36.1	347.7	1.9
	Minimum	4.8	103.2	-0.2	181.7	86.0	1.0	14.6	4.1	10.7	29.9	259.0	1.2
	Maximum	9.6	117.3	4.8	244.8	135.7	1.5	20.1	4.9	14.9	43.3	416.3	3.6
	Mean of QPM check	7.2	113.6	2.1	225.6	137.2	1.2	18.1	4.7	13.3	39.7	386.7	2.6
	Mean of normal check	8.6	102.7	2.8	208.8	101.6	1.2	20.7	4.6	13.7	40.1	392.0	1.0
	F test (Hybrids)	**	**	**	**	**	**	**	**	**	**	**	**
	F test (Hybrids x location)	**	**	**	**	**	**	ns	*	ns	*	**	ns
	SE(m)	0.11	0.5	0.1	0.8	0.4	0.01	0.1	0.0	0.03	0.2	1.2	0.1
	CV (%)	10.4	1.35	41.1	2.67	5.50	12.6	5.17	3.1	5.37	5.21	3.19	20.53
	% high yielding hybrids	6.1											
	Maximum MPH (%)	171.2	4.6	268.9	64.1	103	29.2	68.8	30	9.4	75.6	86.7	-
	Minimum MPH (%)	64.5	-6.6	-515	28.3	27.7	-20.5	17.8	9.6	-4.7	23.5	17.9	-
	Mean MPH (%)	121.3	-0.9	14.3	47.1	53.9	6.5	38.2	19	2.9	45.0	46.6	-

* *P*< 0.05; ** *P*< 0.01; ns= non-significant; GY= grain yield (t ha^{-1}); AD= days to anthesis; ASI= anthesis-silking interval (d); PH= plant height (cm); EH= ear height (cm); EPP= ears per plant; EL= ear length (cm); ED= ear diameter (cm); RPE= number of rows per ear; KPR= number of kernels per row; TKW= thousand kernel weight (g); MOD= endosperm modification; SE(m)=standard error of the mean; CV= coefficient of variation; ‡= proportion of QPM hybrids with higher grain yield than the best check (normal maize or QPM); MPH= Mid-parent heterosis.

5.4.2 Correlations of grain yield with other agronomic traits

Phenotypic correlation coefficients between grain yield and agronomic traits at each and across locations are presented in Table 5.3. Six out of the eleven traits exhibited significant correlations (P< 0.05) with grain yield in at least one of the three locations. Two traits (anthesis date and thousand kernel weight) showed non-significant correlation with grain yield during combined analyses. Grain yield had positive and highly significant correlations with ear diameter, number of ears per plant and number of kernels per row at each and across locations. Ear length also showed highly significant positive correlation with grain yield across locations. At Holetta, highly significant negative and positive correlations of grain yield with days to anthesis and thousand kernel weight were observed, respectively.

Table 5. 3 Phenotypic correlation coefficients between grain yield and related traits at three locations and across locations

Traits[†]	Locations			
	Ambo	Holetta	Kulumsa	Across
Days to anthesis (d)	0.28^{ns}	-0.35**	0.21^{ns}	-0.09^{ns}
Ear diameter (cm)	0.39**	0.52**	0.47**	0.54**
Ear length (cm)	0.19^{ns}	0.56**	0.12^{ns}	0.33**
No. of ears/plant	0.57**	0.62**	0.56**	0.45**
No. of kernels/row	0.30*	0.49**	0.39**	0.48**
Thousand kernel wt.	0.10^{ns}	0.42**	0.07^{ns}	0.20^{ns}

[†] Only traits showing significant correlation with yield in at least one site are listed; significant correlation at * *P*< 0.05; ** *P*< 0.01; ns=non-significant

5.4.3 Combining ability analyses

The mean squares from combining ability analyses of twelve traits for each location are given in Table 5.4. Highly significant differences (*P* < 0.01) due to hybrids were observed for all traits considered in this study at each location. Partitioning of hybrid mean squares into general combining ability (GCA) and specific combining ability (SCA) mean squares showed that the variations due to GCA mean squares were highly significant (*P* < 0.01) for all traits at each location. Similarly, the variations due

to SCA mean squares were significant for the majority of the traits except for ASI, EPP, ED and RPE at Ambo; ED and RPE at Holetta; ASI, ED and RPE at Kulumsa. Combined analysis of variance across the three locations revealed that location had significant effect ($P < 0.01$) on hybrid performance for all traits except ED (Table 5.5). Besides, mean squares of hybrids for all traits but EPP exhibited highly significant differences ($P < 0.01$). GCA mean squares were significantly different ($P < 0.01$) for grain yield and all agronomic traits except EPP. Mean squares due to SCA were significantly different for all traits excluding ASI and EPP. The GCA sum of squares of 66% was larger than SCA sum of squares (34%) for GY. Hybrids x location interaction mean squares were also significant for all traits except for RPE and KPR. GCA x location mean squares were significant for all traits excluding RPE but the magnitudes were consistently smaller than the respective GCA mean squares. Also, SCA x location mean squares were significant for all traits except EPP, EL, ED, RPE, KPR and MOD. Similarly, the magnitudes of SCA x E mean squares were smaller than that of SCA for all traits.

Table 5. 4 Mean squares due to hybrids, general (GCA) and specific (SCA) combining ability of grain yield and related agronomic traits evaluated at three locations

	Ambo			Holetta			Kulumsa		
Traits	Hybrids †df=65	GCA df=11	SCA df=54	Hybrids Df=65	GCA df=11	SCA df=54	Hybrids df=65	GCA df=11	SCA df=54
GY	1.4**	5.5**	0.6**	1.5**	4.8**	0.8**	1.4**	5.7**	0.6**
AD	5.5**	23.4**	1.9**	11.0**	41.4**	4.8**	20.8**	95.7**	5.6**
ASI	1.4**	5.7**	0.5	3.3**	9.3**	2.07**	0.9**	2.3**	0.6
PH	279.5**	1275.8**	76.6**	237.8**	941.6**	94.5**	350.9**	1765.7**	62.7**
EH	186.6**	885.5**	44.2**	212.7**	791.7**	94.7**	156.9**	675.8**	51.2**
EPP	0.03**	0.1**	0.01	0.02**	0.04**	0.01**	0.04**	0.2**	0.02*
EL	1.8**	7.1**	0.7*	2.3**	9.6**	0.8*	2.1**	7.6**	1.0**
ED	0.1**	0.3**	0.01	0.1**	0.3**	0.02	0.04**	0.1**	0.02
RPE	1.1**	4.7**	0.3	0.9**	4.0**	0.3	0.8**	3.6**	0.2
KPR	9.7**	41.7**	3.1*	10.7**	48.2**	3.1**	10.3**	39.1**	4.4**
TKW	1100.0**	3152.9**	681.8**	1732.2**	8375.2**	378.9**	1493.9**	4798.8**	820.8**
MOD	0.3**	1.1**	0.2*	0.2**	0.6**	0.1	0.3**	1.0**	0.2**

* $P < 0.05$; ** $P < 0.01$; †df= degrees of freedom; GY= grain yield (t ha^{-1}); AD= days to anthesis; ASI= anthesis-silking interval (d); PH= plant height (cm); EH= ear height (cm); EPP= ears per plant; EL= ear length (cm); ED= ear diameter (cm); RPE= number of rows per ear; KPR= number of kernels per row; TKW= thousand kernel weight (g); MOD= endosperm modification.

Table 5. 5 Mean square values of grain yield and related agronomic traits of QPM hybrids evaluated at Ambo, Holetta and Kulumsa

Source of variation	df	GY	AD	ASI	PH	EH	EPP	EL	ED	RPE	KPR	TKW	MOD
Location (L)	2	68.0**	5973.2**	159.3**	32766.9**	48747.6**	2.9**	48.6**	0.02	2.1**	747.9**	9267.7**	3.6**
Hybrids	65	2.6**	26.2**	3.6**	670.4**	430.4**	0.04	5.1**	0.1**	2.3**	26.2**	3117.3**	0.6**
GCA	11	10.0**	129.9**	14.6**	3322.0**	2039.3**	0.1	21.6**	0.6**	11.7**	120.3**	13305.1**	2.5**
SCA	54	1.1**	5.3**	1.3	130.3**	102.7**	0.02	1.7**	0.02**	0.4*	7.1**	1042.1**	0.3**
Hybrids x L	130	0.9**	5.6**	1.0**	98.9**	62.9**	0.03**	0.5*	0.014*	0.2	2.2	604.4**	0.09
GCA x L	22	3.0**	15.3**	1.4**	330.6**	156.8**	0.1**	1.4**	0.03**	0.3	4.3**	1510.9**	0.2**
SCA x L	108	0.5**	3.1**	0.9*	51.8**	43.7**	0.01	0.4	0.01	0.2	1.8	419.7**	0.1
Pooled error	177	0.5	2.2	1.4	32.2	35.1	0.02	0.8	0.02	0.5	3.4	124.2	0.16

* *P*< 0.05; ** *P*< 0.01; GY= grain yield (t ha^{-1}); AD= days to anthesis; ASI= anthesis-silking interval (d); PH= plant height (cm);EH= ear height (cm); EPP= ears per plant; EL= ear length (cm); ED= ear diameter (cm); RPE= number of rows per ear; KPR= number of kernels per row; TKW= thousand kernel weight (g); MOD= endosperm modification

5.4.4 General combining ability effects

Significant variation was observed among the twelve QPM inbred lines for GCA effects of all traits evaluated across locations (Tables 5.6, 5.7, 5.8 and 5.9). Estimates of the GCA effects of lines for grain yield and related traits at Ambo are presented in Table 5.6. At Ambo, the GCA effects for GY varied from –1.09 to 1.28. Inbred lines KIT32, FS67, FS60, and 142-1-EQ showed highly significant positive GCA effects for grain yield. Conversely, SRSYN48, FS68, FS59-4, FS232, and CML144 had highly significant negative GCA effects for grain yield. Significantly high negative GCA effects were observed for inbred lines SRSYN48, FS67, FS60 and F7215Q for AD while significantly high positive GCA effects were observed for SRSYN20, FS170, 142-1-EQ and CML144. The GCA effects for ASI ranged between -1.8 to 1.07. Inbred lines KIT32, SRSYN48, FS60 and FS232 had highly significant negative GCA effects for plant and ear heights except inbred lines SRSYN20, FS67, FS59-4, F7215Q, and 142-1-EQ which had highly significant positive GCA effects for the same traits. GCA effects for EPP varied from -0.19 to 0.15. The majority of the inbred lines that had positive GCA effects for GY also had positive GCA effects for EPP except inbred line 142-1-EQ. GCA effects varied between -1.11 to 1.11 for RPE as well as between -2.21 to 5.22 for KPR among the inbred lines. All inbred lines except SRSYN20, FS68, FS170, 142-1-EQ and CML144 had significantly positive GCA effects for TKW. Six inbred lines had negative GCA effects while the other six (including KIT32 and 142-1EQ with high positive GCA effects for GY at Ambo) had positive GCA effects for MOD, showing high endosperm modification scores and hence undesirable in respect of this trait.

The estimates of GCA effects of the QPM inbred lines evaluated for various agronomic traits at Holetta are presented in Table 5.7. Inbreds FS59-4, FS60, F7215Q and 142-1-EQ had highly significant positive GCA effects for GY while FS67, FS68, FS232 and CML144 had highly significant negative GCA effects. KIT32, SRSYN48, FS232, FS7215Q and 142-1-EQ had highly significant negative GCA effects for AD. Also FS67, FS59-4 and FS60 had significant negative GCA effects for the same trait. In contrast, SRSYN20, FS170 and CML144 had highly significant positive GCA effects for this trait. Highly significant negative GCA effects for ASI were observed for FS67 and CML144 while highly significant positive GCA effects were

observed for FS68 and 142-1-EQ. Inbred lines KIT32, SRSYN48, FS60 and FS232 showed significant negative GCA effects for PH and EH while SRSYN20, FS67, FS59-4 and 142-1-EQ had significant positive GCA effects for these traits. FS68 and CML144 showed contrasting GCA effects for PH and EH. GCA effects for EPP were positive and highly significant for FS67, FS68 and FS60. GCA effects for EL varied from highly significant negative value (-1.54) for inbred line FS67 to highly significant positive value (1.69) for inbred line 142-1-EQ. In case of ED, inbred lines KIT32, FS59-4, FS170 and FS60 had positive and highly significant GCA effects, whereas SRSYN48, FS67 and FS68 had negative and highly significant GCA effects.GCA effects ranged between -1.29 (FS232) to 1.04 (CML144) for RPE as well as between -2.80 (CML144) to 5.89 (142-1-EQ) for KPR. For TKW, 6 inbred lines had negative and highly significant GCA effects while the rest had positive and highly significant GCA effects. Six inbred lines had highly significant GCA effects of which SRSYN20, FS232 and CML144 exhibited negative effects and FS59-4, F7215Q and 142-1-EQ had positive effects of MOD.

The estimates of GCA effects of the QPM inbred lines evaluated for different agronomic traits at Kulumsa are presented in Table 5.8. KIT32, SRSYN20, FS170 and 142-1EQ showed highly significant positive GCA effects for GY while SRSYN48, FS68, FS59-4 and F7215Q had significantly lower GCA effects. Inbred lines FS67, FS59-4, FS60, FSFS232 and FS7215 showed highly significant negative GCA effects for AD while KIT32, SRSYN20 and CML144 showed highly significant positive GCA effects. Inbred lines with highly significant negative GCA effects for ASI were FS67 and CML144, and lines with significantly positive GCA effects were SRSYN48, FS68, FS59-4 and 142-1-EQ. Inbred lines KIT32, SRSYN48, FS170, FS60 and CML144 showed highly significant negative GCA effects for PH and EH though some of the effects were not significant. Among the twelve inbred lines, only four (KIT32, FS170, FS232 and CML144) had highly significant and positive GCA effects for EPP. Although most inbreds showed contrasting GCA effects for EL and ED, only 142-1-EQ showed positive effects for both traits. Like the EL and ED, most inbreds exhibited contrasting effects of GCA for RPE and KPR. The only six inbred lines with highly significant and positive GCA effects for TKW were SRSYN48, FS67, FS60, FS232, F7215Q and 142-1-EQ. The inbreds SRSYN20, SRSYN48, FS67, FS232 and CML144 showed highly significant negative GCA effects for MOD.

Table 5.9 and Figure 5.1 summarize the estimates of GCA effects of inbred lines for grain yield and agronomic traits combined across three locations. Inbred line 142-1-EQ that showed consistently highly significant and positive GCA effects for GY at each location had also highly significant and positive GCA effect across locations. In addition, KIT32 and FS60 which showed highly significant positive GCA effects at two locations had highly significant and positive GCA effects in the combined analysis for GY (Table 5.9 and Figure 5.1). Except FS60, the other two lines (142-1-EQ and KIT32) exhibited positive GCA effects for AD. Also, the three inbred lines had positive GCA effects for ASI although only significant for 142-1-EQ.

Overall, inbred lines showed similar trends of significant GCA effects for both PH and EH traits in the combined analysis. For example, inbred KIT32 had highly significant and negative GCA effects for both PH and EH, while 142-1-EQ had highly significant and positive GCA effects for the same traits. FS67 and FS68 exhibited negative GCA effects for both PH and EH, while FS60 and 142-1-EQ exhibited positive effects in both traits. Inbred lines KIT32, SRSYN20, FS170 and CML144 had negative GCA effects for EL and positive GCA effects for ED. However, the opposite held true for inbred lines SRSYN48, FS232 and F7215Q for same traits.

Highly significant and positive GCA effects for RPE were expressed by SRSYN20, FS59-4, FS60 and CML144 although all had negative GCA effects for KPR. Only three inbreds (SRSYN48, F7215Q and 142-1-EQ) had significant GCA effects for KPR. The maximum significant positive GCA effects for TKW were observed in FS60 followed by FS59-4, FS232, F7215Q and KIT32. On the contrary, the minimum and significant negative GCA effects for TKW were shown by CML144, FS68, 142-1-EQ and SRSYN20. Among the three inbreds (KIT32, 142-1-EQ and FS60) with highly significant and positive GCA effects for GY, the first two had highly significant positive GCA effects for MOD while FS60 had positive but non-significant effect.

Table 5. 6 Estimates of general combining ability (GCA) effects of 12 QPM inbred lines for grain yield (t ha^{-1}) and related traits at Ambo

Inbred line	GY	AD	ASI	PH	EH	EPP	EL	ED	RPE	KPR	TKW	MOD
KIT32	1.28**	0.05	-0.06	-5.78**	-7.48**	0.15**	-0.08	0.15**	-0.56**	-0.49	16.32**	0.50**
SRSYN20	-0.13	2.54**	-0.19	10.84**	12.42**	-0.10**	-0.74**	0.07**	0.40**	-1.60**	-3.48	-0.33**
SRSYN48	-1.09**	-0.85**	0.33	-9.13**	-7.33**	-0.19**	0.76**	-0.23**	-0.33**	1.23**	6.32**	-0.32**
FS67	0.50**	-1.20**	-1.80**	2.06*	4.27**	0.14**	-1.49**	-0.13**	-0.64**	-1.66**	4.32*	0.07
FS68	-0.79**	-0.26	1.07**	7.02**	-7.53**	0.09**	-0.37*	-0.24**	-0.73**	1.00**	-20.0**	0.06
FS59-4	-0.46**	-0.54*	0.13	8.69**	10.47**	-0.07*	0.18	-0.01	0.60**	-2.21**	23.83**	0.06
FS170	-0.15	0.84**	-0.33	-1.13	-5.48**	-0.05	0.07	0.20**	-0.01	0.15	-0.34	-0.18*
FS60	0.58**	-2.30**	0.50**	-26.23**	-11.38**	0.05	-0.23	0.25**	0.73**	-0.63	11.91**	-0.04
FS232	-0.44**	-0.27	0.20	-8.40**	-8.53**	-0.01	0.04	-0.15**	-1.11**	-0.04	8.01**	-0.19*
F7215Q	0.02	-1.53**	0.15	10.93**	9.42**	-0.001	0.97**	-0.05	0.52**	1.06**	4.68*	0.61**
142-1-EQ	1.15**	0.68**	0.83**	12.59**	14.02**	-0.01	1.65**	0.02	0.06	5.22**	-8.36**	0.23**
CML144	-0.51**	2.83**	-0.79**	-1.50	-2.83*	0.02	-0.76**	0.10**	1.11**	-2.03**	-43.19**	-0.51**
$SE_{(gi)}$	0.13	0.23	0.19	1.04	1.12	0.03	0.16	0.03	0.12	0.36	2.20	0.08

* *P*< 0.05; ** *P*< 0.01; GY= grain yield (t ha^{-1}); AD= days to anthesis; ASI= anthesis-silking interval (d); PH= plant height (cm); EH= ear height (cm); EPP= ears per plant; EL= ear length (cm); ED= ear diameter (cm); RPE= number of rows per ear; KPR= number of kernels per row; TKW= thousand kernel weight (g); MOD= endosperm modification; $SE_{(gi)}$= standard error of GCA.

Table 5. 7 Estimates of general combining ability (GCA) effects of 12 QPM inbred lines for grain yield (t ha^{-1}) and related traits at Holetta

Inbred line	GY	AD	ASI	PH	EH	EPP	EL	ED	RPE	KPR	TKW	MOD
KIT32	-0.07	-0.74**	0.23	-15.39**	-12.56**	0.02	-0.58**	0.10**	-0.57**	-0.32	9.02**	0.10
SRSYN20	-0.07	3.17**	0.03	8.14**	13.51**	-0.04*	-0.50**	0.01	0.35**	-1.81**	-7.14**	-0.30**
SRSYN48	-0.16	-1.79**	-0.08	-8.33**	-5.44**	-0.04*	0.92**	-0.17**	0.09	1.55**	-8.31**	-0.12
FS67	-0.59**	-0.71*	-2.08**	10.43**	8.11**	0.05**	-1.54**	-0.22**	-0.55**	-1.41**	-20.28**	-0.10
FS68	-0.40**	0.31	1.78**	9.88**	-2.43*	0.11**	-0.94**	-0.23**	-0.40**	-1.25**	-16.98**	0.01
FS59-4	0.34**	-0.63*	0.43	5.89**	7.78**	0.03	0.57**	0.07**	0.29**	-0.74*	40.95**	0.16*
FS170	-0.07	0.89**	0.38	0.77	-7.73**	-0.05**	-0.20	0.22**	0.05	0.03	7.13**	0.02
FS60	1.50**	-1.51*	0.33	-9.90**	-6.82**	0.10**	0.66**	0.28**	0.51**	0.61	31.07**	0.05
FS232	-0.79**	-1.59**	-0.43	-15.13**	-10.19**	-0.08**	0.32	-0.05	-1.29**	-0.12	27.19**	-0.24**
F7215Q	0.50**	-1.03**	-0.33	1.43	6.24**	0.04*	0.79**	0.03	0.60**	0.41	20.50**	0.51**
142-1-EQ	0.78**	-1.17**	0.88**	9.13**	10.86**	-0.04*	1.69**	0.01	-0.14	5.89**	-19.16**	0.28**
CML144	-0.99**	4.76**	-1.13**	3.04**	-1.35	-0.06*	-1.23**	-0.04	1.04**	-2.80**	-63.99**	-0.37**
$SE_{(gi)}$	0.12	0.28	0.26	0.99	1.02	0.02	0.17	0.03	0.11	0.32	2.19	0.07

* *P*< 0.05; ** *P*< 0.01; GY= grain yield (t ha^{-1}); AD= days to anthesis; ASI= anthesis-silking interval (d); PH= plant height (cm); EH= ear height (cm); EPP= ears per plant; EL= ear length (cm); ED= ear diameter (cm); RPE= number of rows per ear; KPR= number of kernels per row; TKW= thousand kernel weight (g); MOD= endosperm modification; $SE_{(gi)}$= standard error of GCA.

Table 5. 8 Estimates of general combining ability (GCA) effects of 12 QPM inbred lines for grain yield (t ha^{-1}) and related traits at Kulumsa

Inbred line	GY	AD	ASI	PH	EH	EPP	EL	ED	RPE	KPR	TKW	MOD
KIT32	0.39**	3.29**	-0.06	-7.12**	-6.27**	0.15**	-0.20	0.05	-0.59**	-0.12	1.80	0.61**
SRSYN20	0.35**	5.33**	-0.32	10.93**	3.17**	0.03	-0.83**	0.09**	0.64**	-1.36**	-1.57	-0.36**
SRSYN48	-1.27**	-0.26	0.39*	-3.57**	-1.53	-0.24**	0.87**	-0.17**	-0.36**	0.68*	8.10**	-0.29**
FS67	0.15	-2.34**	-0.70**	-2.22*	2.16*	-0.10**	-0.88**	-0.02	-0.30*	-0.17	14.42**	-0.20**
FS68	-0.90**	0.21	0.52**	8.53**	-0.80	-0.10**	0.27	-0.17**	-0.56**	0.83*	-13.27**	-0.05
FS59-4	-0.76**	-1.33**	0.41*	9.83**	5.97**	-0.08*	-0.40*	-0.07*	0.44**	-2.38**	-4.22**	0.08
FS170	0.38**	0.28	0.06	-6.07**	-9.95**	0.11**	-0.54**	0.12**	0.17	-0.46	-6.57**	-0.11
FS60	-0.01	-5.66**	0.02	-32.97**	-14.94**	-0.02	-0.37*	0.23**	0.32*	-1.11**	34.55**	0.26**
FS232	0.24	-1.16**	0.18	0.08	4.14**	0.09**	0.82**	-0.06*	-1.04**	-0.05	11.75**	-0.26**
F7215Q	-0.47**	-2.97**	-0.31	7.93**	9.67**	-0.07*	0.83**	-0.06*	0.26	0.52	5.45**	0.37**
142-1-EQ	1.58**	0.63*	0.70**	19.38**	13.76**	-0.04	1.61**	0.04	-0.07	5.42**	7.37**	0.32**
CML144	0.30*	4.00**	-0.85**	-4.77**	-5.34	0.26**	-1.22**	0.04	1.07**	-1.78**	-57.85**	-0.33**
$SE_{(gi)}$	0.13	0.30	0.17	1.05	1.07	0.03	0.16	0.03	0.14	0.33	1.60	0.06

* *P*< 0.05; ** *P*< 0.01; GY= grain yield (t ha^{-1}); AD= days to anthesis; ASI= anthesis-silking interval (d); PH= plant height (cm); EH= ear height (cm); EPP= ears per plant; EL= ear length (cm); ED= ear diameter (cm); RPE= number of rows per ear; KPR= number of kernels per row; TKW= thousand kernel weight (g); MOD= endosperm modification; $SE_{(gi)}$= standard error of GCA.

Table 5. 9 General combining ability effects (GCA) of 12 QPM inbred lines for grain yield (t ha^{-1}) and related agronomic traits combined across three locations (Ambo, Holetta and Kulumsa)

Inbred line	GY	AD	ASI	PH	EH	EL	ED	RPE	KPR	TKW	MOD
KIT32	0.54**	0.87**	0.03	-9.43**	-8.77**	-0.28	0.10**	-0.57**	-0.31	9.05**	0.40**
SRSYN20	0.05	3.68**	-0.16	9.97**	9.70**	-0.69**	0.05*	0.46**	-1.59**	-4.06*	-0.33**
SRSYN48	-0.84**	-0.96**	0.21	-7.01**	-4.77**	0.85**	-0.19**	-0.20	1.15**	2.04	-0.24**
FS67	0.02	-1.41**	-1.53**	3.43**	4.85**	-1.30**	-0.12**	-0.50**	-1.08**	-0.51	-0.08
FS68	-0.69**	0.09	1.12**	8.48**	-3.59**	-0.34*	-0.21**	-0.56**	0.19	-16.75**	0.01
FS59-4	-0.29*	-0.83**	0.32	8.14**	8.07**	0.12	-0.003	0.44**	-1.78**	20.19**	0.10
FS170	0.06	0.67*	0.03	-2.14*	-7.72**	-0.22	0.18**	0.07	-0.10	0.07	-0.09
FS60	0.69**	-3.15**	0.28	-23.03**	-11.05**	0.02	0.25**	0.52**	-0.38	25.84**	0.09
FS232	-0.33*	-1.00**	-0.02	-7.81**	-4.86**	0.40*	-0.09**	-1.15**	-0.07	15.65**	-0.23**
F7215Q	0.02	-1.84**	-0.16	6.77**	8.44**	0.87**	-0.03	0.46**	0.66*	10.21**	0.50**
142-1-EQ	1.17**	0.05	0.80**	13.70**	12.88**	1.65**	0.02	-0.05	5.51**	-6.71**	0.28**
CML144	-0.4**	3.86**	-0.92**	-1.07	-3.18**	-1.07**	0.03	1.07**	-2.21**	-55.01**	-0.40**
$SE_{(gi)}$	0.13	0.27	0.21	1.03	1.07	0.16	0.03	0.13	0.34	2.02	0.07

* *P*< 0.05; ** *P*< 0.01; GY= grain yield (t ha^{-1}); AD= days to anthesis; ASI= anthesis-silking interval (d); PH= plant height (cm); EH= ear height (cm); EL= ear length (cm); ED= ear diameter (cm); RPE= number of rows per ear; KPR= number of kernels per row; TKW= thousand kernel weight (g); MOD= endosperm modification; $SE_{(gi)}$= standard error of GCA.

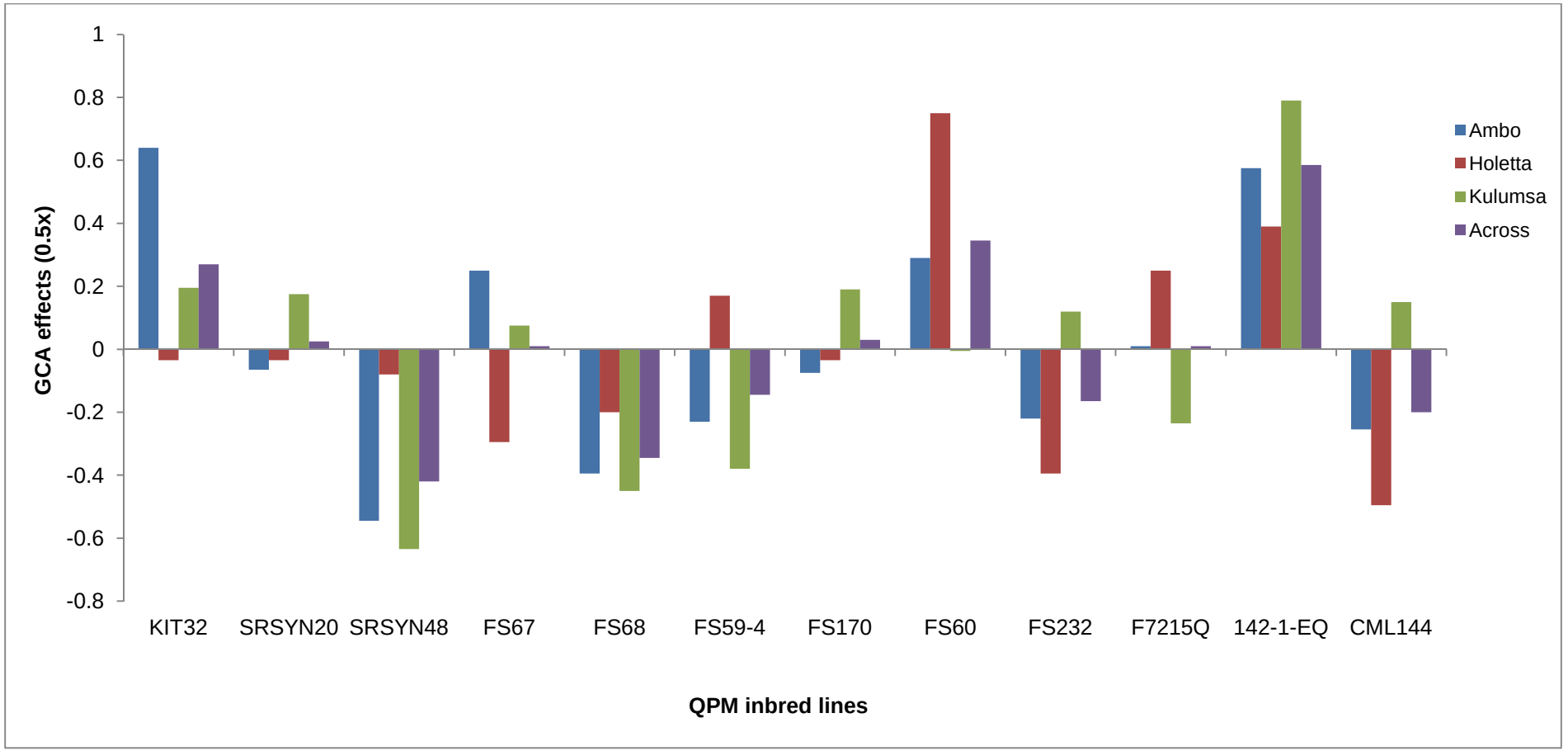

Figure 5. 1 The general combining ability effects of 12 QPM inbred lines for grain yield (t ha^{-1}) at each and across locations

5.4.5 Specific combining ability effects

Selected diallel crosses showing significant specific combining ability (SCA) effects for at least one agronomic trait during combined analysis are given in Table 5.10. Among the 66 hybrids, crosses KIT32 x FS68, SRSYN20 x FS60 and FS67 x CML144 revealed significant and positive SCA effects for GY. In contrast, hybrids such as KIT32 x CML144, FS67 x FS68 and FS170 x CML144 showed highly significant and negative SCA effects for GY. Cross FS67 x CML144 had significant and negative SCA effect for AD. Other hybrids with significant and negative SCA effects for AD included SRSYN48 x FS232, FS67 x FS59-4 and FS67 x CML144. Whereas hybrids with significant and positive SCA effects for AD were SRSYN20 x FS67, SRSYN48 x 142-1-EQ, FS67 x FS60, FS68 x FS232, FS59-4 x CML144, and FS170 x CML144. Hybrids that exhibited significant negative SCA effects for both PH and EH included KIT32 x CML144, SRSYN20 x F7215Q, FS67 x FS68, FS67 x FS59-4, FS232 x 142-1-EQ. However, KIT32 x 142-1-EQ, SRSYN48 x FS68, FS67 x F7215Q and 142-1-EQ x CML144 had significantly positive SCA effects for both PH and EH. Only four hybrids, *viz*., KIT32 x FS68, SRSYN20 x F7215Q, FS67 x CML144 and 142-1-EQ x CML144 showed significantly positive GCA for EL. Only hybrid FS59-4 x CML144 had significantly negative SCA effect for ED. Only hybrid FS59-4 x FS60 had significantly positive SCA effect for RPE. Crosses SRSYN20 x F7215Q, FS68 x FS60 and FS170 x FS232 had significantly positive SCA effects for KPR. Relatively a large number of crosses displayed significant SCA effects for TKW more than any other trait considered in this study. The maximum positive SCA effect was recorded in cross FS170 x CML144 followed by SRSYN20 x FS59-4, KIT32 x FS170, FS59-4 x F7215Q and FS67 x CML144. Among the four hybrids with significant SCA effects for MOD, FS68 x FS60 and FS59-4 x F7215Q had negative effects while KIT32 x F7215Q and FS60 x F7215Q had positive effects.

5.4.6 Components of variance

Table 5.11 summarizes estimates of variance components for grain yield and related agronomic traits obtained during combining ability analyses. The GCA variances (σ^2_{gca}) were consistently higher than SCA (σ^2_{sca}). Consequently, the corresponding additive variances (σ^2_A) were also higher than dominance variances (σ^2_D) for all the

Table 5. 10 Mean grain yield and related agronomic traits showing significant specific combining ability (SCA) effects of selected diallel crosses generated from 12 inbred lines when tested across three locations

Crosses	GY	AD	PH	EH	EL	ED	RPE	KPR	TKW	MOD
KIT32 x SRSYN48	0.17	-1.50	-9.07*	-6.55	-0.51	-0.03	-0.02	0.29	-2.25	-0.21
KIT32 x FS67	0.68	0.62	7.70*	7.44	0.24	0.11	0.08	0.82	4.00	0.32
KIT32 x FS68	0.93*	0.18	1.88	-3.73	1.58**	0.06	-0.26	1.35	15.74*	0.07
KIT32 x FS170	-0.69	0.87	-4.84	-2.46	-0.31	0.00	-0.29	-2.50*	33.62**	-0.50
KIT32 x FS232	-0.24	1.71	9.94**	4.55	-0.19	-0.10	-0.14	-1.06	17.54*	-0.09
KIT32 x F7215Q	-0.18	0.05	4.92	-2.06	-0.76	0.01	0.55	-0.99	-12.19	0.78**
KIT32 x 142-1-EQ	0.90	0.69	9.59**	9.27*	0.19	-0.07	-0.20	1.96	-2.13	-0.33
KIT32 x CML144	-1.43**	0.48	-16.57**	-10.47**	-0.66	-0.05	0.27	-2.36	-31.60**	-0.22
SRSYN20 x FS67	0.23	2.11*	3.26	7.77**	0.61	-0.05	-0.69	0.93	4.01	-0.08
SRSYN20 x FS59-4	0.01	0.52	2.08	1.78	-0.94	0.13	0.30	-2.67*	35.78**	-0.12
SRSYN20 x FS60	1.00*	-0.22	5.79	-1.80	0.72	-0.06	-0.38	1.56	12.22	-0.21
SRSYN20 x F7215Q	-0.23	-1.70	-13.58**	-13.69**	1.91**	-0.18	-0.55	3.19**	3.22	0.11
SRSYN20 x CML144	-0.05	0.36	2.43	4.86	-0.79	-0.07	-0.03	-0.21	-21.76**	0.15
SRSYN48 x FS67	-0.18	0.82	-1.06	3.34	-0.70	0.00	-0.13	-1.14	17.44*	-0.03
SRSYN48 x FS68	0.07	-1.05	10.56**	9.30*	-0.29	0.09	0.17	-0.98	7.44	0.22
SRSYN48 x FS170	-0.28	-1.37	7.94*	4.40	0.29	-0.04	0.30	-0.03	-7.44	0.25
SRSYN48 x FS232	0.50	-2.79**	-6.32	-10.99**	0.04	0.03	-0.11	0.41	10.45	-0.01
SRSYN48 x 142-1-EQ	0.21	2.32*	-0.03	-1.96	-0.08	0.12	0.56	0.07	-17.42*	0.28
FS67 x FS68	-1.39**	-0.07	-11.81**	-7.64*	-0.63	-0.05	0.40	-3.25**	11.36	-0.22
FS67 x FS59-4	-0.22	-3.25**	-10.77**	-12.27**	-0.30	0.01	-0.11	1.12	-25.01**	-0.21
FS67 x FS170	0.06	-0.02	-2.26	-5.44	-0.02	-0.11	-0.23	-0.63	-16.29*	0.01
FS67 x FS60	-0.34	2.24*	-4.70	-7.05	0.43	0.02	0.08	-0.11	4.04	-0.13
FS67 x F7215Q	0.50	1.73	13.70**	10.06**	-0.34	0.07	-0.29	0.35	-17.10*	0.36
FS67 x CML144	1.35**	-1.94*	3.61	-1.12	1.16*	0.04	-0.04	1.61	29.05**	-0.14
FS68 x FS59-4	-0.04	-0.22	-0.06	-2.17	0.55	-0.03	0.19	1.68	-19.50**	0.37
FS68 x FS60	-0.42	-0.70	-2.19	2.05	0.18	0.01	0.02	2.48*	-20.13**	-0.52*
FS68 x FS232	-0.01	2.05*	-3.17	1.63	0.80	-0.08	-0.55	-0.06	21.37**	-0.20
FS68 x CML144	0.23	-0.35	1.56	1.54	-1.30*	-0.10	0.10	-1.99	-15.58*	0.14
FS59-4 x FS60	0.24	0.29	1.12	3.22	0.65	0.10	0.91*	-0.05	24.07**	-0.08
FS59-4 x F7215Q	-0.25	0.51	-2.01	1.43	-0.86	-0.09	-0.63	-1.76	29.57**	-0.82**
FS59-4 x 142-1-EQ	0.23	-0.08	1.49	6.43	-0.02	0.13	0.01	-0.50	14.93*	0.34
FS59-4 x CML144	-0.27	2.11*	-0.10	-2.72	-0.10	-0.21*	0.09	0.84	-35.94**	0.15
FS170 x FS60	-0.04	-0.54	-2.77	1.58	-0.01	-0.02	-0.28	-0.33	-19.31**	0.08
FS170 x FS232	0.44	0.01	2.72	5.00	0.78	0.09	0.08	2.56*	-20.05**	0.20
FS170 x F7215Q	0.37	0.54	5.57	6.23	0.88	0.03	0.68	2.06	-25.81**	-0.46
FS170 x CML144	-1.62**	2.34*	-6.89	-0.32	-0.42	0.10	0.26	0.03	50.60**	0.07
FS60 x FS232	0.21	-0.70	-1.89	1.32	-0.36	0.05	0.37	-0.92	-17.32*	0.09
FS60 x F7215Q	-0.33	-1.17	-3.77	0.62	-2.17**	0.12	0.06	-3.29**	20.58**	0.69**
FS60 x CML144	0.35	-0.47	9.10*	-1.56	0.63	0.03	0.35	0.31	5.60	0.09
FS232 x F7215Q	-0.35	0.65	1.84	0.53	-0.34	-0.04	-0.11	-1.60	-15.19*	0.25
FS232 x 142-1-EQ	-0.60	0.60	-16.23**	-13.97**	0.04	0.01	0.10	0.72	5.03	-0.33
FS232 x CML144	0.23	-0.35	2.55	9.78*	-0.27	0.00	-0.29	-1.36	3.93	0.21
142-1-EQ x CML144	0.30	1.52	12.85**	13.37**	1.20*	0.07	-0.50	2.31	-6.39	-0.25
$SE_{(sij)}$	0.47	0.99	3.74	3.90	0.59	0.09	0.45	1.22	7.34	0.26

* *P*< 0.05; ** *P*< 0.01; GY= grain yield (t ha^{-1}); AD= days to anthesis; PH= plant height (cm); EH= ear height (cm); EL= ear length (cm); ED= ear diameter (cm); RPE= number of rows per ear; KPR= number of kernels per row; TKW= thousand kernel weight (g); MOD= endosperm modification; $SE_{(sij)}$= standard error of SCA.

traits. The narrow sense heritability was higher for number of rows per ear (0.78) followed by ear diameter (0.75), plant height (0.75), days to anthesis (0.74), and

number of kernels per row (0.73). However, relatively lower heritability estimates were observed on grain yield (0.47) and anthesis-silking interval (0.50). The ratios of dominance genetic variance to additive genetic variances for all traits were small (0.04-0.28) except grain yield at 0.33.

Table 5. 11 Estimates of genetic parameters for grain yield and related traits among 66 single crosses generated from a diallel crosses of 12 QPM inbred lines when evaluated across three locations

Traits	σ^2_{gca} ± SE	σ^2_{sca} ± SE	σ^2_A	σ^2_D	σ^2_e	h^2 (%)	σ^2_D / σ^2_A
Grain yield	0.24 ± 0.14	0.16 ± 0.05	0.48	0.16	0.38	0.47	0.33
Days to anthesis	4.83 ± 2.22	0.42 ± 0.28	9.66	0.42	2.97	0.74	0.04
Anthesis silking interval	0.45 ± 0.21	0.12 ± 0.07	0.90	0.12	0.77	0.50	0.13
Plant height	100.63 ± 47.31	23.85 ± 6.88	201.26	23.85	42.29	0.75	0.12
Ear height	63.19 ± 29.02	19.18 ± 5.64	126.38	19.18	35.68	0.70	0.15
Ear length	0.68 ± 0.31	0.38 ± 0.09	1.36	0.38	0.31	0.66	0.28
Ear diameter	0.02 ± 0.01	0.003 ± 0.001	0.04	0.003	0.01	0.75	0.08
No. of rows/ear	0.38 ± 0.17	0.05 ± 0.02	0.76	0.05	0.17	0.78	0.07
No. of kernels/row	3.88 ± 1.71	1.42 ± 0.34	7.76	1.42	1.49	0.73	0.18
Thousand kernel weight	397.3 ± 189.60	173.6 ± 52.60	794.6	173.6	346	0.60	0.22

σ^2_{gca} and σ^2_{sca} = General and specific combining ability variances, respectively; σ^2_A and σ^2_D = Additive and dominance genetic variances, respectively; h^2= narrow sense heritability; SE= standard error.

5.4.7 Parents vs. hybrid per se performances and heterosis for grain yield

Mean performances of inbred lines, their hybrids and heterosis for grain yield across three locations are summarized in Table 5.12. The percentage mid-parent heterosis (MPH) for grain yield ranged from 64.5 to 171.2% with a mean of 121.3%. Generally, about 86% of the crosses showed ≥100% positive MPH values for grain yield. The maximum MPH (171.2%) was recorded for the cross between P2 and P8 followed by crosses between P5 and P7, P5 and P10, P4 and P12, and P8 and P9. However, the maximum grain yield (9.6 t ha^{-1}) was obtained for the cross involving P1 x P11 followed by P7 x P11, P2 x P8, P8 x P11, P11 x P12 and P1 x P8. The best performing hybrids had either P1 or P8 or P11 in the parentage. The *per se* performance and GCA effects of grain yield were also good for these three parents (Table 5.12).

Table 5. 12 Mean performances of inbred lines (diagonal) and hybrids (below diagonal), and mid-parent heterosis (above diagonal) for grain yield (t ha^{-1}) when evaluated across three locations in 2012

‡Inbred line	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12
P1	4.1	107.0	110.0	112.2	148.3	96.7	91.1	125.5	102.6	94.9	125.9	64.5
P2	7.5	3.1	116.0	112.8	137.6	121.3	136.7	171.2	119.1	115.6	103.3	120.9
P3	6.8	5.9	2.4	99.9	139.3	130.8	118.4	113.8	145.6	128.9	126.4	129.0
P4	8.2	7.2	6.0	3.6	67.3	99.3	113.4	111.7	113.2	120.1	85.5	149.6
P5	7.8	6.3	5.4	4.8	2.2	130.7	156.2	134.3	140.4	149.9	123.7	144.0
P6	6.9	6.7	6.1	6.5	5.9	2.9	140.8	141.2	115.8	112.1	120.0	107.2
P7	6.9	7.3	5.9	7.1	6.7	7.2	3.1	138.6	142.4	136.5	137.9	72.8
P8	8.4	8.8	6.1	7.4	6.4	7.6	7.7	3.4	149.0	119.6	125.2	143.6
P9	7.0	6.5	6.3	6.8	5.9	6.1	7.1	7.6	2.8	105.2	99.8	131.8
P10	7.2	6.9	6.4	7.6	6.8	6.6	7.5	7.3	6.2	3.3	96.1	127.6
P11	9.6	7.6	7.6	7.4	7.3	8.0	8.9	8.7	7.1	7.5	4.4	135.4
P12	5.7	6.5	5.9	8.0	6.0	5.9	5.1	7.5	6.4	6.9	8.4	2.8
GCA ($SE_{(gi)}$= 0.13)	0.54**	0.05	-0.84**	0.02	-0.69**	-0.29*	0.06	0.69**	-0.33*	0.02	1.17**	-0.4**

‡ See Table 1 for designations of inbred lines from P1 to P12; * *P*< 0.05; ** *P*< 0.01; $SE_{(gi)}$= standard error of GCA

5.5 Discussion

Significant differences were observed among hybrids from individual and across locations analyses. Similar results of variation among QPM hybrids evaluated under different environments for grain yield and other agronomic traits were reported (Pixley and Bjarnason, 1993; Bhatnagar et al., 2004; Musila et al., 2010; Elmyhum, 2013). Hybrid by location interactions were significant for most traits resulting in rank differences of the genotypes. Genotype-environment interactions observed for most traits in the current study are not uncommon in the tropics. Miranda-Filho (1985) pointed out that apart from great variations due to latitude, day length and temperature in the tropical regions, there is also a wide variation among locations, even if they are geographically closer, which makes genotype by environment interaction an important source of variation. Genotypes evaluated in this study were single crosses which, in turn, contributed for the significantly high interaction of hybrids by locations. Single-cross hybrids reportedly interact more with the environment than other types of hybrids (Hallauer and Miranda, 1988; Troyer, 1996).

As opposed to Dagne's (2008) and Bhatnagar et al. (2004) findings, the majority of the QPM hybrids in the present study were high yielders than both normal and QPM commercial checks. For example, the highest mean grain yield (11.3 t ha^{-1}) was obtained in cross KIT32 x FS59-4 at Kulumsa, with low coefficient of variation (CV) compared to the best non-QPM check (AMH850) that yielded 9.5 t ha^{-1}. With regards to days to 50% tasseling, all hybrids were relatively late (103 to 117 days) compared to the non-QPM check (102 days). This result agreed with Elmyhum (2013) who reported that all QPM hybrids were late when compared to the non-QPM hybrid check. With the prevalence of frost and inadequate amount and distribution of rainfall in tropical highlands such as in Ethiopia, breeders should focus on developing early flowering maize varieties with suitable grain yield. Earliness is one of the main objectives in locations with limited water resources and in climates with short growing seasons (Estakhr and Heidari, 2012).

Assessments of plant and ear heights of the present experimental hybrids showed that hybrids were taller than the non-QPM and shorter than the QPM checks. However, genotypes with tall plant and ear heights are not desirable due to susceptibility to lodging. When selecting for a high yielding genotypes, therefore, maintaining a balance between higher yield and shorter stature is critical. The overall mean values for other traits (ear length, ear diameter, number of rows per ear, number of kernels per row, and thousand kernel weight) appeared smaller than the checks.

The present study also indicated the existence of variation among the hybrids for endosperm modification, which would enable selection for stability of this parameter across locations. Moreover, the performance of the hybrids was consistent across locations for this trait as evident from the non-significant hybrid × location interaction. Previous researchers reported contradicting results with the current finding for endosperm modification evaluations at contrasting environmental conditions (Vasal et al., 1993; Pixley and Bjarnason, 2002; Wegary et al., 2011).

The correlation analysis between grain yield and related agronomic traits indicated that ear characters well-correlated with grain yield. This result was in agreement with Hallauer et al. (2010) who demonstrated that average genetic correlations with yield

were larger for ear traits than for plant and ear heights, days to flower, and tiller number. According to the authors, genetic correlations are useful if indirect selection gives greater response to selection for a trait than direct selection for the same trait. Mode and Robinson (1959) investigated the concept of genetic correlations for traits of maize under the assumption that genes exhibit pleiotropic effects. Huang et al. (2013) also investigated that pleiotropic effect existed among maize traits, *viz*., yield, ear length, kernel number per row, plant height or ear height. It is, therefore, important to observe for desirable associations among traits during selection and testing of genotypes, because success of selection also relies on association between both traits. From the present study, highly significant and positive correlations of ear diameter and length as well as number of ears per plant and kernels per row with grain yield indicated that increases in these traits may result in increased grain yield, thereby revealing the possibility of simultaneous improvement of these traits and grain yield. Among the yield components, number of ears per plant has been proposed as an important component for potential improvement of yield by indirect selection (Singh et al., 1986; Subandi, 1990; Hallauer et al., 2010).

Combining ability analysis showed the existence of considerable variability among parents and their crosses at each and across locations. The mean squares due to GCA and SCA were highly significant for most traits. The SCA mean squares were not significant for anthesis-silking interval, number of ears per plant, ear length and diameter, and number of rows per ear, number of kernels per row and endosperm modification at individual or across locations analyses. Similarly, previous investigations reported that both GCA and SCA interact significantly with environments (Matzinger et al., 1959; Pixley and Bjarnason, 1993; Everett et al., 1995; Pswarayi and Vivek, 2008; Musila et al., 2010; Wegary et al., 2011; Estakhr and Heidari, 2012). Significant GCA x location interaction is an indication of variation of GCA of lines under different environments suggesting the need for selecting different parental lines for hybrids at specific environments. Significant SCA x location interaction, on the other hand, suggests that hybrids preformed differentially across different environments. Furthermore, significance of GCA and SCA at each and across environments indicates the importance of genes having largely additive effects as well as genes having dominance and epistatic effects, respectively (Baker, 1978). Consequently, it would be necessary to consider both the average performance of a

line in hybrid combinations and the specific hybrid combinations. This finding is in line with previous diallel studies on QPM inbred lines (Pixley and Bjarnason, 1993; Bhatnagar et al., 2004; Wegary et al., 2011).

Across locations combining ability analysis indicated that inbred lines KIT32, FS60 and 142-1-EQ were good general combiners showing positive and highly significant GCA effects for grain yield. This indicates that on average these parents contributed to increased yield in crosses. However, KIT32 and FS60 had crossover interaction (i.e. rank differences) at Holetta and Kulumsa in their GCA effects for grain yield. These parents could then make a complementary and well-buffered single cross enabling it to perform as a seed parent for three-way or double cross hybrid development under diverse environments. Similar results were also reported by Pswarayi and Vivek (2008) using different set of parents. Although 142-1-EQ and KIT32 had relatively the highest GCA effects for grain yield, they were not good general combiners for days to anthesis. Maturity and yield are positively correlated (Hallauer et al., 2010). It is known that an earlier maturing variety, owing to its shorter life cycle, is predisposed to lower yield. On the contrary, a late maturing variety has the opportunity to exploit the growth factors and prolonged photosynthesis over a longer period during the growing season. Therefore, whilst selecting for early maturing genotypes maintaining a balance between earlier maturity and higher yield is worth considering. To this end, inbred FS60 with significantly positive GCA effect for grain yield, negative GCA effects for days to anthesis, plant and ear heights, positive GCA effects for ear length and diameter and thousand kernel weight can complement both inbreds (KIT32 and 142-1-EQ) to develop high yielding, early maturing and short statured QPM cultivars.

The combining ability analyses also showed that hybrids: KIT32(P1) x FS67(P4), KIT32(P1) x FS68(P5), KIT32(P1) x 142-1-EQ(P11), SRSYN20(P2) x FS60(P8), and FS67(P4) x CML144(P12) were the best crosses with favourable SCA estimates for grain yield. Non-additive gene effects seem to be small on the average, but they may be important for specific cross combinations (Hallauer and Miranda-Filho, 1988). These crosses however contain at least one parent with high GCA effect for grain yield, indicating the increased concentration of favourable alleles. Crosses KIT32(P1) x 142-1-EQ(P11), SRSYN20(P2) x FS60(P8) were constituted from two parents of

same heterotic group with high GCA. These crosses could thus be used as single cross testers. Baker (1978) suggested that the performance of a single-cross progeny can be adequately predicted on the basis of GCA, if SCA is not significant. On the other hand, Han et al. (1991), Vasal et al. (1992), and Gama et al. (1995) reported that, on average, hybrids produced by crossing inter-population lines have more positive SCA effects than those produced by crossing intra-population lines which tend to have more negative SCA effects. Crosses KIT32(P1) x FS68(P5) and FS67(P4) x CML144(P12) were also good specific combiners for days to anthesis, ear height, ear length and thousand kernel weight.

Estimates of components of variances revealed the overwhelming contributions of GCA variance than SCA variance to the total genetic variances for most of the traits. Estimates of genetic parameters showed that the ratio of dominance to additive variance was relatively large for yield when compared to other traits, showing that dominance variance seems important in the expression of grain yield. This finding is in agreement with several previous experimental results (Hallauer et al., 2010). However, narrow sense heritability estimates for the different traits in the present study were inflated than reported by these authors. This could be attributed, in part, to the analysis of results from small number of experiments compared to the average results of large number of experiments such as presented by Hallauer et al. (2010).

Mid-parent heterosis (MPH) analyses of grain yield in the present study showed that almost all hybrids were superior to their parents. This suggests the potential of the inbred lines for hybrid development to exploit heterosis or hybrid vigour. The ranges of heterotic responses observed were on average higher than that reported by Dagne (2008). However, Tollenaar et al. (2004) observed higher mean grain yield MPH of 167% and Betran et al. (2003e) reported mean MPH of 157% compared to 121.3% observed in this study. Two issues are worth noting from the results of the current study about interrelationships among GCA of parents, *per se* hybrid performance, SCA of crosses and MPH (Table 5.12). Firstly, sufficient MPH exists between parents of high GCA that belong within heterotic groups. In general, tropical maize germplasm is known to have an intra-group diversity (Han et al., 1991) that is sufficient to exploit heterosis contributed by additive genetic effects (Pswarayi and Vivek, 2008). Secondly, the crosses with significant SCA effects had also relatively high percent

MPH and good hybrid *per se* performances. Interesting to note here is that these crosses had, at least, one parent with either negative or non-significant positive GCA effect, and the other parent with highly significant and positive GCA effect. The extent of heterotic response of the F_1 hybrids largely depends on the breeding value and genetic diversity of the parents included in crosses, and on the environmental conditions under which hybrids are grown (Hallauer and Miranda, 1988; Glover et al., 2005). To exploit hybrid vigour, therefore, *per se* performance, SCA effect and the extent of heterosis in hybrids were important parameters and selection based on anyone of these parameters alone may not be effective. Because hybrids with high *per se* performance need not always reveal high SCA effect and vice-versa (Premlatha and Kalamani, 2010; Patil et al., 2012).

5.6 Conclusions

The results of this experiment indicated the presence of high variability for grain yield and agronomic traits, and thus the possibility of selection among the QPM hybrids that are adapted to the highlands of Ethiopia and other similar agro-ecologies in east African countries. Most of the QPM single-cross hybrids performed better than both normal and QPM commercial checks. Inbred lines: KIT32, FS60 and 142-1-EQ were found good general combiners for grain yield; while FS60 exhibited good general combining ability for days to anthesis, plant and ear height. Inbred line FS60 can be genetically complementary to KIT32 and 142-1-EQ for QPM hybrid cultivar development. In general, the new QPM single-cross hybrids performed better than the checks. The best crosses identified were KIT32 x 142-1-EQ and SRSYN20 x FS60 that yielded 9.6 t ha^{-1} and 8.8 t ha^{-1}, respectively. These hybrids would be used as potential single-cross testers for the development of three-way QPM hybrids for the highland ecologies. Although sufficient variation exists within heterotic groups of tropical maize germplasm including the genotypes used for the current study, the putative heterotic groups of the inbreds perhaps need further refinement through molecular techniques.

5.7 References

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CHAPTER 6

Genotype-by-environment interaction and yield stability of quality protein maize hybrids adapted to tropical-highlands

6.1 Abstract

Maize yields are significantly low in the highland agro-ecologies of east and central African region (ECA) due to the lack of improved cultivars and genotype by environment (G x E) interaction, among others. Multi-environment trials (METs) are important for proper separation and ranking of superior genotypes and to identify representative environments for reliable estimation of yield potential and heritability of traits and for competitive seed production. The objectives of this study were to determine G x E interaction and yield stability of recently developed quality protein maize (QPM) single-cross hybrids and to identify representative test and/or production environments. The study was conducted at seven environments representing the highland sub-humid maize growing agro-ecology of Ethiopia. Sixty-six QPM hybrids and two commercial hybrid checks were evaluated in the study. Data were analysed using the Additive Main and Multiplicative Interaction (AMMI) and genotype and genotype by environment (GGE) biplot methods. Hybrid 10 (KIT32 x 142-1-eQ) followed by hybrid 66 (142-1-eQ x CML144) and hybrid 59 (FS60 x 142-1-eQ) with yield levels of 10.3, 9.6 and 9.4 t ha^{-1}, respectively, were selected as the best performers and hence desirable hybrids. Kulumsa was the most suitable environment identified by both analyses. The GGE analysis divided the highland test environments into two mega-environments.

Keywords: Additive Main and Multiplicative Interaction, GGE biplot, Genotype-by-environment interaction, Multi-environment trails, Quality protein maize, yield stability.

6.2 Introduction

Over the past 30 years an increasing production and consumption trend of maize has been observed in Africa. In eastern and southern Africa maize is the dominant staple crop grown by the vast majority of rural households (DeVries and Toenniessen, 2001). In east Africa, the importance of maize is growing with time (CIMMYT, 1999; Thorne et al., 2002; Krivanek et al., 2007) accounting for over 25% of the total calories consumed (FAOSTAT, 2012). The demand for maize in sub-Saharan Africa is projected to increase nearly twofold by the year 2020 (Bigirwa et al., 2003).

In eastern Africa, major agro-ecological zones up to 2400 meters above sea level (masl) are considered favourable for maize cultivation (Twumasi et al., 2002). The highland maize growing agro-ecological zone stands second in maize production next to the mid-altitude zone in the region (Kassa et al., 2013). The highland agro-ecology is distinguished by high rainfall, seasonal cool temperatures, high population density and high levels of poverty (Banziger and Diallo, 2000). In this agro-ecology most of the maize is produced and consumed by resource poor farmers who have limited access to protein sources such as animal products. This leaves the majority to depend on conventional maize, which is deficient in two essential amino acids (lysine and tryptophan), as the main source of protein leading to malnutrition and protein deficiency. Quality protein maize (QPM), a nutritionally enhanced variants of normal maize, may alleviate undernourishment leading to healthier population, improved productivity, and increased income for enhanced livelihoods (Kassa et al., 2013).

Maize yields are considerably low in the highland agro-ecologies of the region partly because of the lack of improved cultivars and genotype by environment (G x E) interaction. Moreover, there was no research interest on highland maize improvement until it was accelerated in 1998 as part of the Highland Maize Gene Pool Project initiated by the International Maize and Wheat Improvement Centre (CIMMYT). The aims of the project were to develop and release improved normal and nutritionally enhanced (QPM) highland maize varieties adapted to the highlands of ECA (Twumasi et al., 2002). A regional nursery was established at Ambo, Ethiopia to run the breeding project. Improved genotypes (QPM and non-QPM inbred lines, hybrids and open pollinated varieties) developed by the project continued to be evaluated in multi-

environments across various locations in the region. Hence, yield stability and wider adaptation of the genotypes being evaluated are of vital importance in these environments where growing conditions are variable. Yan et al. (2007) reported the importance of properly examining environments for uniqueness and for information that would enable the separation and ranking of promising genotypes. This allows stratification and identification of core locations where testing of cultivars can be done without losing information about genotypes. Stratification of maize evaluation environments can help increase heritability of measured traits, accelerate selection gains, strengthen the potential competitiveness for seed production and maximize yield grains for farmers (Gauch and Zobel, 1997).

Multi-environment trials (METs) are important to identify superior genotypes, estimate the genotype by environment (G x E) interaction, and to evaluate and select test environments (Cooper et al., 1996; Yan et al., 2007). Appropriate choice of test environments can reduce cost and improve breeding efficiency (Yan and Holland, 2010). Various methods were developed to analyze data from METs of which the additive main and multiplicative interaction (AMMI) (Zobel et al., 1988) and genotype and genotype by environment (GGE) biplot (Gabriel, 1971) methods are the most widely used multivariate approaches. The AMMI model combines analysis of variance for the genotype and environment main effects with principal components analysis of the G x E interactions (Gauch and Zobel, 1996). The GGE, on the other hand, integrates the genotypic main effect with the G x E interaction effect of a G x E dataset (Yan et al., 2000). A GGE biplot is helpful to: i) facilitate grouping of mega-environments (Gauch and Zobel, 1997), (ii) identify better environments for cultivar evaluation (Cooper et al., 1997), and (iii) compare genotypes and genotype ranking on both mean yield and stability (Yan, 2001).

The highland maize breeding program in Ethiopia has developed a number of promising QPM and non-QPM maize inbred lines and single cross hybrids for use in highland agro-ecologies of ECA. However, there is limited information on the G x E interaction and grain yield stability of the hybrids developed from these inbred lines across target environments. The objectives of this study were, therefore, to determine G x E interaction and yield stability of recently developed quality protein maize (QPM)

single-cross hybrids and to identify representative test and/or production environments using AMMI and GGE biplot models.

6.3 Materials and methods

6.3.1 Description of study sites

The study was conducted in 2012 and 2013 rainy seasons at three and four locations (Table 6.1), respectively, making a total of seven environments. The study sites represent the highland sub-humid maize growing agro-ecology of Ethiopia. Agro-climatic descriptions of the experimental locations used for the study are presented in Table 1.

Table 6. 1 Descriptions of locations used for testing the hybrids in 2012 and 2013

Environment code	Site	Season	Geographic position			Annual rainfall[$] (mm)	Temperature (0^oC)		Soil type
			Longitude	Latitude	Altitude *(m.a.s.l.)		Min.	Max.	
AMB12	Ambo	2012	$38^o07'$ E	$8^o57'$ N	2225	1071	10.4	26.6	Heavy Vertisol
AMB13		2013				1260	9.8	27.5	
						(1115)	(11.7)	(25.4)	
HOL12	Holetta	2012	$34^o48'$ E	9^o 00' N	2400	933	5.3	23.6	Nitosol
HOL13		2013				760	6.2	24.4	
						(1065)	(6.4)	(22.1)	
KUL12	Kulumsa	2012	$39^o13'$ E	$8^o13'$ N	2180	765.2	11.4	23.7	Eutric Vertisol
KUL13		2013				958.8	12.8	22.2	
						(824)	(10.0)	(23.0)	
HAR13	Haramaya		$42^o02'$ E	$8^o37'$ N	2050	(820)	(8.9)	(23.4)	Entisol

*= (m.a.s.l.) = meters above sea level; $ = Long term mean weather data is shown in parenthesis.

6.3.2 Germplasm

The study used recently developed 66 QPM experimental hybrids derived from the diallel crosses involving 12 selected elite inbred lines. Two standard check three-way-cross commercial hybrids: one QPM (AMH760Q) and the other normal maize (AMH850) were included as comparative controls. Further details of the genotypes are given in the materials and methods section of Chapter 4.

6.3.3 Experimental design, field management and data collection

These are also presented in the materials and methods sections of Chapter 4.

6.3.4 Data analysis

Grain yield data were subjected to combined analysis of variance using PROC GLM procedure in SAS 9.1 (SAS, 2002). It was done to test the significance of G x E prior to detailed analyses. The F_1 hybrids were treated as fixed effects and environments (both spatial and temporal), replications within environments and blocks within replications were considered as random effects. The AMMI and GGE biplot models were computed sequentially to analyze G x E interaction and yield stability of genotypes.

The AMMI analysis was performed using the raw data from seven environments for the 68 hybrids using GenStat® Release 14 statistical software (Payne et al., 2007). The model first fits additive effects for the main effects of genotypes and environments followed by multiplicative effects for G x E by principal component analysis (Zobel et al., 1988). The AMMI model as proposed by Zobel et al. (1988) is:

$$Y_{ge} = \mu + \alpha_g + \beta_e + \sum_{n=1}^{N} \lambda_n \gamma_{gn} \eta_{en} + \theta_{ge},$$

where Y_{ge} is the yield of genotype, g, in environment, e; μ is the grand mean; α_g is the genotype mean deviation; β_e is the environment mean deviation; λ_n is the Eigen value of the principal component (PCA) axis, *n*; γ_{gn} and η_{en} are the genotype and environment PCA scores for the PCA axis, *n*; N is the number of PCA axis retained in the model; and θ_{ge} is the residual. The AMMI stability value (ASV) was used to compare stability of genotypes as described by Purchase (1997):

$$\text{ASV} = \sqrt{\left[\frac{IPCA1SS}{IPCA2SS}(IPCA1score)\right]^2 + [\text{IPCA2score}]^2},$$ where, ASV= AMMI stability value; SS= sum of squares; IPCA1 and IPCA2= the first and the second interaction principal component axes, respectively. Hybrids with lower ASV values were considered more stable (Purchase, 1997).

The variation due to genotypes and G x E for grain yield (t ha^{-1}) was explained using GGE biplot based on the principal component analysis (PCA) of environment centred data (Yan et al., 2000). The GGE biplot was also performed with GenStat® Release 14 statistical software using the model based on singular value decomposition (SVD) of the first two principal components (Yan, 2002) as follows:

$$Y_{ij} - \mu - \beta_j = \lambda_1 \xi_{i1} \eta_{j1} + \lambda_2 \xi_{i2} \eta_{j2} + \varepsilon_{ij} ,$$

where Y_{ij} is the mean yield of i^{th} hybrid in j^{th} environment, μ is the grand mean, β_j is the main effect of environment j, μ + β_j is the mean yield across all hybrids in environment j, λ_1 and λ_2 are the singular values (SV) for the first and second principal component (PC1 and PC2), respectively, ξ_{i1} and ξ_{i2} are the Eigen vectors of hybrid *i* for PC1 and PC2, respectively, η_{j1} and η_{j2} are the Eigen vectors of environment *j* for PC1 and PC2, respectively and ε_{ij} is the residual associated with hybrid *i* in environment *j*. Adjusted means of 17 hybrids (25% out of the total 68 hybrids), majority of them selected from the first four AMMI selections per environment, were used to run GGE biplot analysis. Because it would be difficult to visualize the entire 68 hybrids together if included in the different GGE biplot graphs. The genotype x environment relations as represented by the which-won-where pattern (Gauch and Zobel, 1997; Yan, 2002), the interrelationships among test environments (Cooper et al., 1997) and genotypes (Yan, 2001) were visualized using the various GGE biplot graphs. An average environment coordinate (AEC) was drawn on the genotype-focused biplot to visualize the mean and stability of the hybrids (Yan and Kang, 2003). Furthermore, ideal environments and hybrids were also identified using the AEC.

6.4 Results

6.4.1 Analysis of weather data

The weather data in Table 6.1 revealed that there were limited rainfall and high temperature conditions at some of the testing environments, such as Holetta in 2013. At this site there was a reduction of rainfall by 29% and a 10% rise in air temperatures when compared to the long term means of these variables. The weather data further showed an overall trend of rise in temperatures and decrease in amount of rainfall across sites.

6.4.2 Combined analysis of variance

The combined analysis of variance of the 68 maize hybrids evaluated in seven environments is presented in Table 6.2. The analysis showed highly significant differences ($p< 0.01$) of grain yield among hybrids. There were also significant differences among environments ($p< 0.01$) and their interaction with genotypes (GEI) ($p< 0.01$). This implied that there was genotype x environment (G x E) interaction affecting the performance of the hybrids, which necessitated further analysis of the nature of GEI.

Table 6. 2 Combined analysis of variance of grain yield of 68 maize genotypes tested across seven environments in Ethiopia

Source of variation	df	Sum of squares	Mean squares
Environments (E)	6	2336.99	389.50**
Replications (R)	1	0.001	0.001^{ns}
Blocks within (E x R)	48	72.07	1.50**
Genotypes (G)	67	896.83	13.39**
Genotypes x Environments (G x E)	402	778.34	1.94**
Error	427	296.92	0.70
	R^2	CV (%)$^{\psi}$	Mean$^{\varepsilon}$
	0.93	11.35	7.35

ψ = coefficient of variation; ε= Grand mean; ns non-significant (P > 0.05); ** Significant at P ≤ 0.01; df= Degrees of freedom

6.4.3 AMMI Analysis

The AMMI analysis of variance showed highly significant effects ($p< 0.01$) of genotypes, environment and the genotype by environment interaction, GEI (Table 6.3). The relative magnitude of the different sources of variation varied greatly (Table 3). Of the total variation, 51.8% was contributed by the main effect of the environment. The GEI contributed to 40.1% of the total variation of which 21.8% was due to genotypic effect and 18.3% by the interaction effect. Hence, AMMI model explained 92% of the total variation in the study.

The GE interaction effects were further partitioned into interaction principal component axes (IPCA): IPCA1, IPCA2, IPCA3, IPCA4 and the G x E residual effects. All the IPCAs were highly significant (Table 6.3) accounting to 41.5, 24.1, 13.9, and

8.6% of the total GEI variations by IPCA1, IPCA2, IPCA3, and IPCA4 in that order. The residual effect contributed to 11.8% of the variation. Based on IPCA1 scores, QPM hybrids KIT32 x FS170, KIT32 x 142-1-eQ, KIT32 x CML144, SRSYN20 x 142-1-eQ, SRSYN20 x CML144, FS67 x CML144, FS68 x CML144, FS170 x 142-1-eQ, FS170 x 142-1-eQ and FS232P9 x CML144P12 had relatively high positive interaction with the environment. Whereas hybrids SRSYN48 x FS67, SRSYN48 x FS170, SRSYN48 x F7215Q, FS67 x FS68, FS67 x F7215Q, FS68 x FS170, FS68 x FS232, FS68 x F7215Q, FS59-4 x F7215Q, FS170 x F7215Q and FS60 x F7215Q had high negative interaction with the environment. Hybrids FS67 x FS60, F7215Q x CML144, FS59-4 x FS232 and Check-1 (AMH760Q) showed the lowest positive interaction while FS67 x 142-1-eQ and KIT32 x FS59-4 showed the lowest negative interaction (Table 6.4).

Table 6. **3** AMMI analysis of variance for grain yield of 68 maize genotypes tested across seven environments in Ethiopia

Source of variation	df[$]	Sum of squares	Mean squares	Total variation explained (%)	G x E explained (%)
Environments (E)	6	2337	389.52***	51.76	-
Genotypes (G)	67	984	14.69***	21.79	-
*Blocks	7	33	4.66***	0.73	-
Interactions (G x E)	402	825	2.05***	18.27	-
IPCA1	72	342	4.75***	-	41.45
IPCA2	70	199	2.85***	-	24.12
IPCA3	68	115	1.69***	-	13.94
IPCA4	66	71	1.07**	-	8.61
(G x E) Residuals	126	97	0.77^{ns}	-	11.76
Pooled error	469	337	0.72	7.46	-

*= The block source of variation refers to blocks within environments; $df= Degrees of freedom.

ns = non-significant (P > 0.05); **, *** Significant at P ≤ 0.01 and P ≤ 0.001, respectively

AMMI stability value (ASV) of each genotype revealed variations among the 68 hybrids in yield stability (Table 4). According to Purchase (1997), a stable variety is defined as one with ASV value of zero. Consequently, hybrid FS67 x FS60 with ASV value of 0.01 was the most stable while hybrids SRSYN20 x CML144, FS68 x CML144, FS59-4 x CML144 and FS232 x CML144 were the least stable. The mean grain yield performance of 18 hybrids was ≥ 8.0 t ha^{-1} including check-2 (AMH850). Overall, the test hybrids displayed a mean grain yield of 7.4 t ha^{-1}. Fifteen genotypes

including check-1 had mean grain yields closer to the grand mean indicating an average yield performance, while the remaining 35 hybrids had poor yield performance across environments (Table 4). The hybrid that combines both high yield and average stability across environments was FS67 x FS60. Hybrid SRSYN20 x F7215Q was also a stable genotype identified with average yield performance. The AMMI analysis identified the first four best performing hybrids for each environment (Table 6.5).

Table 6. 4 AMMI adjusted mean grain yield (t ha^{-1}), IPCA scores of genotypes and AMMI stability value (ASV) of 68 maize hybrids tested across seven environments in Ethiopia

No.	Genotype	Mean	IPCA1	IPCA2	ASV	No.	Genotype	Mean	IPCA1	IPCA2	ASV
1	P1 x P2	8.09	0.10	1.09	1.37	35	P4 x P9	7.33	-0.12	0.32	0.30
2	P1 x P3	7.51	0.19	-0.21	0.38	36	P4 x P10	7.57	-0.56	-0.03	0.96
3	P1 x P4	8.82	0.35	0.57	0.92	37	P4 x P11	7.70	-0.03	0.41	0.22
4	P1 x P5	7.68	-0.28	0.21	0.52	38	P4 x P12	8.85	0.60	-0.05	1.03
5	P1 x P6	7.30	-0.05	-0.54	0.38	39	P5x P6	6.03	-0.22	-0.44	0.57
6	P1 x P7	7.83	0.73	0.24	1.32	40	P5x P7	6.80	-0.71	0.51	1.48
7	P1 x P8	9.01	0.31	-0.13	0.56	41	P5x P8	6.94	-0.44	0.14	0.78
8	P1 x P9	7.50	0.16	0.57	0.60	42	P5x P9	6.03	-0.54	0.01	0.93
9	P1 x P10	7.36	-0.34	0.06	0.59	43	P5x P10	6.76	-0.58	0.16	1.02
10	P1 x P11	10.30	0.73	0.44	1.45	44	P5x P11	8.40	0.49	0.31	0.94
11	P1 x P12	6.50	0.52	0.59	1.24	45	P5x P12	7.12	0.84	-0.63	1.85
12	P2 x P3	6.61	0.16	-0.02	0.27	46	P6x P7	7.03	-0.39	0.05	0.67
13	P2 x P4	7.97	0.39	0.44	0.87	47	P6x P8	7.67	-0.26	-0.25	0.51
14	P2 x P5	6.40	-0.09	0.00	0.15	48	P6x P9	6.60	0.03	-0.17	0.07
15	P2 x P6	6.76	-0.15	0.32	0.36	49	P6x P10	6.31	-0.56	-0.54	1.26
16	P2 x P7	7.64	0.17	-0.22	0.34	50	P6x P11	7.82	-0.30	-0.47	0.74
17	P2 x P8	8.41	-0.13	-0.73	0.76	51	P6x P12	5.94	0.30	-1.15	1.84
18	P2 x P9	7.29	0.26	0.37	0.58	52	P7x P8	7.56	-0.41	-0.15	0.73
19	P2 x P10	7.46	-0.07	-0.03	0.12	53	P7x P9	6.97	-0.18	0.54	0.59
20	P2 x P11	8.63	0.53	0.10	0.92	54	P7x P10	6.93	-0.66	0.12	1.14
21	P2 x P12	7.16	0.68	-0.83	1.85	55	P7x P11	8.85	0.55	-0.37	1.08
22	P3 x P4	5.91	-0.60	0.16	1.05	56	P7x P12	6.29	0.90	0.11	1.56
23	P3 x P5	6.05	-0.30	-0.32	0.62	57	P8x P9	8.11	0.18	0.11	0.32
24	P3 x P6	6.04	-0.50	-0.66	1.29	58	P8x P10	7.05	-0.74	0.23	1.32
25	P3 x P7	5.76	-0.66	-0.04	1.14	59	P8x P11	9.44	0.22	-0.20	0.42
26	P3 x P8	6.36	-0.38	0.08	0.66	60	P8x P12	7.73	0.16	-0.51	0.53
27	P3 x P9	6.30	-0.40	0.20	0.73	61	P9x P10	6.62	0.30	0.27	0.58
28	P3 x P10	6.28	-0.65	-0.06	1.12	62	P9x P11	7.45	0.42	0.03	0.72

Table (Cont'd)

29	P3 x P11	8.30	0.10	-0.37	0.31	63	P9x P12	7.99	1.04	-0.08	1.80
30	P3 x P12	6.34	0.02	-0.24	0.08	64	P10x P11	8.42	0.42	0.31	0.82
31	P4 x P5	4.86	-0.55	0.34	1.06	65	P10x P12	7.29	0.01	-0.24	0.08
32	P4 x P6	6.53	-0.31	-0.15	0.55	66	P11x P12	9.61	0.47	0.23	0.86
33	P4 x P7	7.51	-0.11	0.00	0.19	67	Check-1	7.49	0.02	0.37	0.17
34	P4 x P8	7.90	0.00	0.00	0.01	68	Check-2	8.59	-0.12	-0.16	0.23

P1= KIT32, P2= SRSYN20, P3= SRSYN48, P4= FS67, P5= FS68, P6= FS59-4, P7= FS170, P8= FS60, P9= FS232, P10= F7215Q, P11=142-1-eQ, P12= CML144.

Table 6. 5 The first four AMMI selections of maize hybrids per environment

Environment	Mean	Standard deviation	Score	^1st	2nd	3rd	4th
HAR13	9.80	2.08	2.321	10	59	55	63
KUL13	8.78	1.92	1.057	10	1	66	59
KUL12	7.78	1.31	0.438	10	55	3	38
AMB13	7.12	1.33	0.116	66	3	29	38
AMB12	7.34	1.32	-0.65	10	55	68	66
HOL13	4.77	1.01	-1.111	10	66	59	3
HOL12	5.84	1.35	-2.171	59	17	50	7

^= Refer to Table 4 for codes of hybrids denoted in columns 1^{st} to 4^{th}.

6.4.4 GGE biplot analysis

The GGE biplots for grain yield of 17 QPM hybrids evaluated in seven environments are shown in Figures 6.1 to 6.4. The first principal component (PC1) scores were used as the *x*-axis and the second principal component (PC2) scores as the *y*-axis. In Figure 6.1, the percentage of GGE explained by PC1 was at 60.13% and PC2 at 11.43%. The biplot thus explained 71.56% of the total variation relative to G and GEI. Figure 6.1 is referred to as the vector view of the GGE biplot, in which the environments are connected with the biplot origin via the vectors. This view helps to understand the interrelationships among the environments. According to Yan and Kang (2003), the length of the vector, which approximates the standard deviation (SD) within each test environment, is a measure of the environment's ability to discriminate among hybrids. Accordingly the vector repressing the site, Holetta, during 2013 (HOL13) in Figure 6.1 is the shortest relative to the biplot size. Thus, genotypic differences that depend on projections onto vectors of such an environment may not

be reliable; they may only reflect noise. Because of this, the data belonging to HOL13 were not included when running the other consecutive biplot analyses. It was the result of unsuitable weather condition for maize performance during 2013 at Holetta. Considering the length of the environment vectors (Figure 6.1) and standard deviation within each environment (Table 6.5), Kulumsa during 2013 (KUL13) and Haramaya during 2013 (HAR13) sites had long vectors and large SD. These were the most discriminating environments. Conversely, HOL13 was the least discriminating. Other environments had more or less similar vector lengths or discriminating ability.

Figure 6.2 shows the polygon-view of the GGE biplot helpful in visualizing the “which-won-where” pattern of the multi-environment trial (MET) dataset. The principal component (PC) axis 1 explained 61.27% of total variation while PC2 explained 11.93% and, thus these two axes accounted for 73.2% of the total variation for grain yield. The polygon was drawn by connecting hybrids that were furthest from the biplot origin such that all hybrids were enclosed within the polygon. Perpendicular lines were then drawn to each side of the polygon starting from the biplot origin. Hybrid 3 (KIT32 x FS67), hybrid 10 (KIT32 x 142-1-eQ), hybrid 59 (FS60 x 142-1-eQ), hybrid 50 (FS59-4 x 142-1-eQ), hybrid 52 (FS170 x FS60) and hybrid 4 (KIT32 x FS68) located at the corner of the polygon are the vertex genotypes with the longest vectors. They were, therefore, among the most responsive genotypes to environments in their respective directions, while all other hybrids were less responsive. Hybrid 68 (check-2) located very close to the origin and with similar rank in all environments was the least responsive to the environments.

It is worth noting that the five environments (KUL13, KUL12, HAR13, AMB13 and AMB12) fell in the same sector, separated from the rest of the biplot by two perpendicular lines drawn to the respective sides of the polygon. Hybrid 10 involving cross KIT32 x 142-1-eQ is the highest-yielding vertex hybrid in all the test environments that share the sector with it. Hybrid 59 was the highest yielding that fell in a separate sector (Figure 6.2) at the Holetta site during 2012 growing season (HOL12). Apart from identifying the best hybrid in a given test environment, the polygon view also divides the test environments into groups. From Figure 6.2, two groups of environments are visible: KUL13, KUL12, HAR13, AMB13 and AMB12 in the hybrid 10 sector, and the other one environment (HOL12) in the hybrid 59 sector.

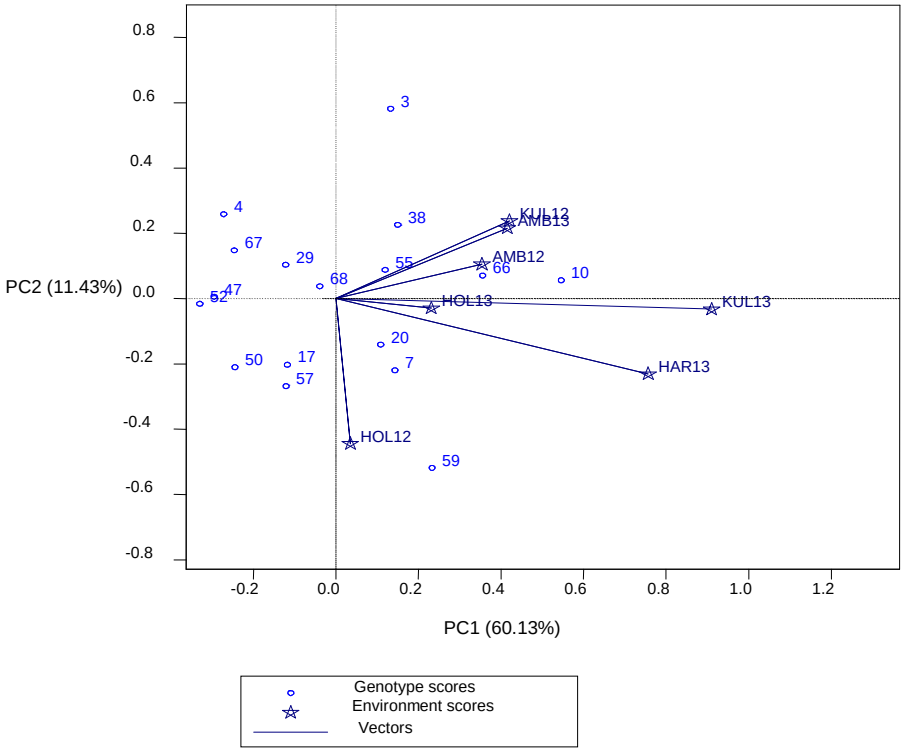

Figure 6. 1 The vector view of the GGE biplot based on environment-focused singular-value partitioning showing the discriminating power and representativeness of the test environments. See codes for environments in Table 6.1.

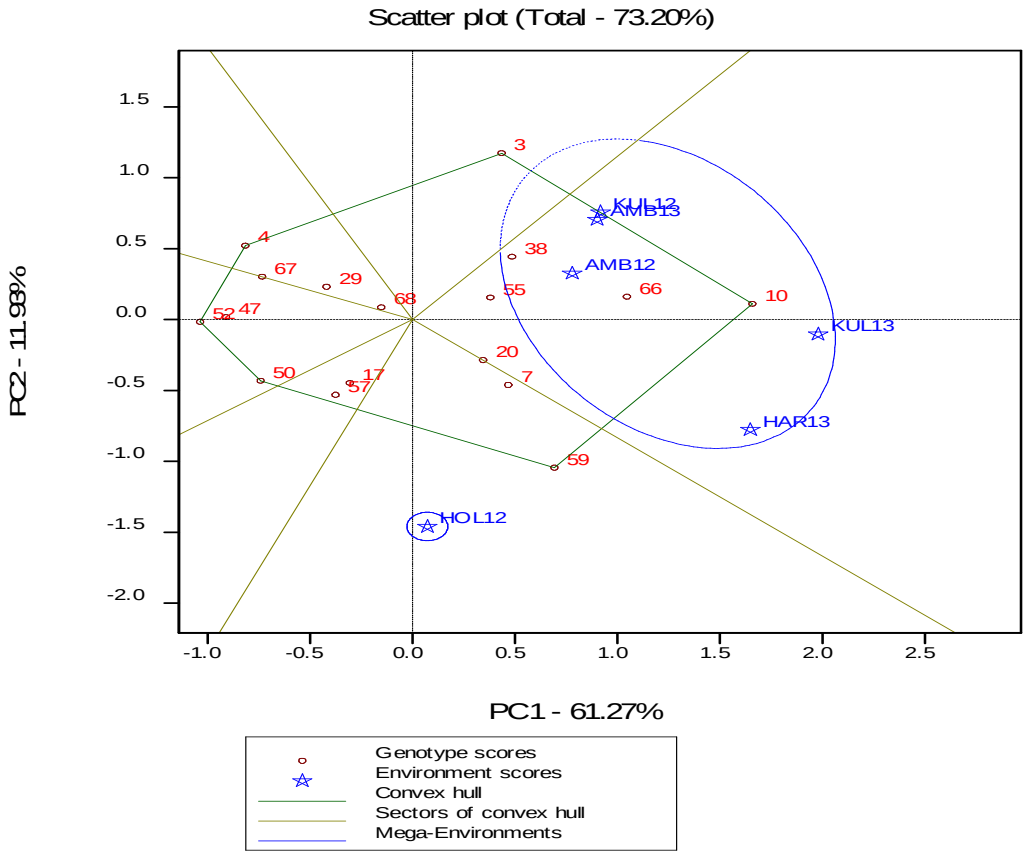

Figure 6. 2 The "which-won-where" view of the GGE biplot under each mega-environment constructed based on environment-centred and symmetrical singular-value partitioning. See codes of environments in Table 6.1 and genotypes in Table 6.4.

Figure 6.3 shows the GGE biplot based on the principal component analysis (using genotype-focused scaling). It provided the best means for recognizing mean performance and stability of the tested hybrids simultaneously. Visualization of the mean and stability of the hybrids was achieved by drawing an average environment coordinate (AEC) on the genotype-focused biplot. The ideal hybrid in Figure 6.3 is represented by the small circle located on the AEC abscissa and with an arrow pointing to it. It had the highest yield of all cultivars under evaluation and absolutely stable. Hence, hybrid 10 has close proximity to the ideal genotype, and therefore, most desirable of all the tested hybrids followed by hybrids 66 and 38. On the other hand, the small circle on the AEC axis, with an arrow pointing to it and drawn based on the environment-focused biplot, represents the ideal environment within a mega-environment (Figure 6.4). According to Yan and Kang (2003), an ideal test environment should be both discriminating and representative. Hence, in Figure 6.4, the ideal environment was used as the center of a set of concentric lines used to measure the distance between each environment and the ideal environment.

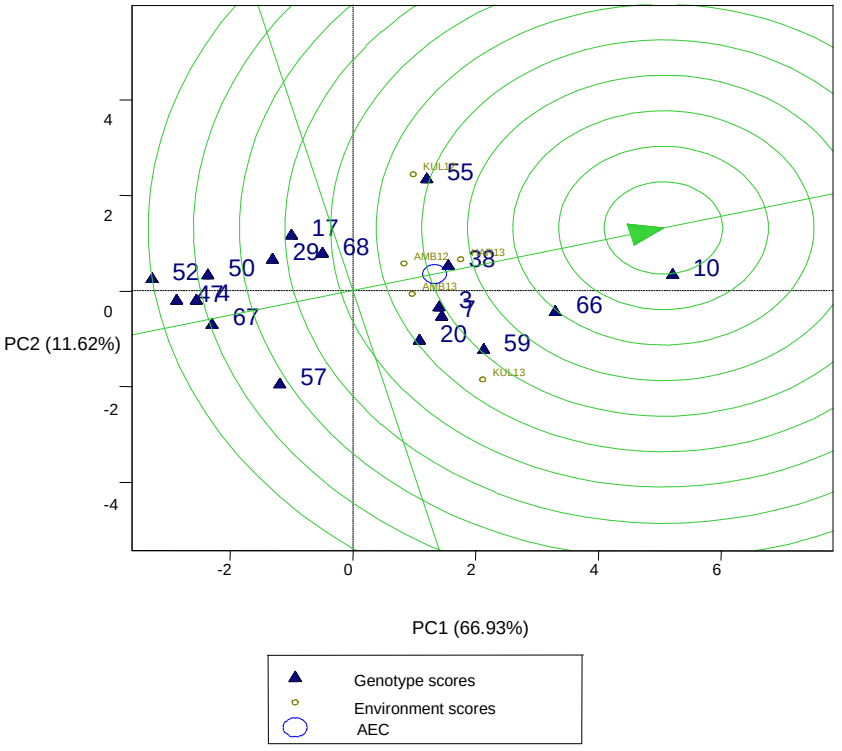

Figure 6. 3 Biplot showing comparison of all genotypes with the ideal genotype, constructed based on environment-centred and genotype-focused singular-value partitioning. See codes of environments in Table 6.1 and genotypes in Table 6.4.

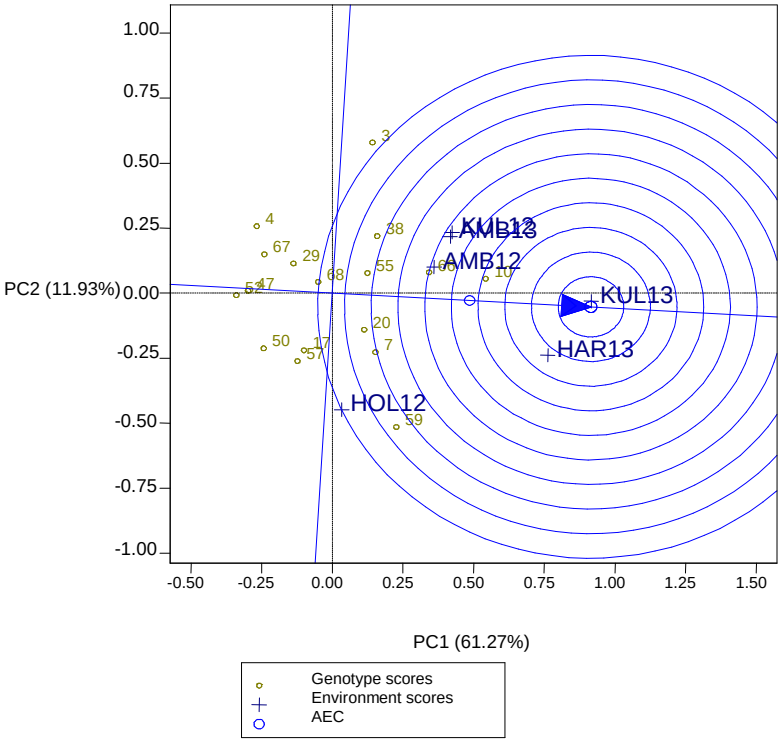

Figure 6. 4 Biplot for comparison of all environments with the ideal environment, constructed based on environment-centred and environment-focused singular-value partitioning. See codes of environments in Table 6.1 and genotypes in Table 6.4.

6.5 Discussion

The large sum of squares for environments in the combined analysis of variance indicated that the environments were diverse with large differences among environmental means resulting in significant yield variations among the experimental hybrids. This showed that environmental conditions accounted for most of the total variation and the presence of wide variation and potentials for maize production in the highland agro-ecologies. A large contribution of the environment affecting yield stability was reported in several studies (Taya et al., 2000; Yan and Kang, 2003; Sibiya et al., 2012; Kassa et al., 2013; Nzuve et al., 2013). As expected, the yield response of the hybrids was different in the seven test environments due to the highly significant G x E. Single crosses are more sensitive to environments than other types of crosses and open pollinated cultivars (Pixley and Bjarnason, 2002; Hallauer et al., 2010). Significant G×E interactions for grain yield in maize were also reported in

several studies (Makumbi, 2005; Menkir and Ayodele, 2005; Derera et al., 2008; Sibiya et al., 2012; Nzuve et al., 2013).

From the AMMI analysis, the first two IPCAs accounted for more than 50% of the G x E interaction and, therefore, the best-fit model for AMMI can be predicted by using the first two PCs to explain interpretable patterns of the interactions (Gauch and Zobel, 1997; Yan et al., 2000). The analysis also showed the first four selections of hybrids per environment with hybrid 10 (KIT32 x 142-1-eQ) being the best performer in five of the seven environments while other hybrids exhibited rank differences. The difference in ranking of the AMMI selected hybrids in the different environments also implied differential yield performance as a result of the significant genotype by environment interaction. This is also referred to as crossover GEI (Yan and Kang, 2003; Frashadfar et al., 2012; Nzuve et al., 2013) suggesting that different hybrids could be selected for the different highland areas. Hybrids such as KIT32 x FS170, KIT32 x 142-1-eQ, KIT32 x CML144, SRSYN20 x 142-1-eQ, SRSYN20 x CML144, FS67 x CML144, FS68 x CML144, FS170 x 142-1-eQ, FS170 x CML144 and FS232 x CML144 were thus identified for specific adaptations exhibiting high positive interactions (positive IPCA1 scores) with environments. The IPCA scores of genotypes in AMMI analysis are indicators of stability or adaptation over environments (Gauch and Zobel, 1996; Purchase, 1997). This genotypes showing high positive interaction in an environment has the ability to exploit the agro-ecological condition of the specific environment and therefore best suited to that environment (Kandus et al., 2010). However, to find which hybrid won in which environments and to display mega-environments, GGE analysis was the model of choice.

From the GGE biplot (Figure 6.1), it was possible to visualize the interrelationships among the environments. The cosine of the angle between the vectors of two environments approximates the correlation coefficient between them (Yan, 2002). The angles between all the six environments in this study were less than 90°, indicating high correlations amongst them. Whereas the angle between environment HOL12 and three other environments (KUL12, AMB12 and AMB13) was slightly greater than 90°; therefore, they should be slightly negatively correlated. Furthermore, Kulumsa and Ambo sites were identified as redundant test environments because they had

small angles revealing strong positive association across the two years evaluations. A single environment could have then been sufficed to obtain information on the hybrid genotypes to reduce the cost and increase breeding efficiency. It is possible that fewer but better test locations can provide equally or more informative data for cultivar evaluation (Yan and Kang, 2003).

Figure 6.2 shows that the rays of the biplot divided the plot into six sections, with five environments all appearing in one sector and the remaining one appearing in a different sector. These sectors had a different high yielding vertex hybrids each. Similar to AMMI analysis, this is an indication of a cross over GEI observed in the two years evaluation. The figure, thus, suggests that there are two highland maize mega-environments (shown in blue circles) for highland maize testing sites in Ethiopia. According to Yan et al. (2007), when different environments fall into different sectors, it implies that they have different high yielding cultivars for those sectors and it shows crossover G x E, suggesting that the test environments could be divided into mega-environments. Dividing the target environment into different mega-environments (Gauch and Zobel, 1997) and deploying different hybrids in different mega-environments is helpful to make use of GEI.

Cultivar evaluation within a mega-environment should, therefore, be based on both mean performance and stability to avoid the random GEI rather than trying to exploit it (Yan and Kang, 2003). This could be done by identifying the ideal genotype. Yan and Kang (2003) defined an ideal cultivar by two criteria: (1) it has the highest yield of the entire set of genotypes; and (2) it is absolutely stable, as indicated by small circle being located on the AEC abscissa and with an arrow pointing to it (Figure 6.3). Although such an ideal genotype exists rarely in reality, it can serve as a reference for cultivar evaluation. Therefore, as indicated in the AMMI analysis, hybrid 10 was identified as desirable of all the hybrids for being nearby to the ideal cultivar which fulfils the abovementioned criteria in the GGE analysis (Figure 6.3). As Figure 6.3 was used to identify the ideal genotype, Figure 6.4 was used in a similar way to indicate an ideal environment. According to Yan and Kang (2003), the main focus in Figure 6.4 was environment and the environment-focused scaling. This was shown using big size fonts for environment descriptors while small font sizes were used to show hybrid genotypes. This helped identify the best environment (KUL13) that had close

proximity to the ideal environment both in discriminating among the hybrids and representativeness as suitable environment in the present study.

6.6 Conclusion

The analysis of variance using the AMMI model for grain yield of the QPM hybrids revealed that mean squares of genotypes, environments, and GEI were all significant. The study further indicated that the QPM hybrids' yield performance was greatly influenced by the environment main effect while the GEI contributed the least to total variation. Both AMMI and GGE analyses showed that G x E influenced the ranking of the QPM genotypes in the different environments with some locations better for effective evaluations of genotypes than others. This was an indication of a crossover type of GEI. The QPM hybrid 10 (KIT32 x 142-1-eQ) was identified as a responsive genotype in AMMI analysis. Hybrid 10 followed by hybrid 66 (142-1-eQ x CML144) and hybrid 59 (FS60 x 142-1-eQ) with yield levels of 10.3, 9.6 and 9.4 t ha^{-1}, respectively, were selected as the best performers and hence desirable hybrids identified by both AMMI and GGE biplot analyses. Further testing of these QPM hybrids in multi-location and -season trials and promoting to three-way hybrids for wide adaptation could be the way forward to improve food and nutritional security of the highland farmers in Ethiopia. The GGE analysis divided the highland test environments into two mega-environments, viz., Holetta, which is a cooler maize growing environment, as one mega-environment, and the other group including Ambo, Kulumsa and Haremaya as the second mega-environment. However, these divisions should be confirmed after undertaking further METs. The present study also identified the locations which optimized genotype selection on the basis of their discriminating ability and representativeness. Thus, the Kulumsa site during 2013 season (KUL13) was the most suitable environment in discriminating the QPM hybrids and being a representative test environment.

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CHAPTER 7

Overview of research findings

7.1 Introduction and objectives of the study

Agro-ecological zones up to 2400 meters above sea level (masl) are considered suitable for maize cultivation in east Africa. The highland maize agro-ecological zone is one of the major maize growing areas which stands second in maize production next to the mid-altitude zone in the region. Maize yields, however, are significantly low in east and central African region (ECA) due, in part, to the lack of improved normal and nutritionally enhanced cultivars. In this agro-ecology, maize is produced and consumed by resource poor farmers who have limited access to protein sources, such as animal products. Consequently, majority of the farm community depend on conventional maize, which is deficient in two essential amino acids (lysine and tryptophan), as the main source of protein leading to malnutrition. A strategic research is required to develop nutritionally enhanced maize cultivars (QPM) to alleviate food insecurity and nutritional problem of millions of people who depend on this staple food crop. This chapter highlights the study objectives with subsequent summary of the core findings of each objective, and their implications toward high yielding and nutritionally enhanced maize breeding for tropical-highland environments.

This study was pursued with the following objectives in place:

- To assess the magnitude of maize production, the systems used and the farmers' production constraints, and their implications for the adoption of new maize cultivars in selected areas representing the Highland sub-humid agro-ecology of Ethiopia.
- To determine genetic variation among quality protein and normal maize inbred lines adapted to tropical-highlands using phenotypic traits for breeding and cultivar development.

- To examine genetic variability among quality protein and normal maize inbred lines adapted to tropical-highlands using SSR markers.
- To determine the level of heterosis and combining ability of elite QPM inbred lines and their hybrids for cultivar development for tropical-highland environments.
- To evaluate genotype-by-environment interaction and yield stability of QPM single-cross hybrids and to identify representative test and/or production environments.

7.2 Research findings in brief

Farmers' perceptions of maize production systems and breeding priorities, and their implications for the adoption of new varieties in selected areas of the highland Agro-Ecology of Ethiopia

A participatory rural appraisal (PRA) was conducted using semi-structured interview and focus group discussions involving 160 experienced maize farmers sampled from four districts of two administrative zones. The results of this study revealed:

- Maize is increasingly being grown to the highlands of Ethiopia where it used to be considered a homestead crop of minor importance.
- Very few adopted highland maize cultivars were available in the study area. Instead, a two-decade old cultivar, 'BH660', originally released for the mid-altitude agro-ecology, has been widely adopted.
- Grain yield followed by maturity and plant height were the most important traits in maize cultivar selection by farmers.
- Major production constraints identified were limited access to inputs, late on-set and inadequate rainfall, and inadequate extension service.
- Recommendations for future institutional directions to facilitate the adoption of new maize cultivars should focus on reducing input costs by making sustainable improvements in infrastructure, transportation, credit availability, and markets.

Phenotypic variation among quality protein and normal maize inbred lines adapted to Tropical-highlands

Thirty-six maize inbred lines (30 QPM and six normal) adapted to Tropical-highlands were phenotyped under alpha lattice design (0, 1) with two replications using 18 traits at two locations in Ethiopia. The following outputs were obtained:

- Significant phenotypic variation observed among inbred lines for all measured traits.
- Principal component and unweighted paired group method using arithmetic averages (UPGMA) cluster analyses revealed the presence of three distinct cluster groups.
- Seven inbred lines [KIT32Q, 142-1eQ, SRSYN20Q, FS67(BC_2), FS170Q, FS60, and F7215] with complementary phenotypic traits and relatively better yield performance were selected for making crosses for further genetic analysis and breeding.
- Therefore, the phenotypic traits were found useful for primary characterization of the maize inbred lines, although correlating variation at the molecular level to phenotypic diversity is an important prerequisite for future breeding and conservation plans.

Genetic purity and patterns of relationships among highland adapted quality protein and normal maize inbred lines using microsatellite markers

The 36 phenotyped maize inbred lines (30 QPM and six normal) adapted to Tropical-highlands were also genotyped using 25 polymorphic SSR markers and the results were exhibited as follows:

- 55.6% of the inbred lines showed higher than the expected 6.25% mean residual heterogeneity after five generations of selfing.
- Nearly 98% of the Roger's pairwise comparisons had a genetic distance between 0.30 and 0.78, which indicates large genetic differences among most lines.

- The model-based population structure, principal coordinate and neighbor-joining cluster analyses revealed the presence of 3 groups, which is generally consistent with pedigree information and partly with heterotic grouping.
- Analysis of molecular variance indicated that difference among heterotic groups explained 8.6 to 15.4% of the total SSR variance, indicating the presence of moderate to great genetic differentiation among heterotic groups.

Combining ability and heterosis analyses of quality protein maize genotypes adapted to Tropical-highlands

Sixty-six QPM experimental hybrids derived from a 12-parent diallel crosses and two commercial hybrid checks were field evaluated for grain yield and related agronomic traits at three sites representing highland sub-humid agro-ecology in Ethiopia. The findings are presented below.

- There was significant variation among the hybrids in terms of all measured traits, and generally, the new QPM single-cross hybrids performed better than the checks.
- Inbred lines KIT32, FS60 and 142-1-EQ were found good general combiners for grain yield, while FS60 exhibited good general combining ability for days to anthesis, plant and ear height.
- The best crosses identified with desirable specific combining ability and grain yields of 9.6 and 8.8 t ha^{-1} were KIT32 x 142-1-EQ and SRSYN20 x FS60, respectively.

Genotype-by-environment interaction and yield stability of quality protein maize hybrids adapted to tropical highlands

A seven-environment yield data collected from the 66 single-cross hybrids evaluated for combining ability and heterosis in the previous experiment were subjected to G x E interaction (GEI) and yield stability analyses using AMMI and GGE biplot methods. The results indicated:

- The presence of a crossover type of GEI.
- Hybrid 10 (KIT32 x 142-1-eQ) followed by hybrid 66 (142-1-eQ x CML144) and hybrid 59 (FS60 x 142-1-eQ) with yield levels of 10.3, 9.6 and 9.4 t ha^{-1}, respectively, were selected as the best performers and hence desirable hybrids identified by both AMMI and GGE biplot analyses.
- The GGE analysis divided the highland test environments into two mega-environments.
- The Kulumsa site during 2013 season (KUL13) was the most suitable environment in discriminating among the QPM hybrids and being a representative test environment.

7.3 Implications of findings for QPM breeding

- **PRA study:**- In the past, small cereal crops such as tef, wheat and barley were the most dominant food crops in the Highlands of Ethiopia, where maize used to be a homestead crop of minor importance. To date, maize has become the most important cereal crop of the highland farmers in Ethiopia for various reasons. Although maize is being rapidly adopted in the highland agro-ecology, the few highland cultivars that have been released have not been adopted by most highland farmers. This could be partly due to the fact that many maize cultivars have been released in Ethiopia without including inputs from the farmers in the process of developing of the cultivars. Therefore, the overall issues that need to be addressed through breeding interventions included developing a suitable maize cultivar which incorporate the trait preferences of farmers such as high yield, intermediate plant statures and maturity periods, flint textured grains, field and storage pest resistance/tolerance, and enhanced nutritional quality. Some of these traits are already known to be negatively correlated. The breeding process is thus expected to be more complex than usual.

- **Phenotypic characterization:**- Understanding the genetic diversity and relationships that may exist in maize germplasm is an important and a priority task for breeders. The conservation and utilization of useful genetic diversity is fundamental for sustainable genetic gain of economically desirable traits, and can

help prevent losses due to biotic and abiotic stresses. The broad range of phenotypic variation detected in this study implied great potential for the development of improved open-pollinating varieties, inbred lines and hybrids of QPM adapted to the highland agro-ecologies. The ranges in days to anthesis, for example, suggest the possibility to develop cultivars with different maturity groups. Furthermore, the cluster analysis allowed classification of the maize inbred lines into three groups with distinctive morpho-agronomic traits. This will be useful to set breeding and conservation strategies for QPM germplasm adapted to the highland environments.

- **Genotyping:**- Maize breeders are constantly developing genetically complementary inbred lines to develop new hybrids with increased hybrid vigour. Maintenance of the genetic purity and interrelationships among the QPM and non-QPM inbred lines is thus an important quality control function in maize breeding programs. High level of heterogeneity that can possibly change the uniformity and performance of hybrids was observed in some of the maize inbred lines used in the present study. Consequently, additional generations of purification for all lines with higher proportion of heterogeneity are recommended.

The SSR markers used in this study were able to classify about half of the inbred lines into the three putative heterotic groups defined by breeders (Kitale, Ecuador, and Pool9A). Whereas, the other half of the inbreds were spread throughout the three genetic clusters indicating the disruption of the original heterotic system during the conversion process of the inbreds into QPM. The conversions had been carried out using phenotypic selections without monitoring the genetic backgrounds. Consequently, recombinants were selected and very small portion of the genome of the recurrent parents was recovered. This suggests the use of marker assisted backcross or marker assisted selection (MAS) in the future. The existence of greater variation within than between heterotic groups was also noted in the current study. This could be attributed, in part, to the mix up of germplasm during the conversion process which, therefore, necessitates the establishment of new heterotic groups.

- **Combining ability and heterosis studies:**- The results of this study indicated the presence of high variability for grain yield and related agronomic traits, and thus the possibility of selection among the QPM hybrids that are adapted to the highlands of Ethiopia and other similar agro-ecologies in east African countries. The significance of GCA and SCA mean squares at each and across environments indicates the importance of genes having largely additive effects as well as genes having dominance and epistatic effects, respectively. This suggests the importance of considering both the average performance of a line in hybrid combinations and the specific hybrid combinations in the process of cultivar development. Inbred lines KIT32, FS60 and 142-1-EQ were good general combiners for grain yield; while FS60 exhibited good general combining ability for days to anthesis, plant and ear height. Hence, inbred line FS60 could complement KIT32 and 142-1-EQ for QPM hybrid development. Hybrids: KIT32 x 142-1-EQ, SRSYN20 x FS60, and FS67 x CML144 were the best crosses with favourable SCA estimates for grain yield. These hybrids could be used as potential single-cross testers for the formation of three-way QPM hybrids. The crosses with significant SCA effects had also relatively high percent mid-parent heterosis and good hybrid *per se* performances. To exploit hybrid vigour, therefore, *per se* performance, SCA effect and the extent of heterosis in hybrids were important parameters and should be considered simultaneously during selection.

- **Genotype-by-environment and stability analyses:**- Although the highland maize breeding program in Ethiopia has developed a number of promising QPM and non-QPM single cross hybrids, there is very limited information on the G x E interaction, appropriate choice of test environments and yield stability of the hybrids developed. The AMMI and GGE biplot analyses of the data from the Multi-environment trials in this study helped the selection of superior hybrids and suitable test environments to make appropriate breeding and evaluation plans in the future. Particularly the GGE analysis made possible the division of the highland test environments into two mega-environments which, in turn, simplified the choice of appropriate test environments and desirable genotypes within a mega-environment. These findings, therefore, can un-doubtfully reduce cost of genotype evaluation and improve breeding efficiency of the highland program.