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GENETIC VARIABILITY AND COMBINING ABILITY OF HIGHLAND QUALITY PROTEIN MAIZE (*Zea mays* L.) INBRED LINES

A THESIS SUBMITTED TO COLLEGE OF NATURAL SCIENCES IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN BIOLOGY (APPLIED GENETICS)

BY

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LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of variance
AFLP	Amplified fragment length poly-morphism
BecA	Biosciences for Eastern and Central Africa
CSA	Central Statistical Authority
CML	CIMMYT maize line
CLA	Cluster analysis
CV	Coefficient of variation
EM	Endosperm modification
F1	First filial generation
fl2	Floury-2 allele
GCA	General Combining Ability
GBS	Genotyping by sequencing
IITA	International Institute for Tropical Agriculture
CIMMYT	International Maize and Wheat Improvement Centre
LxT	Line by Tester
NARS	National Agricultural Research System
O2	Opaque-2 allele
PCA	Principal component analysis
PROC	Procedure
PCs	Principal components
QPM	Quality protein maize.

QTL	Quantitative trait loci
RFLP	Restriction fragment length polymorphism
SSR	Simple sequence repeats
SNP	Single nucleotide polymorphism
SCA	Specific Combining Ability
SE	Standard Error
SE (d)	Standard Error of differences
SAS	Statistical analysis software
TRP	Tryptophan
UPGMA	Unweighted pair group method with arithmetic averages

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Abstract

Genetic variability and combining ability of highland quality protein maize (Zea mays) inbred lines

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Information on genetic diversity and combining ability of quality protein maize (QPM) inbreds is important to analyze their potential in forming superior hybrids and to assess the gene action involved in various characters. This study was conducted to assess genetic variability and relationships among elite QPM inbred lines using morphological data and SNP markers and estimate their combining ability to determine their usefulness in developing superior QPM hybrid combinations as well as heterosis. Twenty six QPM inbred lines were assessed for morpho-agronomic traits at two locations in Ethiopia in Alpha lattice design twice replicated during 2014 main season as Set-I experiment. These inbred lines were genotyped using single nucleotide polymorphism (SNP) at BecA. SNP markers and morphological distance estimates showed moderately distance among the inbred lines studied. Cluster analysis based on the two distance measures grouped the 23 parental lines differently. Based on morphological data, lines were clustered into five distinctive groups. Cluster analysis based on SNP marker produced four clusters which showed association of inbred lines that more reliably and efficiently concurred relationship with pedigree data. According to diversity study, promising inbred line was identified with superior characteristics (L20). The information generated from this study is important in the development of maize varieties with high grain yield and nutritional enhanced quality traits. In a spate Set-II experiment24 of the QPM inbred lines were crossed in line x tester mating design to estimate combining ability and gene action of grain yield and its components. A total of 48 hybrids and two checks were evaluated in alpha-lattice design *replicated twice at APPRC, HARC, KARC and AARC in 2014 main season. Data on grain yield, agronomic traits and disease were recorded. The highest GY was 8.74t ha*$^{-1}$ *(L8 x T1.) Mean squares due to GCA and SCA effect were also significant except GCA for ASI, RL EPO, which indicate both additive and non-additive gene action was important. However, in all traits, the proportion of GCA was higher than SCA. Parents L1, L17 and L18 are best for grain yield and most traits as well. Best cross combinations were also identified as breeding material. Morphological distance was of low important in predicting heterosis of grain yield.*

Key words: Inbred lines, testers, general combining ability(GCA), specific combining ability (SCA), heterosis.

1. Introduction

Maize (*Zea mays* L. 2n=20) is an important cereal crop of the world, belonging to the tribe *Maydeae* of the grass family poaceae. It has great worldwide significance as human food, animal feed and as a source of industrial products. Maize also occupies an important position in world economy and trades as a food, feed and industrial grain crop (Vasal, 2000). Apart from manufacturing of mixed feed, maize is a row material to produce corn starch, corn oil and corn syrups (Troyer, 2004; Selvi *et al.,* 2013). It is considered as the first most-produced cereal followed by rice and wheat, but in terms of dietary intake, it is third after rice and wheat (FAOSTAT, 2014). It is an important source of protein accounting for up to 60% of the daily human protein supply in sub-Saharan Africa (Musila *et al*., 2010). The United States produces almost half of the worlds maize production and other top maize producing countries are China, Brazil, Mexico, Indonesia, India, France and South Africa (Nadagoud, 2008). It is estimated that in 2012, the total world production of maize was about 875.2 million tons, with the United States, China, and Brazil harvesting 31%, 24%, and 8% of the total production, respectively (Peter, *et al*., 2014).

It is believed that maize was introduced to Ethiopia in the 16th or 17th century (Haffnagel, 1961). Though it is introduced early, maize research in Ethiopia started in 1950s with the evaluation of introduced materials focusing mainly on grain yield, early maturity, decreased plant height, lower ear placement and resistance to major biotic and abiotic stresses (Kebede *et al*., 1993). The production of maize spears out all over the country from moisture stress areas to high rainfall areas and from lowlands to the highlands (Kebede *et al*., 1993). Highland Maize Breeding Program was established aimed at developing and popularizing improved Highland maize cultivars, and enhancing their crop management technological packages in collaboration with the International Maize and Wheat Improvement Center (CIMMYT), in 1998.

In Ethiopia, Maize is one of the important cereal crop grown for food in the country, and is the first in total production (more than 72 million tons) and yield per unit area (of about 3.4 tons per hectare), and second in area coverage (1.1 million ha) among all the cereal crops in 2014/15 cropping season (CSA, 2015). Maize continues to be a significant contributor to the economic and social development of Ethiopia. It is the crop with the largest small holder coverage of 8.0 million households as compared to 5.8 million for teff and 4.2 million for wheat, indicating that maize is a critical to small holder livelihoods of the country, IFPRI (2010).

The protein of maize and of most cereals is deficient in essential amino acids lysine and tryptophan. Normal maize lacks lysine and tryptophan, which are vital in the synthesis of proteins by the body (Vivek *et al*., 2008). Due to low content of essential amino acids in grain endosperm, the biological value is very low, which is the main limiting factor of common maize in feeding of mono-gastric animals, including human beings (Denic *et al*., 2012).

The net protein utilization of normal maize is low, with a biological nutritional value that is equivalent to 40% of that of milk (Bressani, 1991) and therefore needs to be eaten with complementary protein sources such as legumes or animal products. The need to improve this deficiency in maize protein has been recognized for a long time (Osborne and Mendel, 1914).

The main aim of maize breeding is to develop new inbred lines and hybrids that will outperform the existing hybrids with respect to a number of characteristics. For attaining this purpose, particular attention is paid to grain yield as the most economically important trait in maize (Vasic *et al*., 2001). Grain yield is a complex quantitative trait which is affected by a number of its components (Zivanovic *et al.*, 2007; Bovanski *et al.*, 2009). Moreover, advances in maize genomics, breeding and production have important role on the lives of a large proportion of the world's population (Xu and Crouch, 2008).

While developing new hybrids with high genetic potential for yield and other positive features that exceed the existing commercial hybrids is the primary goal of maize breeding (Secanski *et al.*, 2005), the identification of parental inbred lines that can be used for breeding superior hybrids is the most costly and time consuming phase in maize hybrid development (Betran *et al.*, 2003). To reduce cost and increase breeding efficiency researchers have been seeking ways and means to identify parental combination with increased yield before making extensive crosses. So far the level of genetic diversity of parents and their combining abilities have been used to predict heterosis at parental generation.

Characterization of crop genetic diversity aids in efficiently exploiting the allelic variation for genetic improvement of economically desirable traits (Goodman and Brown, 1988). Genetic diversity and relationship among breeding materials is useful for the development of new maize inbred lines, forming heterotic groups of maize inbred lines, choosing appropriate testers and identification of promising combinations for exploitation of heterosis (Goodman and Brown, 1988; Melchinger, 1999; Mohammadi and Prasanna, 2003; Xia *et al.*, 2005; Legesse *et al.*, 2007). Established methods for characterizing and measuring genetic diversity have relied on morphological, physiological and cytological markers as well as biometric analyses of quantitative and qualitative traits, heterosis, or the segregation variance in crosses (Melchinger 1999). Applications of molecular markers have also led to new insights into the patterns of genetic diversity in maize, including landraces as well as wild relatives especially teosintes in Latin America (Prasanna, 2012). Genetic diversity analysis at DNA level in plant species is made possible through use of molecular markers (Melchinger and Gumber, 1998). Environmental factors are not capable of influencing DNA markers thus they reflect the actual level of genetic difference existing among the genotypes (Westman and Kresovich, 1997: Legesse *et al*, 2006). Maize breeding potential is increased efficiently by characterizing a large number of inbred lines and assign to heterotic group using molecular markers (Choukan *et al*, 2006: Reif *et al*, 2003).

Shortage of protein is a major problem in the world (WHO 2007). Households' poor access to nutritious food is an underlying cause of malnutrition particularly, in rural areas where diets are often based on one staple. The use of QPM in the highlands of Ethiopia in general can contribute to alleviation leading to healthier population with higher productivity and high income. Because the combining ability effect is an indicator of the relative value of the population in terms of frequency of favourable genes and of its divergence, as compared to the other parents included in the analysis (Viana and Matta, 2003; Viana 2000), GCA and SCA effects are rigorously determined in hybrid breeding program as a means to select parents that potentially give higher heterotic advantage upon F_1 production. Moreover, the GCA and SCA variances provide estimation for additive and non-additive gene actions, respectively (Falconer, 1967). Therefore, estimating combining ability is essential statistical tool that helps to predict the performance of the progenies of the inbred lines in hybrid combinations and understand the nature of gene action involved in the expression of quantitative traits. Adequate information on the new elite highland adapted QPM inbred lines including their genetic diversity, combining ability and magnitude of heterosis are, therefore needed for best hybrid development.

2. LITRETURE REVIEW

2.1. Maize and its socio-economic importance

Maize (*Zea mays* L.) is the world's third most widely grown cereal commercially valued economic crop of global importance widely used in poultry and cereal food industries (Ayisi and Poswall, 1997; Sharma and Misra, 2011). Over 100 million people relay on maize as a staple food in Africa (Magenya *et al*., 2009), making it an essential food crop for global food security. In most of Africa's rural economies, at least 85% of maize is used for human consumption, as compared with the developed world where most maize grain is used for animal feed and manufacturing industries (Oluwafemi *et al*., 2008; Stevens, 2008). It is also an important source of food and nutritional security for millions of people in the developing world, especially in sub-Saharan Africa and Latin America (Shiferaw *et al*. 2011). It provides raw materials for starch, gluten, corn oil, corn syrup, sugar, corn meal and com flour (Selvi *et al*., 2013). However, the average yield per hectare of maize in Africa is the lowest in the world and consequently, food shortages are a perpetual problem in most Sub-Saharan countries (Magenya *et al*., 2008).

Anon (2007) highlighted that about 28% of maize produced is used for food purpose, about 11% as livestock feed, 48% as poultry feed, 12% in wet milling industry (e.g., starch and oil production) and 1% as seed. Farmers harvest some 25 million ha, producing about 35 million tons of maize grain every year in Africa (Abdalla 2013). This accounts for 40% of the region's cereal production. Nine-tenths of the grain goes directly for human consumption (Bänziger and Diallo, 2001). Of the 23 countries in the world with the highest per capita consumption of maize as food, 16 are in sub-Saharan Africa (Abdalla 2013). Maize provides 50% of the calories in diets in southern Africa, 30% in Eastern Africa, and about 15% in West and Central Africa (Bänziger and Diallo, 2001). The Economic Community of West African States (ECOWAS) which covers 17% of the area of the continent is one of the economic communities most populous in Africa with a regional population totaling about 300 million in 2008, of which 57% live in rural areas. Maize production in this region has progressively increased in the last 5 years with an important contribution of Nigeria about 50%. When Nigeria is excluded the average production over the last five years indicate that Burkina Faso is third top country in maize production after Ghana and Mali (FAOSTAT, 2008-2012).

Ethiopia is the fourth largest maize producing country in Africa, and first in the East African region (FAO, 2012). Currently, maize is the cheapest source of calorie intake in Ethiopia, providing 20.6 % of per capita calorie intake nationally (IFPRI, 2010). It is one of the important cereal crop grown for food in the country, and is the first in total production (more than 72 million tons) and yield per unit area (of about 3.4 tons per hectare), and second in area coverage (1.1 million ha) among all the cereal crops in 2014/15 cropping season (CSA, 2015). Maize continues to be a significant contributor to the economic and social development of Ethiopia. It is the crop with the largest smallholder coverage of 8.0 million households as compared to 5.8 million for teff and 4.2 million for wheat, indicating that maize is a critical to smallholder livelihoods of the country, IFPRI (2010).

2.2. Highland maize in Ethiopia

In Ethiopia, highland maize production is estimated to cover around 20% of the total land allotted to maize production and 30% of small-scale farmers in the area depend on it for their livings (Twumasi *et al.*, 2002 and Legesse *et al.*, 2009). Farmers in the highlands of Ethiopia have been producing locally adopted maize varieties for more than 300 years (Yoseph 2005). Maize is increasingly grown in the Highlands of Ethiopia where it has been a minor crop in the past. Farmers in the highland zones of Ethiopia with an elevation of 1,800 m above sea level generally grow tropical-highland adapted local varieties originally sourced from the mid- and low-altitude areas (Demissew *et al*., 2014). These varieties are often tall and have long maturity period, which make them vulnerable to frost damage and lodging (Twumasi *et al*., 2002; Demissew *et al*., 2013). In addition most of the varieties grown in the region are low yielding local cultivars (Gudeta *et al*.,2011) with susceptibility to various leaf diseases such as northern leaf blight (NLB), gray leaf spot (GLS) and common rust (CLR), and are very tall in plant and ear height that result into root and stalk lodging, and also are late in physiological maturity (Legesse *et al*., 2009).

To meet the needs of increasing maize production and due to the socio-economic importance of maize in the highland zones of Ethiopia, the Ethiopian Institute of Agricultural Research (EIAR) and the International Maize and Wheat Improvement Center (CIMMYT) initiated a collaborative research project in Ethiopia to develop varieties adapted to the highland zones of east and central African (ECA) countries in 1998 (Twumasi *et al*., 2002). Ethiopian highland maize breeding program is based at Ambo Plant Protection Research Center of the Ethiopian Institute of Agricultural Research and established with the aims of developing and popularizing improved QPM and normal Highland maize cultivars, and enhancing their crop management technological packages. In line with this, the breeding program released five superior Highland maize hybrids between 1999 to 2011 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’ (Demissew 2014). Over 5.8 million hectares of potential suitable land was identified for the highland maize hybrids in the country (Demeke *et al*., 2012).

2.3. Quality Protein Maize (QPM)

Maize is globally important crop and preferred staple food for more than 1 billion people in Sub-Saharan Africa and Latin America, where animal source of protein is not affordable by the common people (prasanna *et al*., 2001). In Africa, maize supplies at least one fifth of total daily calories consumed and accounts for 17 to 60% of people's total daily protein supply in 12 countries, as estimated by FAO food balance sheets (Krivanek *et al*., 2007). Children being weaned, sick children, sick adults, and everyone, when crop production is low are even more dependent on maize as the major source of dietary protein (Vivek *et al*., 2008). Protein-containing foods are necessary for the rapid growth of children (Millward and Rivers, 1989), and in several countries maize is a primary weaning food for babies. Such dependence on maize as a protein source puts people at risk for dietary protein deficiency because maize protein (as most cereal protein) is deficient in two essential amino acids lysine and tryptophan (Ahenkora *et al*., 1999). Malnutrition is a persistent problem in Africa, especially in rural areas where the poor largely depend on staples and have limited access to a diverse diet (Girma *et al*., 2010).

Since long maize is known to be nutritionally poor (Osborne and Mendel, 1914) and agricultural scientists have long had an interest in improving the protein quality of plants (Ignjatovi *et al*., 2008). Maize, one of the most important staple foods in the developing world (Bressani, 1991), is relatively low in protein content, generally about 10% (Gupta *et al*., 2009). Bulk of the proteins in a mature maize kernel is in the endosperm and germ; but, the germ protein is superior in both quantity and quality (Wilson 1986). In normal maize genotypes, zeins usually account for 50 to 70% of the endosperm protein and are characterized by a high content of glutamine, leucine and proline which are devoid of lysine and tryptophan (Wilson 1986, Nelson, 1969). Roughly half of that protein contains almost no lysine or tryptophan, two amino acids essential for building proteins in humans and mono-gastric animals (Prasanna *et al.*, 2001; Ignjatovi *et al*., 2008). All protein fractions other than zeins are balanced in amino acid content and are quite rich in lysine and tryptophan (Wilson 1986). Suppression of lysine-deficient zein fraction without drastically altering the contribution of other fractions could be, thus, seen as a feasible approach to bring about improvements in the amino acid balance in maize grain (Mertz *et al*., 1964).

Protein content in maize kernel is constituent of endosperm protein which is low in quality while germ protein is high in quality and low in amount however, maize kernel contain endosperm and contributes 80% of the total grain protein (Zuber and Helm, 1972). Approximately 50-60% of the soluble proteins found in the endosperm of normal maize are zein proteins. Zein proteins, particularly the alpha zeins, are poor in lysine and tryptophan and contain lysine levels of, 0.1 g/100 g in normal maize (Gibbon and Larkins, 2005). Alpha-zeins are the most abundant proteins in the grain endosperm of maize (Prasanna *et al*., 2001; Gibbon and Larkins, 2005) but limited in lysine and tryptophan.

The discovery of the biochemical effects of mutant alleles *o2* (*opaque* 2) and floury-2 (fl2) improved protein quality of maize kernel by the researchers at Purdue University (Mertz *et al*., 1964; Nelson *et al*., 1965). Homogenous recessive *o2* allele with two copies of the mutation carries significantly higher lysine content (+69%) compared to normal maize grain (Mertz *et al*., 1964). The presence of the *o2* allele in a homozygous state results in a decrease in the production of alpha zein protein enzymes that are responsible for degrading free lysine. In turn, there is a corresponding increase in non-zein proteins such as glutelins. Glutelins confer higher lysine and tryptophan levels (Gibbon and Larkins, 2005) and contain 2g/100g or more of lysine. This is more than twice the amount found in the zein proteins of normal maize. The mutants (*o2* and *fl2*) alter amino acid profile and composition of endosperm protein in maize kernel and results in two-fold increase in the levels of lysine and tryptophan compared to normal maize genotypes. The mutants derive their name from soft, and floury opaque endosperm, respectively (Mertz 1992 and Villegas *et al*., 1992). Effective and useful utilization of the *o2* mutant for protein quality enhancement were emphasized by researchers in most of breeding programs (Glover, 1992 and Villegas *et al*., 1992). In recent QPM research, it has been concluded that genotypes with *o2* mutations also exhibit a corresponding increase in tryptophan content in maize kernel. Ultimately the enhanced concentration of these two essential amino acids in fact doubles the biological value of maize protein (Bressani, 1992). The biological value of normal maize is about 45% and of QPM about 80%, which is similar to the biological value of milk 90% (FAO, 1992). Besides biological value, QPM has additional improved nutritional advantages over normal maize. As the result of increased concentration of tryptophan QPM has higher concentration of niacin (B3). Also, because of reduced leucine concentration absorption of potassium are improved (Graham *et al*., 1980).

2.4.Genetics and breeding of QPM

Breeding of QPM muddle through the manipulation of three distinct genetic systems (Krivanek *et al*., 2007). The recessive mutant allele of the o2 gene is the first core component (Vasal, 2001; Villegas *et al*., 1992). Characterization of this gene has suggested it as encoding a transcription factor (a gene regulator) involved in zein synthesis (Schmidt *et al*., 1990). The o2 mutant causes a decrease in zeins and resulted an increase in non-zein proteins (Vasal, 2002; Gibbon and Larkins, 2005).

Kernel hardness is the second distinct genetic system managed within QPM breeding, comprised of the alleles for endosperm hardness called modifier genes responsible for conversion of the soft to a hard endosperm (Vasal, 2002). The first report by Paez *et al.,* (1969) on endosperm modification in *o2* kernels was 50% translucent and 50% opaque. Subsequently, modified o2 kernels with different proportions of translucent and opaque have been studied by others (Bjarnason *et al.*, 1976; Lodha *et al.*, 1976). These endosperm modifiers along with the *o2* mutant allele can be used as a rapid and low cost method whereby light is projected through the vitreous grains or blocked by the opaque grains, respectively (Vasal, 2001; Krivanek *et al.*, 2007). Opaqueness is rated on a scale from 1 (completely hard) to 5 (soft/opaque). According to their observation, all grains with a score of 2 to 5 are homozygous for the *o2* allele, but only grains with score 2 - 3 have significantly modified hard endosperm to be selected as QPM grains (Vasal *et al.*, 1997a; Lopes *et al.*, 1995 and Krivanek *et al.*, 2007).

The third and more critical in QPM genetics is the amino acid modifier system. This comprises of a distinct set of amino acid modifier genes, which affect the relative levels of lysine and tryptophan content in the grain endosperm (Villegas *et al.*, 1992 and Krivanek *et al.*, 2007). Several breeding programs throughout the world incorporated the *o2* mutation mainly focusing on conversion of normal endosperm inbred lines to *o2* versions using a direct backcross approach since it posses nutritional benefits (Gevers, 1995; Prasanna *et al.*, 2001). Negative effects associated with *o2* mutation appeared such as reduction in grain yield, low grain density, soft and chalky kernel phenotype, susceptibility to ear rot, lower rate of germination and kernel breakage (Bjarnason and Vasal, 1992; Villegas *et al.*, 1992; Vasal, 2001; Prasanna *et al.*, 2001). Genetic engineering was not used during QPM development it is rather a result of conventional plant breeding (Pixley and Bjarnason, 1993). Selection specifically for hard endosperm modification was swiftly integrated into *o2* breeding to overcome these negative effects.

QPM breeding programs at international maize and wheat improvement center (CIMMYT) were focused on conversion of subtropical and tropical lowland adapted, normal endosperm populations to *o2* versions through a backcross selection technique, with emphasis on accumulation of hard endosperm phenotype, maintaining protein quality with increasing yield and resistance to ear rot (Bjarnason and Vasal, 1992; Villegas *et al*., 1992; Prasanna *et al*., 2001 and Vasal, 2001). Inbred lines developed by CIMMYT with enhanced lysine and tryptophan content but without the negative soft endosperm phenotype were termed as Quality Protein Maize (QPM) (Bjarnason and Vasal, 1992).

Institutions other than CIMMYT that continued persistently to improve the protein quality were the University of Kwazulu-Natal, South Africa and the Crow's Hybrid Seed Company at Milford, Illinois, USA (Prasanna *et al.*, 2000 and Vasal, 2001). QPM hybrid programs at CIMMYT were started in 1985, in response to increasing interests in hybrids among the national programs especially in developing countries (Vasal, 1992; Vasal *et al*., 1993b; Bjarnason and Vasal, 2001). Many benefits favoring QPM hybrids than open pollinated varieties include i) increasing yield performance through exploitation of heterosis; ii) facilitating maintenance of the seed purity of inbred progenitors with respect to agronomic traits, the genetic modifiers and the protein quality; iii) reduce dependence on laboratory facilities for monitoring the protein quality provided the lines are fixed and kept genetically pure; iv) the hybrids will exhibit more uniformity and stability with respect to kernel modification and; v) attracting involvement of the private seed industry in the QPM efforts (Gevers and Lake, 1992; Pixley and Bjarnason, 1993; Vasal *et al*., 1993a; 1993b; CIMMYT, 2000; Vasal, 2001; Hadji, 2004).

A result of comparative study of normal and QPM showed that, the lysine levels in normal and QPM maize average 2% and 4%, and tryptophan average 0.4% and 0.8% of total protein in whole grain flour, respectively (Moro *et al*., 1996). However, lysine levels vary across genetic backgrounds from 1.6 to 2.6% in normal maize and 2.7 to 4.5% in their o2 converted corresponding genotypes, and tryptophan range varies from 0.2 to 0.5% in normal maize and 0.5 to 1.1% in QPM counterparts, thus lysine and tryptophan levels are highly correlated therefore, an assay for either of them can be used for analyzing protein quality (Moro *et al*., 1996; Vasal, 2001; CIMMYT, 2002 and Krivanek *et al*., 2007).

Currently QPM breeding efforts at CIMMYT and national breeding programs in sub-Saharan countries focus on introducing and testing QPM developed elsewhere, conversion of existing adapted genotypes to QPM and pedigree breeding (Krivanek *et al*., 2007; CIMMYT, 2004b). Inbred lines, hybrids, and OPVs are acquired primarily from CIMMYT-Mexico (which has a wealth of QPM germplasm), as well as other breeding programs in Mexico, Ghana and South Africa to identify the most adapted cultivars for direct release. Pedigree breeding is commonly used, whereby the best performing inbred lines, complementary in different traits, are crossed to establish new segregating families. According to (Krivanek *et al*., 2007) three types of crosses provide a choice of breeding strategies QPM x QPM, QPM x normal, and QPM x normal backcross conversion of the normal genotype to QPM using at least three backcross generations. Within each of these methods, successive inbreeding of the material is made in parallel with continual selection on the three important QPM genetic. Quality protein maize breeding involves both the (*o2*) gene and the genetic modifiers of the o2 locus in combination, which leads to development of cultivars with modified endosperm kernel, and increased concentrations of lysine and tryptophan (Dagne *et al*., 2011). Studying a 15-parent diallel cross, under low nitrogen stress and optimal nitrogen environment each at Harare (Zimbabwe) and Bako (Ethiopia). (Dagne *et al.*, 2011) reported that, most of the QPM hybrids expressed higher protein quality levels than the best normal check variety under both conditions.

2.5. The nutritional impact of QPM

The increased protein quality of QPM has enormous nutritional benefits for humans and livestock. (Bressani 1991 and Akuomoa 2002) reported that through increased protein absorption and retention, malnourished children recover quickly when fed QPM than normal maize. Human nutrition studies (Akuomoa, 2002) confirmed that infant children who were fed with QPM had fewer sick days than children fed with normal maize. This demonstrates the value of QPM in feeding poorly nourished children, especially in communities where people cannot afford legumes, or other animal protein sources. Nitrogen equilibrium required for normal maize is about 24 g per body weight while 8g is required for QPM (Bressani,1992). Comparisons between QPM and normal maize revealed that approximately 100g QPM needs to be consumed by children to maintain the recommended daily protein requirements while approximately 500 g is required for adults (Nuss and Tanumihardjo, 2011). Further advantages of QPM were realized in livestock feeding studies (Vander, 1995; Krivanek *et al.*, 2007). Pigs fed on a QPM diet for 60 days gained 18 kgs more live weight compared to those fed on normal maize (Vivek *et al.*, 2008). This implies that QPM can reduce the cost of purchasing protein supplements (soybean meal and fish meal) given to non-ruminants, and can be used as an ingredient in conventional and new animal feeds as it provides a well balanced feed (Van der, 1995; Vasal, 2001). Early studies on *o2* maize, involving children recovering from severe malnutrition in clinical settings in Colombia, Guatemala, and Peru, found that a greater proportion of protein from *o2* maize, compared with conventional maize (Bressani., 1991). Studies involving adults found similar results, with the conclusion that the biological value of protein in *o2* maize was comparable to that of most animal proteins (Kies *et al*., 1972; Clark *et al*., 1977). A study by Singh *et al*., (1980), found that children consuming *o2* maize had weight, height, chest, and mid-upper-arm measurements comparable to or only slightly lower than those of children consuming skim milk and greater than those of children consuming conventional maize. According to Gunaratna report, QPM leads to a 9% increase in the rate of growth in height and a 12% increase in the rate of growth in weight in infants and young children with mild to moderate under nutrition in populations in which maize is a significant part of the diet (Gunaratna *et al*., 2010).

The study conducted by Girma *et al* (2010), on nutritional impact of QPM on of young children in the western part of Ethiopia where maize is staple food showed that, children in the QPM group had recovered to height and grew an average of 167 g/month, while children in the conventional maize group grew an average of 146g/month during the13-month study. This is a significant 15% increase in the rate of growth in weight among children in QPM households compared with children in conventional maize households.

Several human nutrition studies were conducted by Akuamoa (2002) in Ghana, where maize is the main staple, was shown that children fed with high lysine/tryptophan maize were healthier, had reduced stunting and better growth enhancing capabilities, compared with children fed normal maize porridge. The conclusion was that high lysine/tryptophan maize holds the promise of improving the nutritional status of vulnerable groups whose main staple is maize and who cannot afford protein-rich foods to supplement the diet.

2. 6. Genetic distance assessment of QPM inbred lines

Genetic diversity is the probability that two randomly chosen alleles are different in a sample (Hallauer *et al*., 2010). Genetic diversity can be determined by measuring the genetic distances among a number of genotypes. The distances reflect the actual level of genetic difference existing among the genotypes. In order to identify heterotic patterns, genetic distance has been used as a tool to group similar germplasm (Lee and Ash, 2007). One way to investigate the relationship among diverse genetic materials, such as maize inbred line is the detection of their similarity or dissimilarity (Schaller., 2006). These measures can be calculated using agronomic or morphological characteristics of each inbred lines and more recently using molecular markers (Schaller., 2006). Genetic gains from phenotypic selection have been assessed for many plant species and the progress has been varied (Volenec *et al*., 2002). The most important factor influencing selection gains is the amount of available genetic variation for general adaptation and traits necessary for improved production under specific constraints (Ceccarelli, 1989; Vasal *et al*., 1997b). It has been recommended to start selection on high performing and agronomically desirable genotypes exhibiting large variation for stress tolerant traits (Vasal *et al*., 1997b). The choice of breeding methods for genetic improvement of a crop depends upon the nature and magnitude of genetic variability present (Singh and Chaudhary, 1985; Hallauer and Miranda, 1988). A primary goal of breeding is to develop and identify high yield ingtransgressive segregants. Kisha *et al*., (1997) reported that, populations with higher genetic variance are more predicted to be high yielder transgressive segregants than populations having lower genetic variance.

QPM research activities in sub-Saharan Africa region led by CIMMYT and the International Institute for Tropical Agriculture (IITA) in collaboration with National Agricultural Research Systems (NARS) are highly coordinated enabling joint development, and broad testing of promising genotypes for all agro-ecological niches (CIMMYT, 2005b). Their goals are focused on broadening genetic base of adapted QPM germplasm to suite their specific biotic and abiotic stresses (Krivanek *et al*., 2007).

The quantification of similarity or dissimilarity between different plant accessions is based on the principle of genetic distance (Reif *et al*., 2005). Summarized the genetic and mathematical properties of a set of distances that are suitable to characterize plant materials.

Assessment of genetic diversity and relationships within a given set of germplasm is important for several reasons including: (i) assisting in the selection of parental combinations for developing progenies with maximum genetic variability (Barrett, 1998), (ii) describing heterotic groups (Benchimol *et al*., 2000; Flint *et al*., 2009; Lu *et al*., 2009), (iii) determining the level of genetic variability when defining core subsets selected for specific traits (Mohammadi *et al*., 2003) and (iv) estimating possible loss of genetic diversity during conservation or selection programs (Reif *et al*., 2005).

2.6.1. Morphological

Characterization involves evaluation of quantitative and qualitative attributes of given genotypes in order to differentiate them and determine their usefulness, structure, genetic variability and relationships among them. In characterization using morpho-agronomic markers, descriptors are used (IBPGR, 1991). Varietal management schemes practiced in subsistence communities are complex and sufficiently sophisticated that differences in heritability among morphological traits are recognized and used to direct management practices (John *et al.*, 2011). According to (Louette *et al.*, 1997) Farmer's classification of maize germplasm using ear characteristics can be equivalent to those obtained from sophisticated statistical analyses.

Pedigree relationships often serve as standards to test the effectiveness of morphological markers in determining relationships among breeding lines and predicting heterosis (Gerdes and Tracy, 1994). Although morphology has proved useful for classifying maize races and populations these markers have limitations in detecting differences among closely related genotypes and elite breeding germplasm (Goodman and Brown, 1988; Smith and Smith, 1989b; Bernardo, 1992). For example, Gerdes and Tracy (1994) grouped only closely related sweet maize inbred lines by morphological data in agreement with pedigree data, but morphological clustering did not provide an accurate assessment of the relationships present in these lines. Morphological data provided the initial basis for taxonomic studies of maize (Sturtevant, 1884) and have remained a mainstay of maize racial taxonomy to current times (Ortiz *et al.*, 2008).

Morphological data also play an important role in the management of genetic resources that are conserved in *ex situ* gene-banks (Sanchez *et al.*, 2000). Yoseph *et al*., (2005) reported highly significant differences among accessions for all of the traits studied suggesting that, there was a high degree of phenotypic diversity among the accessions of highland agro-ecology of Ethiopia. The authors concluded that, morphological dissimilarity covered a greater range, but was significantly skewed towards small values. Studying 35 QPM inbred lines on the basis of morpho-agronomic data, Dagne (2008) classified into four clusters and two outlier inbred lines. Lucchin *et al*. (2003) clustered 20 Italian flint maize landraces into groups using morphological and agronomic traits. Yoseph *et al*., (2006) classified 62 traditional highland maize accessions into three groups using 15 morphological traits. In diversity analysis of 45 maize inbreds, Gerdes and Tracy (1994) grouped closely related inbred lines by morphological data in agreement with pedigree data. However, several reports indicated that morphological markers have shortcomings because of their being influenced by prevailing environmental conditions (Gerdes and Tracy, 1994; Yoseph *et al*., 2005).

2.6.2. Molecular diversity assessment in maize

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 (Bertan *et al*., 2007; Flint *et al*., 2009; Lu *et al*., 2009). Molecular markers are powerful complement to help define heterotic groups and to examine the relationships among inbred lines at the DNA level (Melchinger,1993; Senior *et al*., 1998; Melchinger, 1999). Genetic diversity studies using DNA finger printing more efficient to detect sufficient polymorphisms in various crop species including maize (Pejic *et al*., 1998). 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 (Smith and Smith, 1992; Westmann and Kresovich, 1997; Melchinger, 1999). Various molecular marker types have been used to investigate relationships among maize inbred lines from different heterotic groups (Smith *et al*., 1997; Lu and Bernardo, 2001; Xia *et al*., 2005). The most commonly used marker methods in maize are RFLP, RAPD, AFLP, SSR and SNPs. Some of these markers are co-dominant, highly polymorphic, multi-allelic, and have become the marker of choice for genetic analysis in crops (Gupta and Varshney, 2000). Genetic distance based on molecular markers has been suggested as a tool for grouping of similar germplasm as a first step in identifying promising heterotic patterns (Melchinger, 1999). Knowledge of germplasm diversity and relationships among elite breeding materials is fundamentally important in crop improvement (Melchinger *et al*., 1991; Bernardo 2002). Detailed knowledge regarding genetic diversity and relationship among breeding materials is indispensable for the development of new maize inbred lines, establishment and assignment of maize inbred lines to heterotic groups (Smith and Smith, 1992; Melchinger, 1999; Xia *et al*., 2005; Legesse *et al*., 2007).

Genetic distance can be estimated from various types of molecular markers, including restriction fragment length polymorphism (RFLP), amplified fragment length poly- morphism (AFLP), simple sequence repeats (SSRs) and single nucleotide polymorphisms (SNPs). Advances in molecular technology, however, have produced a shift towards SNP markers (Hamblin *et al*., 2007; Jones *et al*., 2007). Because of their low cost per data point, high genomic abundance, locus specificity, co-dominance, potential for high throughput analysis and lower genotyping error rates (Schlotterer 2004; Chagne *et al*., 2007). Single Nucleotides Polymorphisms have emerged as powerful tool for many genetic applications, including genetic diversity studies, linkage and quantitative trait loci (QTL) mapping, and marker assisted breeding (Zhu *et al*., 2003). Currently, chip based technology is the most high throughput SNP genotyping platform. The Illumina chip-based SNP detection technology is useful for a broad range of applications to genotype samples with different possible levels of multiplexing, from 48 to 384 (BeadXpress) and 1536 (GoldenGate) to 55,000 SNPs (Infinium) (Kassa *et al*., 2012). Such chip based genotyping platforms are suitable for large scale studies that require genotyping of individual samples with thousands of SNPs (Low *et al*., 2006). High levels of multiplexing, high total cost and lengthy process of initial assay development are a drawback of chip based platforms. They may be unsuitable for studies where only a small to moderate number of SNPs are needed over a large number of samples, as is the case in mapping, marker assisted recurrent selection, marker assisted backcrossing, and quality control applications. In such cases, uniplex SNP genotyping platforms are more suitable (Low *et al*., 2006). Furthermore, a significant percentage of the SNPs in highly multiplexed chip based assays generally prove uninformative in any given population (Dvornyk *et al*., 2004). It is therefore necessary to select the best SNPs to provide a good level of discrimination for uniplex assays of each population under study.

Several study have been done using SNP marker for instance, (Lu *et al*., 2009) characterized 770 lines, including 394 tropical/subtropical germplasm from CIMMYT, 14 tropical/sub-tropical and 268 temperate germplasm from China; and 1 temperate and 93 tropical/subtropical germplasm from Brazil, using 1034 SNPs. The result indicates the presence of clear population structure and genetic divergence between temperate and subtropical/tropical germplasm. Similarly, Yan *et al*., (2009) studied 632 inbred lines from temperate, tropical, and subtropical public breeding programs and reported the presence of clear structure between temperate and tropical lines, and also complex familial relationships among global maize collection. An association mapping panel study by Wen *et al*., (2011) consisting of 359 maize inbred lines both from CIMMYT and International Institute for Tropical Agriculture (IITA) breeding programs that have resistance to drought, low nitrogen, soil acidity, pest and disease resistance. The authors pointed out that, the presence of a subgroup that largely consisted of lines developed from LaPosta Sequía. Genotyping 450 maize inbred lines using SNP, a high genetic distance and low kinship coefficients among most pairs of lines were found, indicating the uniqueness of the majority of the inbred lines (Kassa *et al*., 2012). A study by Demissew *et al*., (2014) on QPM inbred lines adapted to tropical highland agro-ecologies of Ethiopia using SSR markers showed a genetic distance ranging from 0.077 to 0.780. Marker-assisted selection (MAS) is a biotechnology research tool adapted to enhance conventional breeding with accuracy and to accelerate variety development (Xu and Crouch, 2008). Parental lines resistance to Downey mildew and maize streak virus was identified through marker-assisted selection (Nothando 2013).

2.7. Heterosis and hybrid performance in maize

Heterosis or hybrid vigor is a phenomenon in which an F1 hybrid has superior performance over its parents. According to Falconer and Mackay, (1996) explanation, heterosis is the difference between the hybrid value and the mean value of the two parents for the same trait. There are two major types of estimation of heterosis namely, mid-parent or average heterosis, which is the increased vigor of the F1 over the mean of two parents; and high-parent or better parent heterosis, which is the increased vigor of the F1 over the better parent (Sinha and Khanna, 1975; Jinks, 1983).

In maize, heterosis is the primary reason for the success of commercial industry (Stuber *et al.* 1992). Three major theories, such as dominance, over-dominance and epistasis, have been proposed as the main theories to explain the genetic mechanisms underlying the phenomena of heterosis. However, it is generally accepted that heterosis, to a large extent, is due to dominance gene action (Hallauer and Miranda, 1988; Singh, 2005).

The dominance hypothesis suggests that the alleles from one parent are dominant over the alleles from the other parent, and due to the cancelation of deleterious effects at multiple loci, the F1 hybrid is superior to the parents (Bruce 1910; Keeble and Pellew 1910). The over-dominance hypothesis assumes that the loci with heterozygous genotypes are superior to both homozygous parents (East 1908; Shull 1908). Epistasis is also frequently mentioned as a possible cause of heterosis (Antonio *et al.*, 2008). Any of a multitude of genetic phenomena known to influence qualitative or quantitative characters is expected to influence heterosis but over the years that dispersion of completely or incompletely dominant genes and over-dominance along with some contribution of non-allelic interactions have been considered to be the main causes of heterosis.

Heterosis breeding has received more attention than other branches of plant breeding in several crop plants and maize is one such cross fertilized crop in which has an array of hybrids have been realized over decades. Bruce (1910) put- forth the support for dominance hypothesis, which suggested that increase in vigour after crossing resulted from the combination of various dominant alleles by each parent. The manifestation of heterosis depends on genetic divergence of the two parental varieties (Moll *et al*., 1965; Hallauer and Miranda, 1988). Low grain yield heterosis is observed for crosses among genetically similar germplasm and for crosses among broad genetic base germplasm (Beck *et al*., 1991; Vasal *et al*., 1992a). Higher levels of heterosis were seen with increased divergence within a certain range, but that heterosis declined in extremely divergent crosses (Moll *et al*., 1965).

Heterosis in maize has been investigated extensively. Hallauer and Miranda (1988) summarized results from studies on heterosis up to 1979. They reported that mid-parent heterosis ranged from -3.6% to 72.0% while high-parent heterosis ranged from -9.9% to 43.0%. The magnitude of heterosis has not been changed during the hybrid era (Duvick, 1999), even though mean commercial maize grain yield has substantially increased during this time. Kara (2001) observed positive heterosis for all characters studied except for days to tasseling with the average hybrid yield being 79.89 per cent above that of the parents. Over dominance may exist at some loci but of minor importance.

Hybrid varieties are the first filial generations (F1) from crosses between two or more pure lines, inbreds, open-pollinated varieties, clones or other populations that are genetically dissimilar

(Singh, 2005). Maize hybrid development began in the early 1900s (Hallauer *et al*., 1988). In maize, hybrid breeding remains the method of choice for attaining maximum genetic gain from the effect of heterosis. According to Singh (2005), most of the commercial hybrid varieties are F1's from two or more inbreds. The success of hybrid maize depends on the ability of the breeding program to rapidly isolate lines that combine well in hybrid combinations and to identify appropriate heterotic combinations to maximize the vigour of the hybrid (Kim and Ajala, 1996). The general process to develop maize hybrids starts with the creation of a source segregating breeding population that is used to develop inbred lines through inbreeding and selection (Betran *et al*., 2004). Selected inbred lines are then evaluated in hybrid combinations across locations to select superior hybrids and to estimate their combining ability. Maize improvement involves formation, evaluation, selection, and recombination of genetically variable families or inbred lines (Pixley *et al*., 2006). Test cross performance of experimental lines is the prime selection criterion in hybrid breeding of maize (Mihaljevic *et al*., 2005). QPM hybrids yield more than open pollinated cultivars, but mean grain yield does not differ for single-, three-way, and double-cross QPM hybrids in multi-location trials (Pixley and Bjarnason, 2002). They suggested that failure of the single cross to yield more than three-way or double cross progeny is due to lack of heterosis among at least some of the parent lines used. They further explained that broader genetic constitution of three-way and double cross hybrids buffer them better than single crosses against the extreme environmental diversity of the trial sites. Development of hybrids and OPVs of different maturity groups are the main focus of the national maize research strategy, particularly for the mid-altitude and highland sub-humid maize growing areas of Ethiopia (Mosisa *et al*., 2011).

2.8. Combining ability and heterosis

Sprague and Tatum (1942) introduced the concepts of general combining ability (GCA) and specific combining ability (SCA) to distinguish between the average performances of parents in cross combinations (GCA) and the deviation of individual crosses from the average performance of the parents involved (SCA). Line × tester mating design developed by Kempthorne (1957), provides reliable information on the general and specific combining ability effects of parents and their hybrid combinations. The mating design can also be used to determine the nature and magnitude of gene action and heterosis for yield and other important traits in maize, for example over two environments GCA enabled breeders to exploit the existing variability in the breeding materials, to identify individual genotypes conferring desirable attributes and to distinguish relatedness among genotypes (Vacaro *et al.*, 2002; Melania and Carena, 2005). In contrast SCA serves to determine heterotic patterns among populations, to identify promising single crosses and to assign inbred lines into heterotic groups (Parentoni *et al.,* 2001; Revilla *et al.,* 2002). Combining ability of inbred lines is the vital factor determining future usefulness of the lines for hybrid development (Hallauer and Miranda, 1988). Generally, heterosis and combining ability is prerequisite for developing a good economically viable hybrid maize variety Krivanek *et al.*, (2007). The choice of the most effective breeding scheme and the rate of the genetic improvement are dependent upon the relative magnitude of various gene effects (Dhillon and Pollmer, 1978). 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; Sughroue and Hallauer, 1997). GCA is recognized primarily as a measure of additive gene action and SCA as an estimate of non-additive gene action such as dominance and epistasis (Sprague and Tatum, 1942; Rojas and Sprague, 1952; Gowen, 1964; Kambal and Webster, 1965). Based on Younes and Andrew, (1989) information, additive gene effects were more important in determining traits in the populations while non-additive gene actions were important in inbred line crosses. Lee *et al*., (2005) reported that although both additive and non-additive genetic effects influence grain yield in inbred line crosses, 74% of the total genetic variance is attributed to the additive genetic component. HAN *et al*., (1991) studied combing ability effects of inbred lines derived from CIMMYT populations and observed significantly positive SCA effects for crosses of inter-population inbred lines and concluded that inbred lines derived from different populations are more likely to show superior yielding performances. On the other hand, Zehui *et al*., (2000) studied inbred lines derived from different populations and found significant GCA effects for a number of yield related and morphological traits. Xingming *et al*., (2004) evaluated combining ability and heterotic groups of yellow QPM inbreds and observed significant differences among the crosses and GCA of lines for grain yield, plant height, rows per ear, kernels per row and thousand seed weight; and non-significant difference in SCA mean squares for all traits. Vasal *et al*., (1993a; b) studied heterosis and combining ability of CIMMYT's tropical and subtropical QPM germplasm and reported maximum high-parent heterosis of 16% and the greater importance of GCA relative to SCA. Singh and Gupta, (2009) studied heterotic expression and combining ability for fifteen yield and related traits in maize involving 66 F1 crosses produced in line x tester design. Pooled analysis of variance revealed significant differences among lines, testers and line x tester crosses except for leaves per plant and ear girth due to testers. Previous reports identified heterotic crosses for sugar content (Qi Xin *et al*., 2008; Asbish Khanduri *et al*., 2010 and Sadaiah *et al*., 2013).

2.9. Genetic distance and hybrid performance

Both molecular and morphological markers allow for extensive data estimation of genetic diversity and the levels of genetic variation in maize to identify elite inbred lines that can be crossed to create superior hybrids (Smith and Smith, 1989; Karanja *et al*., 2009). Maize breeding relies on the available genetic diversity which can be manipulated for maximum heterosis estimation in hybrid breeding programmes (Karanja *et al*., 2009). Several reports have indicated the high correlation between genetic distance and hybrid performance in maize (Betran *et al*., 2003; Xu *et al*., 2004; Makumbi, 2005; Kiula *et al*., 2008). Contrary to these, many other workers reported that genetic distance measures are of limited use in predicting hybrid performance and heterosis (Parentoni *et al*., 2001; Makumbi, 2005; Legesse *et al*., 2008). According to Drinic *et al*. (2002) result, SSR markers provide an effective method for predicting hybrid performance and heterosis. Generally, it is concluded that genetic distance estimate is more efficient for the prediction of hybrid performance between closely related inbred lines than in crosses between distantly related inbred lines (Melchinger, 1999). Genetic diversity in relation to hybrid performance and heterosis has been studied extensively in maize. Moll *et al*., (1965) reported positive correlations between morphological markers based genetic diversity of the parents with heterosis in maize hybrids.

3. OBJECTIVES

3.1. General objective:

To assess genetic diversity and relationships among elite QPM inbred lines using morphological data and SNP markers and estimate their combining ability to determine their usefulness in forming superior QPM hybrid combinations that are adapted to the Ethiopian highlands.

3.2. The specific objectives of the study were:

- To assess genetic diversity and relationships among elite QPM inbred lines using morpho agronomic and SNP markers data;
- To cluster and identify groups of similar inbred lines using both morphological and SNP markers data;
- To determine the association and compare between morpho-agronomic traits and SNPs based distance of elite QPM inbred lines adapted to the highland of eastern and southern African region;
- To estimate combining ability of 24 QPM inbred lines for grain yield and morpho-agronomic traits and determine their usefulness in forming superior QPOM hybrid combinations adapted to the highland maize growing agro-ecology of the country;
- To assess the relationship and distance (as measured by morpho-agronomic traits and SNPs markers) with heterosis effects of parental lines grown at different highland environments of the country.

4. MATERIALS AND METHODS

Two separate but interrelated experiments were carried out in this study. The first set of experiment (Set-I) was on determining variability (based on morphological and molecular markers) and interrelationship among the 24 inbred lines along with two testers. The second set of experiment (Set-II) was evaluating the performance of 48 F_1 hybrids formed by crossing the 24 inbred lines with two QPM testers along with two checks (one QPM and one non-QPM three way commercial hybrids) .

4.1.1. Description of the study area

The first set of experiment was conducted at two locations namely, Ambo Plant Protection Research Center and Holetta Agricultural Research Center, and the second set of experiment was conducted at four locations (Kulumsa and Adet Agriculture Research Centers in addition to the above mentioned two research centers) in the main cropping season of 2014. Important geographic and climatic descriptions of the study areas are given in Table1.

Table 1. Geographic and climatic description of the study area

Name of Research Center	Geographic location	Elevation	Soil type	Mean annual rainfall (mm)	Average mean temperature (ºC)	
					Minimum	Maximum
APPRC	8º59′ N and 37 º 51′ E	2101	blackVertisol	1100	11^{0}C	26^{0}C
HARC	09^{0}03′N and 38^{0}30′E	2390	EutricNitisol	1100	6^{0}C	26^{0}C
KARC	8º2′N and 39º10′E	2200	Clay soil	832	10^{0}C	22^{0}C
AARC	11^{0}17′N and 37^{0}43′E	2240	Nitosol	1091	18.2^{0}C	25.3^{0}C

APPRC= Ambo Plant Protection Research Center; HARC= Holetta Agricultural Research Centers; KARC= Kulumsa Agriculture Research Center; AARC= Agriculture Research Center

4.1.2. Pedigree of the plant materials

A total of twenty four quality protein maize inbred lines from maize breeding program at APPRC (Table 2) formed the core plant materials for this thesis research. These lines were developed by backcrossing three conventional maize inbred lines with two known QPM donor CIMMYT maize lines (CML). Their pedigree is shown in Table 2.

Table 2. List of parental lines used to generate the single cross hybrids using line x tester mating design

Code	Pedigree of the inbred lines
L1	TUXCML159 BC2F41-3-7-B-#-#
L2	TUXCML159 BC2F36-2-2-B-#-#
L3	TUXCML159 BC2F36-2-3-B-#-#
L4	TUXCML159 BC2F18-5-7-B-#-#
L5	SADVLACML176 BC2F6-8-3-B-#-#
L6	SADVLACML176 BC2F6-8-6-B-#-#
L7	SADVLACML176 BC2F6-8-11-B-#-#
L8	SADVLACML176 BC2F56-1-1-B-#-#
L9	SADVLACML176 BC2F51-1-3-B-#-#
L10	SADVLACML176 BC2F51-1-9-B-#-#
L11	SADVLACML176 BC2F16-4-5-B-#-#
L12	SADVLACML154 BC2F7-1-1-B-#-#
L13	SADVLA/CML154 BC2F54-4-1-B-#
L14	SADVLACML154 BC2F37-3-5-B-#-#
L15	P502 SRCML 384X176…..98-2-1-2 BC2F6-2-4-B-#-#
L16	P502 SRCML 384X176…..98-2-1-2 BC2F4-1-3-B-#-#
L17	P502 SRCML 384X176…..98-2-1-2 BC2F30-1-6-B-#-#
L18	P502 SRCML 384X176…..98-2-1-2 BC2F30-2-2-B-#-#
L19	P502 SRCML 384X176…..135-2-2-2 BC2F30-4-2-B-#-#
L20	P502 SRCML 384X176…..135-2-2-2 BC2F30-4-4-B-#-#
L21	P502 SRCML 384X176…..135-2-2-2 BC2F30-4-9-B-#-#
L22	P502 SRCML 384X176…..135-2-2-2 BC2F21-3-4-B-#-#
L23	P502 SRCML 384X176…..135-2-2-2 BC2F20-1-4-B-#-#
L24	P502 SRCML 384X176…..135-2-2-2 BC2F2-1-1-B-#-#
T1	CML144
T2	CML159
Check	FS67

4.1.3. Experimental Design

The experiment for morphological characterization for 24 parental QPM inbred lines and two testers (CML144 and CML159) was planted at APPRC and HARC. The design used was alpha lattice 13x4 with two replicates (Patterson and Willianms, 1976). Each entry was planted with two seeds per hill with two-row plot having 3.75 m long and 0.75 m apart with a distance of 0.25 m between plants in a row. The seedlings were thinned to one plant per hill at three weeks after emergence.

4.1.4. Molecular characterization of parental QPM inbred lines using SNP markers

A total of twenty four QPM inbred lines seeds were sent for molecular characterization at Biosciences for Eastern and Central Africa (BecA) hub in Nairobi, Kenya, using a modified version of CIMMYT protocol. Seedlings were raised on plastic trays at the Biosciences Eastern and Central Africa (BecA) hub screen-house in Nairobi, Kenya. A single leaf from each of 10 plants per sample were piled together, the tips trimmed off and approximately equal amount of leaf segment cut at once to make a bulk, and transferred into 1.2 mL strip tubes that contained two 4-mm stainless steel grinding balls (Spex CetriPrep, USA).

Genomic DNA was extracted using a modified version of the CIMMYT high throughput mini-prep Cetyl Trimethyl Ammonium Bromide (CTAB) method as described by Kassa (2014). This extraction protocol has longer steps but provides good quality DNA for different purposes, including GBS that involves restriction digestion. DNA concentration was measured using the Quant-iT™ PicoGreen® dsDNA assay kit (Invitrogen™, Paisley, UK) and the Tecan Infinite F200 Pro Plate Reader (Grödig, Austria), and normalized to 50 ng/µL. The quality of the extracted DNA was checked by digesting 250 ng of the genomic DNA from 8 randomly selected samples with 3.6 units of ApeKI restriction enzyme (New England Biolabs, Boston, USA) at 75 °C for three hours. DNA samples were shipped to the Genomic Diversity facility at Cornell University (http:// www.biotech.cornell.edu/brc/genomic-diversity-facility). DNA samples were genotyped using GBS as described by Elshire *et al.*, (2011). GBS data was generated by the Genomic Diversity Facility, Cornell University using ApeKI as restriction enzyme and 96-plex multiplexing.

4.1.5. Trials Management

In all sites, trials were hand planted 11/06/2014 and 07/07/2014 at APPRC and HARC respectively with two seeds per hill, which were later thinned to one seed per hill after three weeks to get a total plant population of 53, 333 per hectare. Reliable moisture level of soil was in place before starting planting so as to ensure good germination and seedling development and to avoid the occurrence of dry spell after planting. Urea and DAP Fertilizers were applied at the rate of 200kg/ha and 150kg/ha, respectively. All the DAP was applied at planting, while urea was applied twice, half at planting and the remaining was divided in to two equal parts and applied 45 days after planting and at flag leaf stage.

All recommended crop management practices including: land preparation, planting time, planting depth, weed control, disease and insect pest management were done following recommended management practices of the research site. Pre-emergence herbicide, primagram-gold 660SC was applied at the rate of three liters per hectare ($3ha^{-1}$) at planting to control weeds. The rafter weeds were controlled by hand weeding and slashing.

4.1.6. Endosperm modification and laboratory analysis of tryptophan and lysine level

The extent of opaqueness was used to assess endosperm modification for each genotype. Endosperm modification was scored in the maize breeding laboratory at APPRC. Forty eight QPM F1 hybrids along with two standard check and 26 parental inbred lines were scored on a 1-5 scale following the method described by Pixley and Bjarnason (2002) and Vivek *et al*. (2008). Seed sample of 100 kernels were used to determine endosperm modification. The kernels were sorted into the following classes under a light table:

1 = completely modified/ 100% translucent normal phenotype

2 =75% modified

3 =50 % modified

4 =25 % modified

5 = 100% opaque

After visual scoring of light table, the seed sample of 26 inbred lines containing of 20 seeds with the 2 and 3 scores were sent to Mexico CIMMYT laboratory for chemical analysis to determine tryptophan and lysine amino acids levels.

4.1.7. Measurements

Qualitative and quantitative data were collected independently at specific plant stage as descriptive developed by IBPGR (1991).

4.1.7.1. Qualitative traits

1. Leaf orientation (LO): After flowering,(1 erect, 2 semi-erect, 3 pendant and 4 semi-pendant).

2. Silk colour (SKL): At flowering; on plot basis (1 whitish purple, 2 yellowish white, 3 purple, 4 pink and 5 white).

3. **Stem colour (SC):** Indicate up to three stem colours in the order of frequency. Observed between the two top most ears. At flowering; (1 Green, 2 Sun red, 3 Red, 4 Purple, 5 Brown)

4. **Tassel size (TS):** Was recorded after milk stage as 3 (small), 5 (medium) and 7 (large).

5. Kernel type (KT): Indicate up to three kernel types in the order of frequency (1 Floury, 2 Semi-floury, 3 Dent, 4 Semi-dent, 5 Semi-flint, 6 Flint, 7 Opaque 2/QPM)

6. Kernel color (KC): Indicate up to three kernel colors in the order of frequency (1 White and 2 Yellow)

7. Tassel colour (TC): At flowering; on plot basis (1 purple, 2 whitish purple, 3 white, 4 gray and 5 yellowish white).

4.1.7.2. Quantitative traits

1. Number of leaves per plant (NL): Number of leaves of ten randomly sampled plants per plot was counted at mid-silking stage.

2. Leaf length (LL): This was recorded as the length of the leaf from ligule to apex. Measurement was taken after flowering from the leaf that subtends the uppermost ear from five randomly sampled plants.

3. Leaf width (LW): Measurement was taken from the same leaf as the leaf length at mid-way along its length.

4. Leaf area (LA): Measurements taken on leaf length and width was used to calculate leaf area. The leaf area represented the area of the upper most ear leaf computed as maximum width x length x 0.75 in centimeter squares.

5. Tassel peduncle length (TPL): After milk stage; measured from the last leaf sheath up to first branch of the tassel.

6. Tassel length (TL): After milk stage; measured from the base of the first branch up to the tip of the tassel.

7. Number of primary branches on tassel (NPBT): total number of primary branch on tassel were counted on plant basis.

8. Ear diameter (ED): This was measured at the mid-section along the ear length, as the average diameter of five randomly taken ears from each experimental plot in centimeters using calliper.

9. Number of rows per ear (NRE): This was recorded as the average number of kernel rows per ear from the five randomly taken ears for ear length and ear diameter measurements.

10. Number of kernels per row (NKR): This was also recorded as the average number of kernels per row from the five randomly taken ears.

4.1.8. Data Analysis

The collected data were subjected to analysis using the PROC PRINCOMP of SAS version 9.1.3. (SAS 2004) and MINTAB (MINTAB, 2003). The same results were obtained by using these two statistical packages. Multivariate analysis such as, principal component and cluster analysis were done. The components which had eigen values equal to or greater than 1 were retained and considered worthy of interpretation according to the method by Kaiser (1960). The standardized mean values (mean of each trait was subtracted from the data values and the result divided by the standard deviation) were used to perform principal component analysis. Following the average linkage cluster analysis and the determination of the number of clusters was estimated based on the Pseudo-F and Pseudo-T^2 options. Correspondence between Euclidean distance and Roger's genetic distance was determined by the product moment correlation derived from mantel Z test using(NTSYS-pc version 2.1).

4.1.9. SNP Genotyping

Raw reads from the sequenced GBS library were called in the GBS analysis pipeline TASSEL version 4.0.28, an extension to the Java program TASSEL (Bradbury *et al.*, 2007). The filtered sequences were aligned to the maize reference genome B73 RefGenv1 (Schnable *et al.*, 2009) using the Burrows–Wheeler alignment tool. This procedure provided a total 3, 825 SNPs covering all the 10 chromosomes of the maize genome. SNP loci, having not less than 0.05 allele frequency and no missing value, were selected and used to analyze the genetic diversity of the inbred lines. The SNP marker was distributed throughout the sample uniformly and polymorphic information content is achieved in the current study.

Roger's genetic distances between each pair of inbred lines were computed using TASSEL (Bradbury 2007) software. Based on genetic similarity, a dendrogram was constructed from the genetic distance matrix using the unweighted pair group method with arithmetic average (UPGMA) and the resulting tree was visualized using MEGA version 6.0.

4.2.1. Experimental design for the set-II experiment

Twenty four quality protein maize inbred lines were crossed in line x tester mating design during the main season of 2013. The inbred lines were crossed with two testers (CML 144 and CML 159) at APPRC to generate 48 hybrids. The resulting 48 testcrosses along with two standard check varieties: one QPM and the other conventional maize (AMH760Q and AMH800) were planted using 10x10 alpha lattice designs with two replications at four locations during 26/05/2014, 07/06/2014, 11/05/2014 and 17/06/2014 at Ambo Plant Protection Research Center, Holetta Agricultural Research Center, Kulumsa Agricultural Research Center and Adet Agricultural Research Center respectively under rain fed condition. Each entry was sown in one row having 0.75 x 0.25m crop geometry with plot size of 5.25m length. The trials were managed in similar way as described in experiment one above in section 4.1.5. Kernel endosperm modification was also done in the same way described in section 4.1.6 of experiment one.

4.2.2. Measurements on grain yield and other agronomic traits

4.2.2.1. Qualitative traits

1. Plant aspect (PA): Overall phenotypic appearance of the plant; where 1= excellent and 5= poor.

2. Husk cover (HC): (3 Poor, 5 Intermediate, 7 Good)

3. Root lodging (RL): Percentage of plants root-lodged. Two weeks before harvest.

4. Stalk lodging:(SL): Percentage of plants stalk-lodged. Two weeks before harvest

5. Ear aspect (EA): It is the visual evaluation of harvested ears for general performance with regard to diseases and uniformity. Ear aspect was scored on 1-5 scale.

6. Disease score: Major diseases at the experimental locations Gray leaf spot (GLS), Turcicum leaf blight (TLB) and Common leaf rust (CLR) were recorded using 1-5 visual scale, where 1 indicated highly resistant and 5 highly susceptible in terms of reaction to the particular disease assessed.

4.2.2.2. Quantitative trait

1. Days to taselling (DT): The number of days from planting to when 50% of the plants in a plot shed pollen.

2. Days to silking (DS): The number of days from planting to when 50% of the plants in a plot emerge 2-3 cm long silk.

3.Anthesis –silking interval (ASI): This parameter was recorded as the difference between anthesis date and silking date (ASI= SD- AD)

4. Days to maturity (MD): The number of days from planting to when 50% of the plants in a plot form black layer at the tip of each kernel.

5. Number of ears per plant (EPP): The total number of ears harvested from a plot divided by the number of plants in that particular plot at harvest.

6. Thousand Kernel weight (TKW): After shelling, random kernels from the bulk of shelled grain in each experimental unit was taken and thousand kernels were counted using a photoelectric seed counter and weighed in grams and then adjusted to 12.5% grain moisture.

7. Grain yield (GY): During harvesting only ears were removed from all plants in each plot leaving other crop residues (husk, leaf, stem and tassel) intact. The total field weight from all the ears of each experimental unit was recorded. This was adjusted to 12.5% moisture (electronically determined) level and 80% shelling percentage to estimate grain yield in tons per hectare for each genotype.

8. Ear height (EH): The height from the ground level to the upper most ear-bearing node of ten randomly taken plants from each experimental unit was measured in centimeters. The measurement was made two weeks after pollen shedding ceased.

9. Plant height (PH): The height from the soil surface to the first tassel branch of ten randomly taken plants from each experimental unit was measured in centimeters. Like ear height, this was also measured two weeks after pollen shedding had ceased from the same plants that EH measured.

4.2.3. Data analysis

The data obtained for different traits from field measurements analyzed using SAS statistical package version 9.1.3 (SAS, 2004). Accordingly, the significance of each trait in each location, combining ability for individual locations and across locations was determined. The details of data analysis procedures and formulas used are presented below. Before data analysis some parameters root lodging, stem lodging, husk cover and ear rot were transformed using square root transformation, $x'=\sqrt{x+0.5}$), as most of the plots had zero values (Gomez and Gomez, 1984). ASI was transformed using $ln\sqrt{(ASI +10)}$ Bolanos and Edmeades, 1996. During the data analysis, entries were used as fixed factor while location, replication and incomplete blocks within replication were considered as random factors. Entry means adjusted for block effects that generated from individual location analysis according to lattice design (Cochran and Cox, 1957) were used to perform the combined. Traits that showed significant differences among crosses, further analysis was done according to the 'line x tester' analysis to partition the mean square due to crosses into lines, tester and line by tester effects (Singh and Chaudary, 2005) for individual location and combined across locations.

The combined analysis was done for the significant trait in individual location analysis after testing the homogeneity of error variances. Whenever traits were found to be significant only in three locations out of the four, the data of the three locations were combined given that the variances are homogeneous when tested using variance ratio (Gomez and Gomez, 1984). The mean squares of crosses and location were tested against the mean squares of their corresponding interaction with location as error term while their interaction with location were tested against their corresponding pooled error.

Table 3. Skeleton of ANOVA for combining ability across locations

Source of variation	Degree of freedom	Mean square
Crosses	(LxT) -1	MSlxt
Lines	L-1	MSl
Testers	T – 1	MSt
Lines X testers	(LxT) (L-1) (T-1)	MSlxt
Crosses x locations	(Cxloc) (LxT) (Loc-1)	MScxloc
Lines x location	(Lxloc) (L-1) (Loc-1)	MSlxloc
Testers x location	(Txloc) (T-1) (Loc-1)	MStxloc
Lines x testers x location	(LxT)xloc(L-1) (T-1) (loc-1)	MS(lxt)xloc
Pooled error	Loc (r-1) (LT-1)	MSpooled error

4.2.3.1. Estimation of combining ability effects

Genotypic mean of individual locations were used for the determination of GCA (general combining ability) and SCA (specific combining ability). The two standard checks were excluded while analyzing combining abilities. The GCA effect of lines and tester, the SCA effect of lines x tester, and their interactions with the environment were determined following the lines x tester analysis of Kempthorne (1957), assuming the following model.

$Y_{ijk}=\mu + g_i + g_j + s_{ij} + e_k + (ge)_{ik} + (ge)_{jk} + e_{ijk} + (se)_{ijk} + \delta_{ijk}$, Where Y_{ijk} = the performance of the hybrid, made with ith line and jth tester, in the kth location, μ= the overall mean, g_i= the effect of the ith line, g_j= the effect of the jth tester, s_{ij}= the interaction of the ith line with the jth tester, e_k= the effect of the kth environment, $(ge)_{ik}$= the interaction of the g_i and e_k, $(ge)_{jk}$= the interaction of the g_j and e_k, $(se)_{ijk}$= the interaction of s_{ij} and e_k.

The proportional contributions of Lines (GCAL), Testers (GCAT), and their interaction (SCALxT) to the sum square of crosses were calculated as the ratio between sum of squares of each component and the cross sum of squares as given by Singh and Chaudary (1985) and Sharma (1998) as follows.

Contribution of lines SS(L) X100/SS (crosses)
Contribution of testers SS(T) X100/SS (Crosses)
Contribution of (lxt) SS (lxt) X100/SS(lxt)

4.2.3.2. Estimation of general combining ability effects

The GCA due to lines and testers was calculated as a deviation of line mean from all hybrids mean.

a. Lines; g_i= x_i/tr-x/ltr b. Tester; g_j=x_j/tr-x/ltr

Where, g_i = gca effect for ith line, g_j = gca effect for jth tester, $X_{i..}$ = sum of the ith line, X.j.= sum of the jth tester, X… = grand sum, l = number of lines, t = number of testers, r = number of replications. For GCA effects of lines and tester, the restriction $\sum g_i = \sum g_j = 0$ was imposed.

4.2.3.3. Estimation of specific combining ability effects

SCA effects were calculated as a deviation of each cross mean from all hybrids mean adjusted for corresponding GCA effects of parents.

$S_{ij}=x_{ij/r}-x_{i/tr}-x_{j/lr}$ +x/ltr..,where, S_{ij} = SCA effect of the ij^{th} cross, $X_{ij.}$ = i x j cross sum, X_i = i^{th} line sum, $X_{.j.}$ = j^{th} tester sum, l = number of lines, t = number of testers and, r = number of replications. Here also the restriction $\sum S_{ij}=\sum S_{ij}=0$ was imposed.

4.2.3.4. Standard errors for combining ability effects

In order to test the significance of GCA and SCA effects or that of the difference between any two GCA and SCA effects, the SE and SEd were calculated as follows.

1. Standard error for general combing ability effects

a) Line: SE(gca for line)= $\sqrt{(l\text{-}1)\text{eMS}/l\text{rt}}$

b) Tester: SE(gca for tester)= $\sqrt{(t\text{-}1)\text{eMS}/l\text{rt}}$

2. Standard error for specific combining ability effect

SE (sca effct)= $\sqrt{(l\text{-r})(\text{t-r})\text{eMS}/l\text{rt}}$

The significance of GCA and SCA effects was tested by dividing the GCA effects of a particular line or tester and SCA effects of particular cross by its respective standard error. Then, this ratio was used as calculated t and compared with tabular t-value at error degree of freedom.

3. Standard error of the differences between combining ability effects

a) Standard error of the differences between general combining ability effects

SE $(g_i\text{-}g_j)$ line=$\sqrt{2\text{eMS}/\text{rt}}$

SE $(g_i\text{-}g_j)$ tester=$\sqrt{2\text{eMS}/\text{r}l}$

b) Standard error of the differences between specific combining ability effects

$SE(s_{ij}-s_{kl}) = \sqrt{2eMS/r}$

4.2.4. Heterosis

Heterosis was estimated only for two locations (APPRC and HARC) as the inbred lines were not evaluated at the other two sites. Mid- parent heterosis (MPH), better parent heterosis (BPH) and standard heterosis in percent were calculated following the method suggested by Falconer and Mackay (1996).

BPH (%) = (F1-BP) /BP *100

MPH (%) = (F1-MP)/MP*100

SH(%)=(F1-SH)/SH*100

Where, F1 Mean value of the cross

MPV= mean value of the two parents (line and testers)

BPV= mean value of the better parent

SH= mean value of standard heterosis

Test of significance of heterosis was made using the t-test. The critical differences (CD) for testing the significance of mid parent (MP) and high parent (HP) were calculated using the following formulas:

1. Critical difference for heterosis over mid-parent (MP):

CD (MP) $= \pm \sqrt{3eMS/2r} \times t$

2. Critical difference for heterosis over better parent

CD (BP/SH) $= \pm \sqrt{2eMS/r} \times t$

Where eMS is the error mean square, r is the number of replication and t is the table value at 5% and 1%.

5. Results

5.1. Agro-morphological trait variation

5.1.1.Performance of inbred lines

Analysis of variance (Table 4) showed that the 24 lines and two testers were highly significantly different for all traits evaluated ($P \leq 0.01$). Grain yield ranged from 1.13 (L17) to 4.04t ha^{-1}(L5) with mean of 2.40 t ha^{-1}. Number of ears per plant ranged from 1 (L17) to 2 (L20) with mean of 1.13 (Table 4). The anthesis date ranged from100 (L5) to 112 (T1) with the mean value of 104 days. Ear position of the lines ranged between 0.33 (L18) to 0.53 cm (L1) with mean value of 0.41cm. L22 measuring 147.3cm was tallest while L16 with height of 96.75 cm was the shortest. The biggest ear height (65.76 cm) was from L22, which also scored the highest for plant aspect, plant height and ear height. L16 was the shortest plant (96.75 cm) and had the smallest measurement for ear height (33.39cm).

Maize line L20 showed the highest number of kernel row per ear (16) while L17 showed the lowest (11). In the case of number of kernels per row, L7 exhibited the highest (28) and high yielder as well. In respect to thousand kernel weight L23 stood 1st with 314g, while L16 scored the least (142.5 g) and L23 had the thicker ear diameter (44.15cm) whereas, L3 had the thinnest diameter. Traits showed a wide range of variability particularly for TKWT, LA and LL traits. The lowest range of variations were recorded for characters such as LW, TL, AD and EPO. Means that were calculated for each trait showed considerable diversity among the inbred lines (Table 4).

Table 4. Means of morpho-agronomic traits of 26 highland QPM inbred lines (24 lines and 2 testers) evaluated at APPRC and HARC

Code	GY	AD	EPO	EPP	PH	EH	NKRE	NKR	TKWT	ED	**LL**	**LA**	**LN**	**LW**	**TL**	**TPL**	**NPBT**
L1	2.06	108.5	0.53	1.01	122.25	64.41	13.33	22.08	229	40.75	64.8	450.88	14	9.27	27.35	12.5	8.95
L2	2.97	102	0.48	1.35	120.4	58.51	12.16	25.41	246	36.13	63.35	406.04	13.18	8.55	26.85	14.7	11.45
L3	2.25	106.5	0.46	1.36	118.5	54.51	12.33	22.83	194	29.78	63.45	449.72	13.27	9.44	27.45	14.53	14.4
L4	2.17	101.5	0.48	1.27	110	53.23	11.82	19.66	208	32.5	64.58	511.97	14.31	10.59	25.8	12.9	10.85
L5	4.04	103.5	0.38	1.43	145	56.23	13.17	21.92	248.75	40.25	85.19	579.79	14.6	9.06	25.3	14.43	9.85
L6	2.78	100.5	0.36	0.92	120.25	44.23	14.17	23.33	223	36.98	75.37	482.82	15.32	8.52	28.2	12.7	6.7
L7	3.22	100	0.35	1.3	139.75	48.89	13.67	27.66	219.25	35.05	66.15	446.99	14.75	8.39	24.75	16.65	15.05
L8	3.88	101	0.37	1.15	140.7	53.26	13.67	27.5	266.75	34.88	74.89	543.23	14.5	8.95	28.75	14.88	4.78
L9	3.65	101	0.47	1.32	136.75	65.26	12.83	24.66	247.75	35.9	68.66	415.35	15.33	8.04	24.5	13.1	20.15
L10	3.12	103	0.38	1.14	128.5	48.98	11.67	25.5	195.75	36.83	65.88	486.06	12.37	9.76	30.25	16.6	8.55
L11	1.2	102	0.38	1.04	129.25	49.16	12.66	20.08	181.5	35.53	59.58	365.21	12.45	8.04	25.9	18.1	13.05
L12	2.16	102	0.46	0.91	132.75	62.23	11.5	21.58	236	36.85	55.6	339.14	12.65	8.04	26.65	17.08	12
L13	1.63	102.5	0.43	0.88	133.5	58.14	12.82	24.58	196	41.18	68.31	524.8	13.28	10.24	27.1	15.35	14.85
L14	1.3	101	0.35	0.86	120	41.66	12.83	24.92	180.75	35.88	66.13	565.87	13.2	11.38	25.1	14	15.9
L15	3.38	100	0.46	1.47	135.75	62.16	11.67	22.08	258	35.43	71.93	587.46	12.25	10.92	35.45	14.3	5.95
L16	2.34	101	0.34	1.19	96.75	33.39	14	26.42	142.5	33.53	57.58	362.06	11.97	8.37	27	14.35	6.5
L17	1.13	110	0.36	0.61	130	47.14	10.66	21.08	246.75	37.08	65.54	485.78	13.66	9.87	27.55	17.05	6.05
L18	2.21	105.5	0.33	1.09	112.5	37.48	12.17	26.75	194	34.18	71.7	548.64	11.52	10.19	31.35	15.85	4.75
L19	1.47	109.5	0.4	1.09	113.5	46.01	15.33	25	181.25	37.9	58.9	459.6	14.32	10.39	21.05	10.6	5.35
L20	2.67	106.5	0.45	1.61	115.75	52.39	15.66	25.17	176.25	36.78	61.74	499.19	14.55	10.79	24.35	8.9	6.95
L21	3.47	105	0.47	1.39	101	48.39	13.5	22.33	189.25	40.45	59.72	428.25	14.65	9.55	20.75	11.2	4.85
L22	2.22	106.5	0.44	1.04	147.25	65.76	13.66	19.25	223.5	38.23	71.81	513.36	16.22	9.52	24.55	13.05	10.95
L23	1.48	110	0.43	0.86	129.75	56.39	12.5	19.58	314	44.15	55.83	415.24	15.32	9.91	23.25	8.53	5.05
L24	1.93	103	0.42	0.98	128	54.41	11.66	19	231.5	34.18	71.08	541.82	14.13	10.13	27.2	18.55	12.35
T1	2.18	111.5	0.45	1.26	121.75	54.91	14.83	23.5	162.75	33.78	76.15	632.65	14.65	11.07	29.15	8.85	10
T2	1.57	109	0.36	0.89	114	41.26	12.17	18.16	255.5	33.53	68.57	502.4	12.67	9.76	27.9	12	8
Mean	**2.4**	**104.33**	**0.41**	**1.13**	**124.75**	**52.25**	**12.94**	**23.08**	**217.22**	**36.45**	**66.634**	**482.47**	**13.812**	**9.5669**	**26.6731**	**13.88**	**9.74**
F-test	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**
CV	**26.29**	**15.8**	**10.49**	**14.62**	**20.2**	**7.65**	**11.6**	**10.6**	**13.11**	**11.9**	**5.56**	**12.13**	**5.27**	**7.32**	**5.92**	**12.36**	**21**
Min.	**1.13**	**100**	**0.33**	**0.61**	**96.75**	**33.39**	**10.66**	**18.16**	**142.5**	**29.78**	**55.6**	**339.14**	**11.52**	**8.04**	**20.75**	**8.53**	**5**
Max.	**4.04**	**111.5**	**0.53**	**1.61**	**147.25**	**65.76**	**15.66**	**27.66**	**314**	**44.15**	**85.19**	**632.65**	**16.22**	**11.38**	**35.45**	**18.55**	**20**

GY=-grain yield (t ha^{-1}), AD= anthesis date, EPO= ear position, EPP=number of ears per plant, PH= plant height (cm), EH=ear height (cm),NKRE=number of kernel row per ear, NKR=number of kernels per row ,TKWT=-thousand kernel weight and ED=ear diameter, LL=Leaf length, LA=Leaf area, LN=Leaf number, LW=Leaf width, TL=Tassel length, TPL=Tassel peduncle length , NPB=number of primary branch on tassel

5.1.2. Morphological characterization based on qualitative data

The 10 characters recorded and their variations are presented in Table 5. A narrow range of variation was observed for most traits. Most of the lines, about (88.5%,) showed higher level endosperm modification, scores between 1 and 2 . Only one line (L11) scored greater than 2.0 (75% modified kernels) while two lines (L12 and L13) scored 2. With regards to leaf orientation, the inbred lines were classified in to four groups, 58% had pendant leaves, 12% semi-pendant, 12% erect and the remaining 18% had semi-erect leaf orientation. Husk covering was predominately intermediate to good.

Classification based on kernel type of QPM inbred lines were categorized into two types; flint and semi-flint. There were 21 inbred lines with flint, 5 with semi-flint grain texture (Table 5). Ear aspect of the lines also showed variation from 2.26 to 3.89 with the mean of 2.98 (0-5 scale), the line that was with best ear aspect was L23 while T2 was the poorest. The values for plant aspect ranged from 1.9 to 3.23 (0-5 scale); L22 exhibited good plant aspect while L10 and L13 were the poorest.

Table 5. Means of 10 qualitative traits and tryptophan content measured according to maize descriptor (IBPGR) in 26 QPM inbred lines (24 lines and 2 testers) tested at APPRC and HARC

lines	**EM**	**LO**	**SC**	**SKC**	**TC**	**KT**	**HC**	**TS**	**PA**	**EA**	**TRY %**
1	1.75	2.75	2.5	3.25	1	6	4.6	3.5	2.48	3.16	0.06
2	1.75	1.5	1	4.00	1	5.5	1.9	4.1	2.66	2.41	0.058
3	1.5	3	1	4.00	1.75	5.75	1.3	4.3	1.98	3.04	0.057
4	1.6	2.75	1	2.75	3	6	1.2	3.55	2.23	3.54	0.064
5	1.6	3.25	1.75	2.25	1	6	1.5	2.9	2.47	2.64	0.051
6	1.6	3	1	3.75	1	4.75	4.0	2.2	2.4	2.51	0.043
7	1.88	1.5	1	2.75	3.5	5.25	6.2	3.85	2.97	2.39	0.04
8	1.5	1.75	1	2.50	1	6	6.2	2.15	2.47	2.26	0.034
9	1.5	1.5	1	3.00	2.5	5	12.8	4.05	2.97	2.76	0.042
10	1.75	3	1.75	3.25	1	4.5	5.0	2.95	3.23	3.54	0.043
11	2.13	2.25	1.75	3.50	3.75	5.25	2.8	3.4	1.9	2.76	0.042
12	2	3	1.75	3.00	1	6	1.6	3.25	2.66	2.66	0.046
13	2	3.5	1.75	3.50	1	5.25	6.8	4.7	3.23	2.79	0.071
14	1.88	2.5	2.5	3.50	1	5.5	24.6	4.55	2.23	3.66	0.056
15	1.4	3	1	3.25	1	5.5	4.9	3	2.22	3.01	0.059
16	1.88	2.25	2.75	2.50	1	5.25	6.9	3.05	2.97	3.26	0.091
17	1.63	2.75	1.25	5.00	2	5	6.4	2.5	2.65	3.39	0.049
18	1.88	3	1	2.00	1	6	2.4	2.4	2.15	2.64	0.054
19	1.63	2	1	4.00	3.25	5.5	2.5	2.5	2.15	3.14	0.038
20	1.5	1	1.75	5.00	3.25	4.75	3.2	2.25	2.16	2.91	0.043
21	1.5	1	1	2.25	1	6	3.6	2.05	2.41	3.29	0.035
22	1.63	2.75	1.25	5.00	3.5	6	1.5	2.8	1.91	2.66	0.042
23	1.63	1	2.5	4.25	1.75	6	4.1	2.5	2.15	2.64	0.071
24	1.88	2.75	1.75	3.00	1	5.25	1.9	4.4	2.98	3.29	-
25	1.5	2.75	2.75	3.00	2.75	5.75	0.8	3.6	2.66	3.16	0.061
26	1.75	3.25	1.75	3.25	2	6	3.0	2.65	2.4	3.89	0.07

EM=-endosperm modification, LO= Leaf orientation, SC= Stem color, SKC= Silk color, TC=Tassel color, KT= Kernel type, HC= husk cover, TS= Tassel size, PA=plant aspect and EA=ear aspect, Try=Tryptophan percent.

5.1.3. Principal component analysis

Agro-morphological variability was explained by a total of 23 components. The PCA indicated that the first nine principal components (PCs) with eigenvalue greater than unity accounted for 85.4% of the entire diversity among 26 inbred lines for all the 23 traits(Table 6). Under the first PC (14.8%) the most important traits contributing to the variation were leaf length, tassel size, stem color, tassel length, endosperm modification, thousand kernel weight, number of primary branch on tassel and grain yield. The 2^{nd} PC, on the other hand, which explained 13.9 % of the total variation, resulted predominantly from traits such as ear diameter, ear height, silk color, leaf orientation and number of leaves number of leaves, ear height, silk colour,ear diameter, plant height. The 3^{rd} PC, which accounted for 13% of the total variation, was dominated by characters such as, grain yield, anthesis date, tassel peduncle length, and leaf width. The most discriminating traits under PC four were tassel size, ear aspect and plant aspect which accounted for 10.7% of the total variation. Leaf area, Leaf width, Plant height, Leaf orientation, Endosperm modification and Number of kernel row per ear are important traits contributing to more than one principal components.

Table 6. Eigenvectors, eigen values, individual and cummulative percentage of variation explained by the first nine principal components (PC) for 26 traits in 23 highland QPM inbred lines

Characters	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
Leaf length	-0.32	-0.06	0.16	0.18	0.25	0.24	0.09	0.13	0.05
Leaf area	-0.26	-0.12	0.26	0.22	0.18	0.14	-0.17	-0.03	0.24
Leaf width	-0.05	-0.13	0.27	0.19	-0.10	-0.06	-0.42	-0.17	0.39
Number of leaves	0.08	0.41	0.06	0.03	-0.03	0.16	0.13	-0.09	0.07
Tassel length	0.28	0.09	-0.20	0.25	-0.15	0.27	0.00	-0.21	0.03
Tassel peduncle length	-0.14	-0.17	-0.28	0.13	0.18	0.10	-0.14	0.24	-0.29
Number of primary branch on tassel	0.27	0.07	-0.22	0.25	-0.05	0.35	0.10	-0.07	0.00
Number of kernel row per ear	0.01	0.23	0.17	-0.30	0.04	0.33	0.22	0.04	0.20
Number of kernels per row	-0.06	0.01	-0.06	-0.27	0.42	0.13	0.10	-0.16	0.08
Thousand kernel weight	-0.26	0.17	-0.23	0.15	-0.14	-0.31	0.07	-0.17	0.02
Ear diameter	-0.01	0.28	0.09	0.14	-0.10	-0.25	0.22	0.34	0.14
Anthesis date	0.20	-0.01	0.42	0.02	0.08	0.06	0.04	0.04	-0.23
Plant height	-0.09	0.26	0.01	0.19	0.01	0.07	-0.06	0.45	-0.33
Ear height	0.23	0.33	-0.05	0.07	0.15	-0.24	-0.08	0.15	0.20
Ear position	0.10	0.17	-0.11	0.01	0.38	-0.06	-0.31	0.25	0.29
Ear per plant	0.24	0.02	0.06	0.13	0.43	-0.19	-0.20	-0.03	-0.19
Grain yield	-0.26	0.04	-0.35	-0.05	-0.09	-0.19	-0.12	-0.15	0.05
Ear aspect	0.19	-0.19	-0.05	-0.27	-0.06	-0.01	-0.12	0.13	-0.01
Plant aspect	0.20	-0.20	-0.13	-0.39	-0.03	-0.04	-0.07	0.23	-0.10
Endosperm modification	0.27	-0.21	0.04	0.16	0.13	-0.27	0.12	-0.21	-0.03
Leaf orientation	-0.06	-0.26	0.03	0.23	-0.29	0.20	0.02	0.29	0.08
Stem colour	0.31	-0.15	0.11	0.00	-0.13	-0.17	0.22	0.18	0.34
Silk colour	0.02	0.32	0.03	-0.05	-0.27	-0.02	-0.36	0.05	-0.06
Taselcolour	0.09	0.24	0.20	-0.10	-0.07	0.14	-0.29	-0.30	-0.27
Kernel type	-0.02	0.03	0.18	0.24	0.11	-0.25	0.35	-0.15	-0.26
Tassel size	0.31	-0.12	-0.17	0.33	-0.01	0.13	-0.13	0.01	0.00
Eigenvalues	4	3.76	3.52	2.9	2.4004	2.14	1.62	1.42	1.216
Individual %	14.8	13.9	13	10.7	8.9	7.9	5.9	5.3	5.00
Accumulated variation %	14.8	28.7	41.8	52.8	61.4	69.3	75.2	80.5	85.00

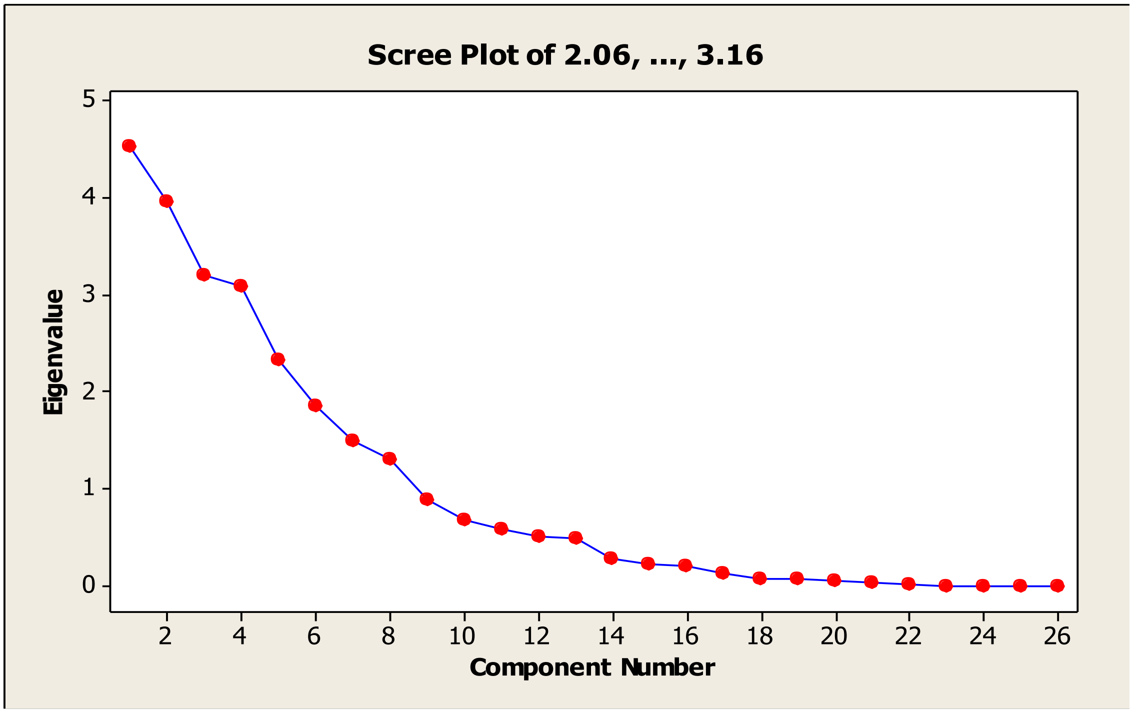

Figure 1. The relative magnitude of contribution of each component to the overall diversity in 23 QPM inbred lines

The relative contribution of the different PCs were plotted and presented (Figure1). There was a sharp decline in contribution from PC1 to PC2 and then from PC2 to PC3 in that order while the rate of decrease in contribution became lower and lower for the remaining PCs. This shows that the first few principal components had the greatest contribution to the overall variation among the inbred lines for the 19 traits considered in this study.

5.1.4. Cluster analysis

Cluster analysis made based on 26 morph agronomic traits grouped the 23 highland QPM lines in to four distinct classes and an outlier (Figure 2). The number of maize genotypes in a particular cluster ranged from two in cluster IV to ten in cluster II. Eigenvectors of each trait in the current study for four clusters are presented in (Table 7). Cluster I consisted of 6 inbred lines were characterized by having lowest leaf orientation, highest number of leaves, maximum anthesis date, tassel size, number of primary branch on tassel, hieghest ear height, highest thausand kernel weight, heighest ear diameter and hiest ear position. On the other hand, cluster II consisted of 10 inbred lines that exhibited the maximum number of leaf orientation, silck colour and maximum plant aspect. Inbred lines in cluster III were characterized by the highest leaf area, leaf width, highest tassel length, plant height, highest number of kernel per row, highest number of ears per plant and highest grain yield. The unique features of inbred line in cluster IV were greatest number of kernels row per ear, tassel color, stem colour, highest tassel peduncle length, ear aspect, highest endosperm modification and highest kernel type. (Table 7). Maize line T2 were observed as an outlier against the remaining lines.

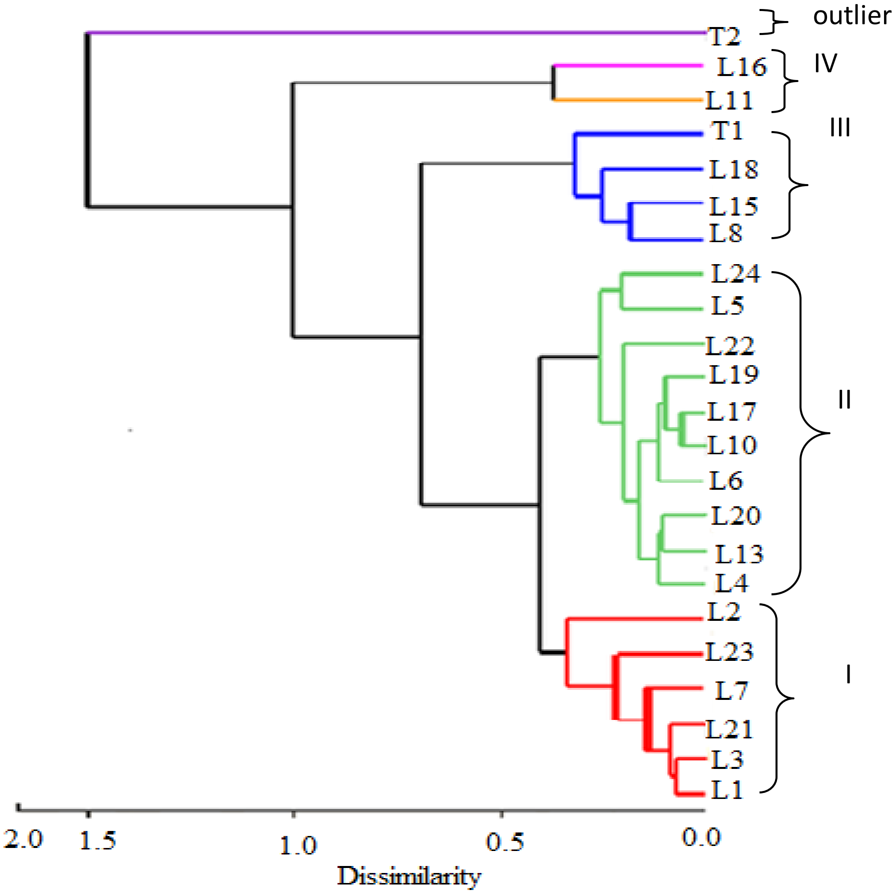

Figure 2. Dendrogram of 23 maize inbred lines revealed by UPGMA cluster analysis based on morpho-agronomic data. Lines L9, L12 and L 14 were missed

Table 7. Clusters mean of the 26 morpho-agronomic traits of the 23 inbred lines (21 lines and two testers) grouped in four clusters

Characters	CI	CII	CIII	CIV
Leaf length	62.22	70.12	73.67	58.08
Leaf area	432.85	517.42	578.00	363.64
Leaf width	9.18	9.87	10.28	8.205
Leaf orientation	1.79	2.78	2.63	2.25
Stem colour	1.50	1.43	1.44	2.25
Silckcolour	3.42	3.55	2.69	3
Number of leaves	14.19	13.99	13.23	12.21
Anthesis date	105.33	104.25	104.50	101.5
Tassel size	3.38	3.07	2.79	3.22
Tasele colour	1.67	1.78	1.44	2.37
Tassel length	25.07	27.17	31.18	26.45
Tassel peduncle length	13.02	14.54	13.47	16.22
Number of primary branch on tassel	9.96	9.19	6.37	9.77
Plant aspect	2.44	2.54	2.38	2.44
Plant height	121.94	127.08	127.68	113
Ear height	55.18	51.80	51.95	41.27
Number of kernel row per ear	12.91	12.75	13.08	13.33
Number of kernels per row	23.32	22.62	24.96	23.25
Thousand kernel weight	231.92	214.35	220.38	162
Ear diameter	37.72	36.82	34.56	34.53
Ear position	0.46	0.40	0.40	0.36
Ear per plant	1.20	1.10	1.24	1.12
Grain yield	2.58	2.39	2.91	1.77
Ear aspect	2.82	2.99	2.77	3.01
Endosperm modification	1.67	1.71	1.57	2
Kernel type	2.82	2.99	2.77	3.01

5.2.Molecular characterization of inbred lines using SNP markers

The genetic distance between pair wise comparisons of all the 23 inbred lines ranged from 0.16 to 0.35 (Table 8), and the overall average genetic distance was 0.32. The highest value of genetic distance was recorded between L1 and L10 (0.35), while L20 and L19 showed higher similarity with value 0.16. The UPGMA tree generated from genetic distance matrix grouped the inbred lines into four major groups and six sub groups (Figure.3). Cluster I consists of nine lines and cluster II consists of six inbred lines while cluster III consists of only one inbred line. Cluster IV consists of a greater number of lines, compared to the other groups.

Table 8. Estimates of genetic distance based on SNP for all pair-wise comparisons of 23 QPM inbred lines

	L1	L2	L3	L4	L5	L6	L7	L8	L10	L11	L13	L15	L16	L17	L18	L19	L20	L21	L22	L23	L24	T1	T2
L1																							
L2	0.30																						
L3	0.31	0.21																					
L4	0.31	0.31	0.31																				
L5	0.34	0.34	0.34	0.34																			
L6	0.34	0.34	0.34	0.34	0.22																		
L7	0.34	0.34	0.34	0.34	0.18	0.18																	
L8	0.34	0.34	0.34	0.34	0.20	0.17	0.18																
L10	0.35	0.34	0.34	0.34	0.32	0.32	0.31	0.31															
L11	0.34	0.34	0.34	0.34	0.31	0.29	0.30	0.29	0.33														
L13	0.35	0.34	0.34	0.34	0.32	0.32	0.32	0.31	0.33	0.31													
L15	0.34	0.34	0.34	0.34	0.35	0.34	0.34	0.34	0.34	0.34	0.34												
L16	0.33	0.32	0.33	0.33	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.30											
L17	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.33	0.34	0.29	0.28										
L18	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.33	0.34	0.31	0.28	0.17									
L19	0.34	0.33	0.33	0.33	0.34	0.34	0.34	0.33	0.34	0.34	0.34	0.28	0.29	0.30	0.29								
L20	0.34	0.33	0.33	0.34	0.33	0.33	0.33	0.33	0.34	0.33	0.34	0.29	0.28	0.30	0.29	0.16							
L21	0.33	0.33	0.33	0.34	0.34	0.34	0.34	0.33	0.33	0.34	0.34	0.30	0.27	0.29	0.28	0.18	0.17						
L22	0.34	0.34	0.33	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.32	0.31	0.32	0.32	0.29	0.30	0.30					
L23	0.34	0.33	0.34	0.34	0.33	0.33	0.33	0.34	0.34	0.34	0.34	0.29	0.27	0.29	0.29	0.28	0.28	0.28	0.31				
L24	0.34	0.33	0.33	0.34	0.33	0.33	0.33	0.33	0.33	0.33	0.34	0.33	0.33	0.34	0.33	0.33	0.33	0.33	0.33	0.33			
T1	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.33	0.33	0.34	0.33	0.34	0.34	0.34	0.34	0.33	0.33		
T2	0.27	0.30	0.28	0.32	0.33	0.32	0.33	0.33	0.33	0.33	0.33	0.32	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.32	0.29	0.32	

*Inbred lines L9, L12 and L14 were missed in the genotyping

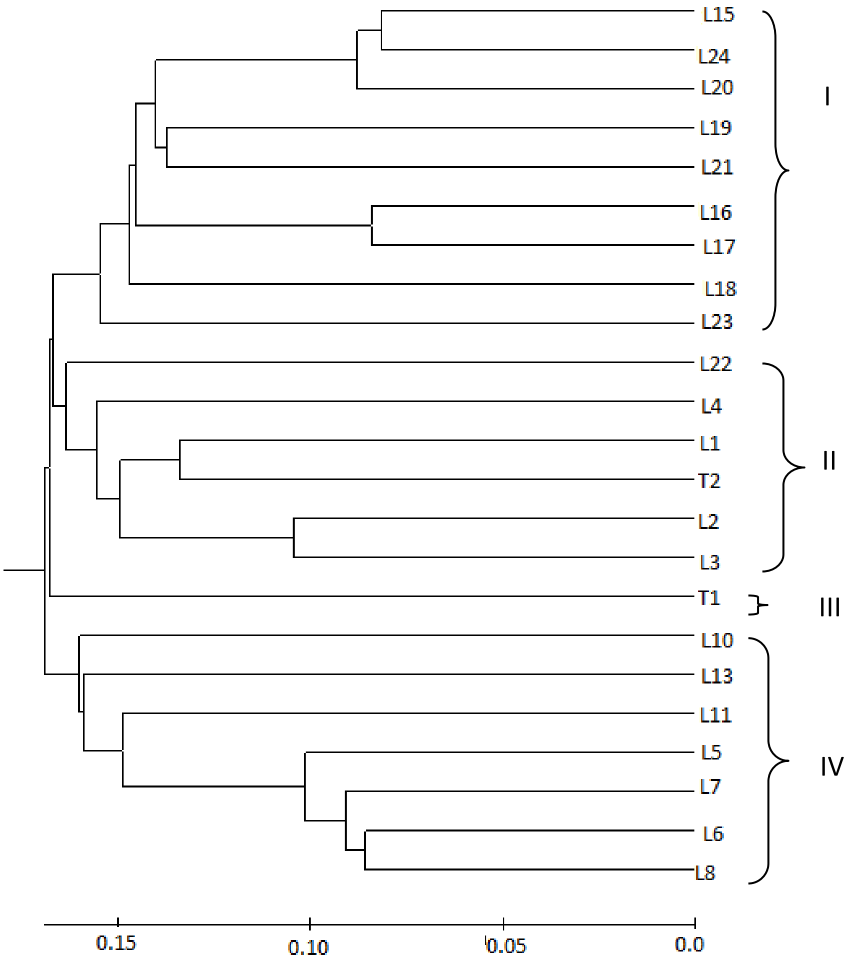

Figure 3. Dendrogram of 23 QPM inbred lines generated by UPGMA cluster analysis based on SNP markers.

Numbers at the side of the figure refers to the inbred lines shown in Table2.

5.3.1. Performance of testcrosses evaluated at four locations

Table 9 presents mean, minimum and maximum values of the16 traits for the 48 hybrid in each of the four locations. Generally mean grain yield showed wide variation among the four test environments. KARC was the highest-yielding environment, with a mean GY of 12.46 t ha^{-1}followed by HARC (10.94 t ha^{-1}), APPRC (9.36 t ha^{-1}) and AARC (5.74 t ha^{-1}) in decreasing order.

At APPRC grain yield ranged from 4.72 to 9.46 t ha^{-1}, with a mean value of 7.07 t ha^{-1}. Hybrids mean grain yield at HARC, KARC and AARC ranged from 2.91 to 10.94, 5.17 to12.5 and 3 to 5.74 t ha^{-1}, respectively. At APPRC, the highest GY of 9.36 t ha^{-1} was recorded for the cross L17 x T1, which out-yielded the high yielding check, AMH760Q (5.57 t ha^{-1}) by 40.5% whereas the lowest yield of 4.77 t ha^{-1} was recorded from the hybrid L3 x T2. The hybrid that exhibited the maximum GY at APPRC was also the top yielder at KARC (12.46 t ha^{-1}), whereas L15 x T2 was the lowest yielder (5.17 t ha^{-1}) which also showed lowest yield at HARC.

At HARC, the hybrid L18 x T1 was the top yielder with a GY of 10.94 t ha^{-1}, whereas L15 x T2 was the lowest yielding hybrid (2.91 t ha^{-1}). At AARC the highest grain yield of 5.74 t ha^{-1} was recorded from the cross L6 x T1. The lowest grain yielder of the site was recorded for the cross L4 x T1 with grain yield of 3 t ha^{-1}.

Table 9. Mean, minimum and maximum values of grain yield and other agronomic traits of 48 highland QPM F1s evaluated at four locations (APPRC, HARC, KARC and AARC)

	APPRC			HARC			KARC			AARC			
Trait	Mean	Min	max	Mean	Min	Max	Mean	Min	Max	Mean	min	max	Overallmean
GY	7.07	4.72	9.46	7.04	2.91	10.90	7.93	5.17	12.50	4.09	3.00	5.74	6.53
AD	94.40	89.50	99.70	109.00	103.40	115.00	99.30	92.70	106.00	96.30	90.50	105.90	99.00
ASI	1.90	-0.20	4.50	2.70	-2.20	4.40	2.40	-2.30	5.10	3.30	0.60	4.70	2.57
PH	230.50	192.80	262.90	197.20	166.70	234.00	191.80	174.40	215.00	/	/	/	206.50
EH	123.40	89.90	153.70	92.70	66.60	125.00	92.50	78.60	107.00	/	/	/	102.87
EPO	0.53	0.45	0.60	0.48	0.33	0.72	0.50	0.42	0.58	/	/	/	0.50
SL	4.20	3.20	7.80	6.80	0.40	35.50	8.05	1.15	17.73	/	/	/	6.35
RL	5.10	2.30	9.50	10.98	0.10	52.50	4.33	2.65	7.15	/	/	/	6.80
EPP	1.47	1.00	2.08	1.53	0.96	2.13	0.95	2.26	0.94	0.94	0.62	1.22	1.38
HC	11.70	3.10	19.10	16.60	1.00	64.30	7.21	3.12	16.02	5.58	0.10	29.40	10.33
ER	4.40	1.90	8.60	0.72	0.00	7.10	4.37	1.92	9.32	61.40	25.60	97.70	17.73
GLS	1.40	1.00	2.00	1.50	0.70	2.60	/	/	/	1.80	1.40	2.70	1.57
CLR	1.70	1.00	2.60	3.50	2.00	5.00	2.00	1.00	3.00	1.80	1.10	2.60	2.25
TLB	1.70	1.00	2.60	2.60	1.70	3.50	1.30	0.90	1.70	1.80	1.20	2.30	1.85
NP	19.90	17.40	21.20	16.90	7.30	21.80	18.80	15.20	21.40	19.50	15.50	22.10	18.77
EA	2.80	2.00	3.50	2.80	1.70	4.10	2.90	2.30	3.50	3.20	1.90	4.40	2.93
PA	2.50	2.10	2.80	2.50	1.20	4.20	2.60	2.10	3.60	3.50	2.60	4.20	2.77

GY=grain yield (t ha^{-1}),PH=plant height (cm), EH=ear height (cm), GLS=gray leaf sptot (1-5 scoring), TLB=turcicum leaf blight (1- 5 scoring), CLR=common leaf rust (1-5 scoring), EPP=ears per plant (number), PA=Plant aspect, EA=Ear aspect, AD=anthesis date, ASI-=anthesissilking interval, EPO=ear position, SL=stem lodging, RL=root lodging, HC= bad husk cover, ER=ear rot, NP=number of plants per plot. /=missing data

Table 10. Mean grain yield and other agronomic traits of the 48 highland QPM F1s grown at four locations (APPRC, HARC, KARC and AARC)

Code	GY	AD	ASI	PH	EH	EPO	RL	SL	EPP	HC	ER	GLS	CLR	TLB	NP	EA	PA
L1XT1	8.59	105.2	3.7	223.5	125.9	0.53	5.25	6.91	1.62	8.45	1.92	1.2	1.5	1.8	19.8	2.8	2.2
L1XT2	5.79	105.7	3.3	201.1	112.9	0.5	4.00	4.47	1.36	13.35	2.66	1.3	1.5	1.8	17.3	3.3	2.5
L2XT1	7.24	99.7	2.3	221.3	120.3	0.5	4.67	3.00	1.58	7.37	4.35	1.5	2.6	1.7	16.7	2.2	2.4
L2XT2	5.91	99.1	3.4	200.8	99.8	0.49	5.74	4.45	1.24	14.01	5.31	1.6	2.2	1.9	16.4	3.1	3.1
L3XT1	7.93	103	3.5	219	124.5	0.56	4.77	7.21	1.6	7.64	2.11	1.2	1.5	1.4	18.2	2.4	2.6
L3XT2	4.49	101.6	4.7	205	104.8	0.5	3.97	8.14	1.3	14.69	4.39	1.6	1.8	1.6	11.9	3.4	3.3
L4XT1	7.16	101.8	3.1	217.8	116.8	0.52	3.80	4.03	1.59	6.26	2.49	1.6	2.6	2	18.8	2.6	2.9
L4XT2	5.39	101.3	3.6	199.3	113.1	0.54	5.49	6.86	1.23	9.85	5.07	1.4	2.6	2	16.4	2.9	3.1
L5XT1	7.57	101.6	1.7	208.7	108.5	0.51	7.40	6.27	1.45	5.15	2.62	1.7	3.1	1.9	21.1	2.9	2.8
L5XT2	6.33	101.5	3.5	211.9	105.1	0.52	5.40	5.75	1.12	7.83	3.65	1.5	2.4	2	20.8	2.9	2.8
L6XT1	7.28	100.2	2.4	205.3	103.2	0.51	3.49	7.22	1.44	6.56	1.91	1.7	2.9	1.9	19.2	2.8	2.5
L6XT2	7.73	98.8	3.4	190.4	102.3	0.5	4.34	4.43	1.27	9.66	3.74	1.2	2	2	20	2.9	2.5
L7XT1	6.86	103.7	3.9	220.2	104.3	0.5	5.97	12.76	1.58	7.39	1.90	1.4	2.8	1.8	16.4	2.9	3.2
L7XT2	7.37	99.9	4.2	206.3	112.9	0.5	5.00	6.16	1.29	16.71	2.56	1.5	2.6	1.7	19.6	3.1	3.4
L8XT1	6.31	102.2	3.1	206.9	103.2	0.49	4.69	4.37	1.53	5.53	2.31	1.6	2.8	2	16.3	3.1	2.8
L8XT2	7	98.3	3.9	208.2	103.1	0.49	3.76	7.93	1.26	14.67	2.95	1.7	2.5	2	17.8	3.3	2.7
L9XT1	5.97	104.3	2.2	218.2	104.7	0.47	5.98	9.79	1.38	8.07	3.91	1.4	1.9	1.6	17.1	3.1	2.9
L9XT2	4.88	102.5	3.1	218.9	110.4	0.49	3.79	4.13	1.2	11.84	3.44	1.4	2.1	1.7	15.7	3.2	3.1
L10XT1	6.16	106.1	2.8	219.2	94.9	0.47	4.58	6.69	1.33	5.49	1.92	2	2.8	1.8	18.9	3	3.5
L10XT2	5.49	101.2	4.7	211.8	104.8	0.49	5.42	10.55	1.27	14.27	3.43	1.7	2.8	1.8	15.1	3.1	3.6
L11XT1	6.15	100.8	4.3	211	108.1	0.47	3.74	4.18	1.38	7.27	2.79	1.7	2.8	2	18	2.8	2.9
L11XT2	5.61	98.5	2.6	213.5	103.6	0.48	4.95	5.90	1.14	7.61	3.19	1.7	2.8	1.7	14.3	2.7	3.1
L12XT1	6.18	101.7	2	215	115.9	0.53	3.48	7.77	1.4	4.17	2.29	2	2.9	2.1	16	2.5	3
L12XT2	6.45	99.6	3.5	202.9	109.2	0.54	4.74	4.79	1.1	5.77	2.42	2	3	1.8	16.9	2.4	2.8
L13XT1	6.49	104.7	4	214.4	120.8	0.53	3.96	4.30	1.44	6.73	1.73	1.5	2.8	2	19.2	3.2	2.7
L13XT2	6.01	103	4.1	205.3	111.2	0.5	4.25	7.32	1.3	9.38	2.85	1.6	2.4	1.9	19.9	3	2.7
L14XT1	5.6	100.1	3.9	207.4	107.3	0.51	5.92	8.00	1.54	11.44	2.63	1.7	3	2.2	16.8	2.7	3.4
L14XT2	4.96	100	3.9	202.8	90.5	0.46	6.46	12.11	1.19	16.35	3.28	1.8	2.9	2	14.7	3.5	3.4
L15XT1	7.07	103	3.2	204.5	115.8	0.49	5.72	11.91	1.68	7.48	2.48	1.8	2.8	1.9	15.8	2.7	3.1
L15XT2	4.87	97.9	2.5	197.6	105.7	0.49	5.40	7.16	1.1	13.84	5.06	2.1	2.7	1.8	13.4	2.9	3.2
L16XT1	5.87	105	4.7	197.8	87.9	0.49	3.95	5.23	1.49	4.35	2.43	1.7	3.5	2.2	19.6	2.7	3.3
L16XT2	5.23	99.5	3.9	186.9	82.1	0.45	3.79	4.30	1.22	14.23	3.50	1.9	3.1	2.1	18.4	3.4	3.4
L17XT1	8.66	106.6	2.9	221.4	113.1	0.51	2.86	6.42	1.66	7.19	2.16	1.5	1.7	1.7	18.6	2.6	2.3
L17XT2	8.05	106.3	4.1	232	112.6	0.52	4.90	6.43	1.29	15.40	2.03	1.2	1.5	1.7	17.7	2.9	2.2
L18XT1	8.74	104.6	0.6	217.7	105.7	0.46	5.12	4.29	1.76	10.44	1.56	1.3	1.6	1.4	20.9	2.7	2.3
L18XT2	6.96	101.1	2.8	205	103.3	0.51	3.28	4.62	1.3	17.56	2.45	1.3	1.7	1.7	20.4	3.3	2.7
L19XT1	6.83	106.8	3.2	207.3	114	0.54	4.96	4.84	1.68	4.50	2.01	1.7	2.7	1.9	18.7	2.9	2.8

L19XT2	5.77	102.2	2.7	199.7	101.6	0.51	4.29	5.74	1.11	12.15	2.77	1.4	2.6	1.6	16.2	3.1	2.9
L20XT1	6.88	103.7	4.2	210.3	111.2	0.51	3.93	5.33	1.55	5.65	2.64	1.9	2.7	1.8	20.9	2.9	2.8
L20XT2	6.68	102.8	3.3	196.8	99.3	0.47	6.81	5.22	1.19	9.08	4.56	1.5	2.6	1.6	19.3	2.9	2.9
L21XT1	6.67	108.1	2.7	215.9	109.1	0.48	4.46	4.46	1.6	9.82	2.09	1.5	2.6	2	18.5	2.7	2.6
L21XT2	6.12	104.3	3.1	205.7	95.3	0.46	4.60	6.50	1.23	8.27	2.16	1.3	2.1	1.5	18.1	3.3	2.8
L22XT1	6.04	107	4.3	224.7	114.3	0.51	3.79	5.98	1.42	7.09	2.82	1.4	3.4	2.4	18.3	3.1	3
L22XT2	6.29	103.2	4	213.2	104.6	0.48	6.23	2.34	1.16	12.83	3.98	1.8	2.7	2.3	17.9	3.3	2.8
23XT1	7.12	110.3	3.1	221	139	0.58	4.89	4.85	1.77	6.20	1.86	1.7	1.9	1.9	17.2	2.7	2.8
L23XT2	6.63	104.4	3.3	223.4	127	0.54	5.25	6.10	1.44	10.27	2.02	1.6	1.9	1.9	15.3	2.9	2.3
L24XT1	7.45	106.4	2.8	215.5	107.3	0.47	3.88	4.69	1.38	6.68	2.67	1.3	2.5	1.8	19.6	2.7	2.6
L24XT2	6.84	101.6	3.3	220.1	93.5	0.46	4.33	3.89	0.98	11.26	2.43	1.8	2.6	1.9	20.2	3.1	2.5
Check 1	6.04	103.5	4	229.6	122.9	0.58	4.35	5.93	1.36	7.59	4.40	1.5	1.9	1.5	19.3	3.5	3.1
Check 2	6.57	99.9	2.9	225	120.8	0.55	3.64	3.75	1.29	8.09	2.98	1.2	1.6	1.4	18.3	3.1	2.8
max	**8.74**	**110.3**	**4.7**	**232**	**139**	**0.58**	**7.40**	**12.76**	**1.77**	**17.56**	**5.31**	**2.1**	**3.5**	**2.4**	**21.1**	**3.5**	**3.6**
CV	**16.28**	**2.6**	**12.3**	**6.5**	**10**	**10.02**	**39.99**	**40.65**	**14.46**	**27.22**	**28.08**	**1.2**	**20.5**	**10.1**	**10.8**	**12.9**	**12.7**
mean	**6.53**	**102.7**	**3.3**	**211.1**	**108.6**	**0.5**	**4.71**	**6.11**	**1.38**	**9.47**	**2.90**	**1.6**	**2.4**	**1.8**	**17.8**	**2.9**	**2.9**
min	**4.49**	**97.9**	**0.6**	**186.9**	**82.1**	**0.45**	**2.86**	**2.34**	**0.98**	**4.17**	**1.56**	**1.2**	**1.5**	**1.4**	**11.9**	**2.2**	**2.2**
Mse	**1.12**	**5.8**	**1**	**108.6**	**110.6**	**0**	**3.29**	**7.92**	**0.05**	**6.06**	**1.59**	**0.1**	**0.1**	**0.1**	**6.1**	**0.2**	**0.2**

GY= grain yield (t ha^{-1}),PH=plant height (cm), EH=ear height (cm), GLS=gray leaf sptot (1-5 scoring), TLB=turcicum leaf blight (1- 5 scoring), CLR=common leaf rust (1-5 scoring), EPP=ears per plant (number), PA=Plant aspect, EA=Ear aspect, AD=anthesis date, ASI-=anthesissilking interval, EPO=ear position, RL=root lodging, SL=stem lodging, HC-=bad husk cover, ER=ear rot, NP=number of plants per plot

Across locations, L18 x T1 had the highest average GY of 8.74 t ha^{-1} followed by L17 xT1 (8.66 t ha^{-1}). Out of the standard varieties used in this study, AMH800 attained the maximum yield (6.57 t ha^{-1}) compared to AMH760Q (6.04 t ha^{-1}) (Table 10). At APPRC, L17 x T1, which yielded the highest, had maximum number of ears and the hybrid with lowest ear number was (L2 xT2). At HARC, the hybrid, L10 x T1, had the lowest ear per plant while hybrid (L23 x T1) had the highest. At KARC, L24 x T2 exhibited the lowest ears per plant whereas L18 x T1 produced the maximum number of ears per plant as well as highest grain yield. The hybrid (L1 x T1) at AARC were the lowest EPP whereas L17 x T1 had the maximum EPP.

L17 x T 2 (2.1) had the best PA and ears per plant at APPRC while L16 x T2 showed poor PA (3.6) with a mean of 2.9. At HARC the best PA was recorded for L13 x T2 while the worst PA (4.2) was recorded from L10 x T2. L22 x T1 was the best at KARC for PA (2.1) while L16 x T2 showed poor PA (3.5) with a mean of 2.6. Cross of L1 x T1 was the best at AARC for ear aspect (2.6) while cross of L14 x T1 showed poor plant aspect (4.2) with a mean of 3.5. The hybrid with best PA across the four environment was a cross between L1 x T1 (2.2) while the hybrid with poor PA was a cross of L10 x T2 (3.6) with a mean PA value of (2.9). At APPRC the best hybrid for EA was a cross made between L17 x T1 (2), this cross scored best for other parameters such as maximum EPP and best in PA while L3 x T2 (3.5) was inferior for EA. At HARC (2.1) L24 x T1 and L14 x T2 (3.6) with a mean value EA of (2.9) were the best and worst for EA for. L4 x T1 (2.3) was best EA at KARC whereas L3 x T2 (3.5) with a mean EA value of 2.9 had the lowest. The best EA at AARC was recorded from L2 x T1 (1.9) while AMH760Q, the check variety, had bad EA score (4.4). L2 x T1 (2.2) showed the best EA across the four testing sites. L14 x T2 showed poor (3.5) EA across the testing environment.

Endosperm modification (EM) score percentages are shown in (Table 11). Range of EM scores were 1 to 5. Based on the mean scores, 95% of both trials evaluated had scores between1 to 3.

Table 11. Percentage of endosperm modification for parental inbred lines and hybrids

Endosperm modification score	percent inbredlines trial	percent hybrid trial
1	68.18	70.19
2	24.77	22.76
3	3.17	2.01
4	1.11	1.69
5	2.77	3.35

5.3.2. Combining ability across locations

Results from the combined analysis of variance over four locations made following the LXT design is given on Table 12. Crosses exhibited was significant (at P<0.01 or P<0.05) mean squares for all the traits except ASI. The interaction between crosses and location was highly significant (P<0.01) for all traits except ASI, TLB, EM, RL and SL. Location effect was significant (at P<0.01 or P<0.05) for all traits indicating the variation in performance of hybrids along the four location. Mean squares due to GCA of both lines and testers were significant (P<0.01 or P<0.05) for all the traits except GY, ASI and EPO for lines and ASI, EPO, GLS, PA, EM and RL for testers.

Interaction between LxE was significant only for five traits (EH, HC, CLR, AD and EA). Mean squares of all traits due to LxT interaction (SCA) were not significant for most of the traits except EA and HC. Mean squares for LxTxE (SCA x E) were not significant for all traits except SL.

Table 12. Combined ANOVA and proportional contribution of GCA and SCA for grain yield and 15 other agronomic traits in 48 highland QPM hybrids evaluated at four locations (APPRC, HARC, KARC and AARC)

Sources of Variation					**Mean square**				
	DF	**AD**	**PA**	**ASI**	**GLS**	**EPO**	**CLR**	**TLB**	**EA**
Location	3	3492.86**	0.15**	0.01**	3.95**	4**	0.12**	17.12**	4.98**
Crosses	47	5091.13**	3.93**	4.77	1.06**	0.18**	2.76**	1.7**	4.55**
Cross x Location	191	6*	4.58**	3.04	0.08**	0.01**	69.69**	12.23	0.17**
Line (Lgca)	23	67.34**	0.81**	0.03	0.36**	0.01	2.01**	0.37**	0.79**
Tester (Tgca)	1	518**	0.01	0.01	0.04	0.02	0.67*	0.03**	4.74**
Lx Location	69	10.69**	0.26**	0.03	0.20	0.01	0.48**	0.14	0.31**
T x location	3	29.09**	1.4**	0.01	0.39	0.01	0.44*	0.15	0.59*
Line x Tester (sca)	23	8.27	0.16	0.04	0.10	0.01	0.23	0.05	0.49**
LxT x location	69	7.56	0.18	0.04	0.10	0.01	0.10	0.09	0.25
Pooled error	**191**	**6**	**0.15**	**0.03**	**0.07**	**0.01**	**0.11**	**0.11**	**0.16**
% GCA (Line)		10.11	82.47	30.13	72	22.22	69.07	82.22	13.12
% GCA (Tester)		88.5	1.03	55.19	8	66.67	23.02	6.67	78.74
% SCA		1.39	16.49	14.79	20	11.11	7.90	11.11	8.14

Sources of Variation					**Mean square**				
	DF	**HC**	**EPP**[†]	**PH**[†]	**EH**[†]	**RL**[†]	**SL**[†]	**EM**	**GY**
Location	3	2.86**	0.86**	43508.8**	18240.38**	4599.19**	33.25.24**	39.6**	287.3**
Crosses	47	195.42*	1.15**	21606**	5358.29**	4.86**	2.26**	2.6*	20.19**
Cross x Location	191	11357.17**	0.07**	165.64**	114.14**	22.2	23.62	3.3	1.47**
Line (Lgca)	23	539.23**	0.17**	724.04**	517.53**	58.53**	70.73**	3.71**	7.44
Tester (Tgca)	1	13390.43**	11.57**	4983.35**	3472.22**	68.39	161.35*	6.9	61.19**
Lx Location	69	150.25*	0.09	194.68	165.02*	45.9	172.24	2.62	2.94
T x location	3	873**	0.35**	147.36	491.40*	43.75	129.45*	5.4	12.87**
Line x Tester(sca)	23	360.04**	0.05	251.06	126.35	69	63.27	2.14	4.36
LxT x location	69	112.40	0.05	202.69	86.37	80.41	115.1*	2.46	2.05
Pooled error	**191**	**97.1**	**0.06**	**165.46**	**114.14**	**77.86**	**94**	**2.6**	**1.46**
%GCA (Line)		3.77	1.44	12.15	12.57	32.93	33.51	29.09	11.49
%GCA (Tester)		93.71	98.13	83.63	84.36	26.85	41.91	54.12	79.54
% SCA		2.52	0.43	0.42	3.07	40.22	24.58	16.8	8.97

*= significant at 0.05 probability level, **= significant at 0.01 probability level

†Data combined over three locations only Ambo, Holetta and Kulumsa (PH, EH, EPP, RL and SL) GY=grain yield (t ha^{-1}),PH=plant height (cm), EH=ear height (cm), GLS=gray leaf sptot (1-5 scoring), TLB=turcicum leaf blight (1- 5 scoring), CLR=common leaf rust (1-5 scoring), EPP=ears per plant (number), PA=Plant aspect, EA=Ear aspect, AD=anthesis date, ASI= anthesissilking interval, EPO=ear position, SL=stem lodging, HC= bad husk cover, NP=number of plants per plot and EM=endosperm modification

5.3.2. Effect of general combining ability

Estimate of GCA effect of lines showed that L17 to be the best general combiner for GY with a highly significant ($P<0.01$) and positive GCA effect of 1.47 t ha^{-1}, followed by L1, L6 and L18 ($p<0.05$) with GCA effect of 0.8, 0.91 and 0.99 t ha^{-1} respectively. L9 and L16 showed highly significant and negative GCA effects of -1.08 and -1.04 t ha^{-1} respectively for GY (Table 13). Both the testers had significant GCA effect for GY but in the opposite direction: T1 with 0.40 and T2 with -0.40t ha^{-1}.L1 and L23 showed highly significant ($P<0.01$) and positive GCA effects of 0.21 and 0.16 for EPP, respectively.

For AD, L2, L6, L7, L8, L11, L12, L14, and L15 showed negative and significant GCA effects across all environments. In contrast, L13, L17, L19, L21, L23, and L24 showed significant and positive GCA effects across all environments. Tester 1 had significant and positive GCA effect whereas Tester 2 showed negative and significant GCA effects across environments (Table 13).

The GCA estimate of parental lines ranged from -8.57 to 19 cm for plant height (PH). L9, L16, L17 and L23 showed positive and significant GCA effects. On the other hand, L6, L14, andL16 showed significant negative GCA effects (Table 13). Regarding the testers, T1, showed positive GCA effects for PH whereas T2 depicted negative and significant GCA effects.

The GCA estimate of parental lines ranged from -14 to 19.33cm for ear EH. L17 and L23 showed positive and significant GCA effects for EH. On contrary L1, L14 and L16 showed negative GCA effect (Table 13).

GCA estimates ranged from -0.48 to 0.43 for PA. L7, L10, L14 and L16 have showed significant and positive GCA effects while L1, L6, L17, L23 and L24 exhibited significant and negative GCA effects. Testers CGA effect for PA was not statistically significant.

Concerning EA, 12 inbred lines exhibited significant GCA effects out of which the GCA effect of the seven lines (L8, L9, L14, and L22) was positive while the other four (L2, L11, 17 and L24) was negative. With equal magnitude of GCA effect T1 contributed negatively and T2 positively for EA. Regarding PA several inbred lines exhibited significant GCA effects such as; (L1, L6, L10, L14, L16, L17 and L24), out those lines three of them were showed with significant and negative effects whereas the other four lines were significant and positive (GCA effects at $P<0.01$ and/or $P<0.05$ (Table 13).

Estimate GCA effect of the 24 lines ranged from -0.24 to 0.29 for GLS and only four lines showed a significant GCA effect. L18 showed negative and significant GCA effect indicating it ability to tolerate GLS. L10, L12, and L15, showed positive and significant GCA effects which imply the susceptibility of crosses where these parents are involved. Nothing is contributed by testers with regard to GLS.

The GCA estimate of lines ranged from -4.15 to 6.43 for SL; L7, L9, L10, L14, and L15 showed positive and significant GCA effects while L18 and L24 showed negative and significant GCA effects for SL. Regarding testers, T1 showed positive and significant GCA effect where as T2 showed negative and significant GCA effect.

General combining ability estimates of parental lines ranged from -3.22 to 4.38 for RL. L5, L14, and L15 showed significant and positive GCA effect whereas L17, L18 and L24 showed negative and significant GCA effects. L17, L18 and L24 are desirable, these parents are good general combiner to develop RL tolerant maize variety. The GCA estimate of parental lines ranged from -11.43 to 12.39 for HC. Parental lines, L7, L8, L14 and L18 showed high and positive significant GCA effects. On the other hand, L5, L11, L12, L19 and L20 revealed negative and significant GCA effect.

Regarding endosperm modification (EM) significantly ($P \leq 0.05$ and $P \leq 0.01$) endosperm modification effect was observed only from four lines. Positive significant GCA effect was recorded for L1 (1.88) and L20 (0.44), while negative effects was observed for two other lines: L3 (-0.65) and L12 (-0.72).

Table 13. Estimates of general combining ability (GCA) effect of lines and tester for grain yield and 15 other agronomic traits combined across four locations (APPRC, HARC, KARC and AARC)

Code	GY	AD	ASI	EA	EH	EPO	EPP	TLB	GLS	HC	PA	PH	CLR	RL	SL	EM
L1	0.8*	1.02	0.04	0.17*	6.16*	-0.02	0.21**	-0.05	-0.21	4.57	-0.35**	5.85	-0.78**	0.05	-2.54	1.88**
L2	-0.02	-1.73*	0.07	-0.24**	-1.26	0	0.03	-0.13	0.01	4.89	-0.04	-0.32	-0.12	-0.28	-2.68	-0.34
L3	-0.33	0.96	-0.01	-0.11	2.83	0.01	0.01	-0.27	-0.15	1.69	0.02	-0.65	-0.59**	0.93	1.46	-0.65**
L4	-0.13	-0.29	0.01	-0.08	5.83	0.01	0.04	0.19*	0.07	-4.66	0.08	-1.32	0.03	0.19	0.72	-0.22
L5	0.27	0.02	-0.04	-0.05	1.41	0.01	-0.12	0.12	0.04	-7.99**	-0.04	-4.49	0.07	3.72**	1.78	0.06
L6	0.91*	-3.35**	-0.06	-0.08	-4.17	0.02	0	0.12	-0.12	-4.73	-0.29**	-8.57*	0.03	-1.58	-0.86	0.22
L7	0.54	-1.6*	-0.02	-0.02	1.74	-0.03	0.11	-0.11	-0.08	8.84**	0.24**	6.85	0.22	1.79	6.42**	-0.19
L8	0.3	-1.85*	-0.08*	0.26**	-5.34	-0.02	0.08	0.12	-0.15	8.97**	0.02	-3.15	0.13	-0.24	1.7	-0.19
L9	-1.08**	0.08	0.05	0.23**	2.33	-0.03	-0.08	-0.1	-0.15	-1.44	0.15	11.26**	-0.31	-2.54	3.85*	0.25
L10	-0.64	1.4	-0.02	0.13	-1.42	-0.01	-0.11	-0.06	0.23*	1.42	0.43**	4.26	0.35*	1	3.6*	-0.12
L11	-0.61	-3.98**	0.04	-0.24**	-2.51	0	-0.16	-0.02	0.17	-7.27**	0.12	-1.07	0.22	0.46	-2.42	-0.22
L12	-0.13	-2.6**	-0.07	-0.52**	4.49	0.04*	-0.18*	0.03	0.29**	-11.43**	-0.04	-0.9	0.41*	0.61	-0.07	-0.72**
L13	-0.45	1.52*	-0.04	0.14	0.41	0.03	-0.08	0	-0.02	-2.97	-0.01	-0.74	0.16	-0.73	-0.63	-0.03
L14	-1.16**	-2.73**	0.05	0.29**	-7.59*	0	-0.05	0.17	0.1	7.84**	0.43**	-8.4*	0.38*	3.94**	6.43**	-0.34
L15	-0.23	-1.92*	0.04	0.01	-3.67	-0.02	0.06	-0.06	0.23*	4.41	0.15	-3.74	0.22	4.38**	5.38**	-0.09
L16	-1.04**	-0.48	-0.02	0.2*	-14.42**	0	-0.09	0.2*	0.17	-0.52	0.43**	19.15**	0.66**	0.45	-1.61	-0.15
L17	1.47**	3.15**	-0.02	-0.27**	9.66**	0.01	0.11	-0.13	-0.15	4.45	-0.48**	17.68**	-0.68**	-2.94*	-2.5	-0.03
L18	0.99**	0.83	0.01	0.04	-2.42	0.01	0.16	-0.25**	-0.24*	12.39**	-0.13	-0.57	-0.72**	-3.22*	-4.15*	-0.09
L19	-0.26	1.83*	0.03	0.14	-0.42	0	0.03	-0.03	0.01	-6.35*	-0.13	-6.82	0.22	0.49	-1.1	0.22
L20	0.37	0.77	0.01	-0.05	-5.17	0	-0.02	-0.08	0.17	-6.85*	-0.1	-6.82	0.19	-0.16	-2.41	0.44*
L21	-0.09	2.58**	-0.04	0.2*	-4.84	0	0.02	-0.1	-0.15	-0.92	-0.13	-1.9	-0.09	-1.7	-1.07	0.1
L22	-0.32	1.4	0.04	0.29**	2.91	-0.02	-0.08	0.33**	-0.02	2.71	0.05	7.01	0.41*	0.19	-3.12	0.19
L23	0.14	3.08**	0.05	-0.14	19.33**	0.08**	0.28**	0.15	-0.02	-2.17	-0.2*	11.01**	-0.47**	-1.95	-2.67	0.13
L24	0.7	1.9*	-0.02	-0.33**	-3.84	-0.07**	-0.17*	-0.06	-0.02	-4.85	-0.32**	4.68	0.07	-2.87*	-3.51*	-0.09
SE	**0.4**	**0.79**	0.04	**0.08**	**3.1**	**0.02**	**0.08**	**0.09**	**0.11**	**2.75**	**0.09**	**3.94**	**0.17**	**1.4**	**1.6**	**0.19**
SEd	**0.11**	**0.85**	0.01	**0.08**	**2.4**	**0.21**	**0.04**	**0.01**	**0.21**	**8.3**	**0.1**	**2.8**	**0.32**	**0.8**	**0.86**	**0.07**
T1	0.40**	1.16**	-0.04	-0.11**	0.01	0.20**	0.20**	0.01	0	-6.63**	-0.03	4.16**	0.07*	0.35	0.67*	-0.13
T2	-0.40**	-1.16**	0.04	0.11**	-0.01	-0.20**	-0.20**	-0.01	0	6.63**	0.02	-4.16**	-0.07*	-0.35	-0.67*	0.13
SE	**0.11**	**0.26**	**0.04**	**0.04**	**0.01**	**0.03**	**0.03**	**0.02**	**0.03**	**1.51**	**0.06**	**0.72**	**0.03**	**0.42**	**0.29**	**0.08**

*= significant at 0.05 probability level, **= significant at 0.01 probability level

GY=grain yield (t ha^{-1}),PH=plant height (cm), EH=ear height (cm), GLS=gray leaf sptot (1-5 scoring), TLB=turcicum leaf blight (1- 5 scoring), CLR=common leaf rust (1-5 scoring), EPP=ears per plant (number), PA=Plant aspect, EA=Ear aspect, AD=anthesis date, ASI= anthesissilking interval, EPO=ear position, SL=stem lodging, HC= bad husk cover and EM=endosperm modification.

5.3.3. Effects of specific combining ability

Estimates of specific combining ability (SCA) effects for GY and other agronomic traits for all hybrids computed across locations are presented in (Table14). Almost half of the crosses (22 of the 48 crosses) showed significant SCA effect; of these 11 were with positive magnitude. The three best crosses among those crosses exhibited significant and positive SCA in increasing order were L3 x T1 (1.25t ha^{-1}), L1 x T1 (1.15t ha^{-1}) and L7xT2 (0.86t ha^{-1}). Concerning AD, cross combinations of L1xT1, L1xT2, L4xT1, L4xT2 , L8xT1, L8xT2, L20xT1, L20xT2, L23xT1 and L23xT2 showed highly significant SCA effect whereas, crosses of L2xT1, L2xT2, L7xT1, L7xT2, L10xT1, L10xT2, L11XT1, L11XT2, L24XT1 and L24XT2 showed significant effect ($P \leq 0.05$). In the case of EA test crosses L2 xT1, L2 xT2, L3xT1, L3xT2, L5xT1,L5xT2, L10xT1, L10XT2, L11xT1, L11xT2, L14xT1, L14xT2, L18xT1, L18xT2, L19xT1, L19xT2, L20xT1 and L20xT2 showed highly significant SCA effects respectively. Whereas, L1xT1 and L1xT2 showed significant ($P \leq 0.05$) SCA effects for this trait. ASI was positive and significant SCA effect for a cross made between L20xT1 while L20xT2 exhibited negative and significant SCA effect for this trait. Tester 2 contributed towards negative SCA effect and T1 contributed toward positive SCA effects (Table 14).

All crosses showed highly significant effects of SCA for ER except crosses of L13xT1, L13xT2, L14xT1, L14xT2, L16xT1, L16xT2, L19xT1, L19xT2, L9xT2, L21xT1, L21xT2, L23xT1, L23xT2, L24xT1 and L24xT2. Crosses L3xT1, L4xT1, L5xT1, L6xT1, L8xT1, L9xT1, L12xT2, L13xT1, L14xT2, L15xT2, L18xT1, L19xT2, L21xT2 and L24xT2 revealed negative and significant SCA effects for TLB.

Table 14. Estimate of specific combining ability (L x T) effects of 48 hybrids for grain yield and 15 other agronomic traits combined across four locations (APPRC, HARC, KARC and AARC)

Cross	GY	AD	ASI	EA	EH	EPO	EPP	ER	TLB	GLS	HC	PA	PH	CLR	RL	SL	EM
L1XT1	1.15**	-0.97**	0.01	-0.14*	4.86**	0.01	0.04	3.07**	0	-0.03	5.45**	-0.06*	4.09*	-0.16**	-2.16	0.89	-1.65**
L1XT2	-1.15**	0.97**	-0.01	0.14*	-4.86**	-0.01	-0.04	3.07**	0	0.03	-5.45**	0.07*	-4.09*	0.16**	2.16	-0.89	1.65**
L2XT1	0.16	-0.72*	-0.03	-0.29**	3.11	0	-0.02	-2.07**	0.02	-0.06*	-6.72**	-0.12**	6.76**	0	2.63*	0.44	-0.12
L2XT2	-0.16	0.72*	0.03	0.29**	-3.11	0	0.02	2.07**	-0.02	0.06*	6.72**	0.14**	-6.76**	0	-2.63*	-0.44	0.12
L3XT1	1.25**	-0.29	-0.02	-0.35**	7.53**	0	-0.09**	1.67**	-0.15**	-0.15**	-1.08*	-0.25**	5.92**	-0.16**	-0.28	-0.84	0.01
L3XT2	-1.25**	0.29	0.02	0.35**	-7.53**	0	0.09**	-1.67**	0.15**	0.15**	1.08*	0.26**	-5.92**	0.16**	0.28	0.84	-0.01
L4XT1	0.32	-1.29**	-0.09	-0.07	-1.64	0.01	0.02	1.86**	-0.04*	0	2.92**	-0.06*	3.26	-0.03	1.31	-2.86**	0.13
L4XT2	-0.32	1.29**	0.09	0.07	1.64	-0.01	-0.02	1.86**	0.04*	0	-2.92**	0.07*	-3.26	0.03	-1.31	2.86**	-0.13
L5XT1	0.23	-0.6	0.03	0.15**	-2.56	0.01	-0.03	3.13**	-0.04*	0.10**	4.07**	0.06*	-5.08*	0.12**	1.57	-1.64	0.17
L5XT2	-0.23	0.6	-0.03	-0.15**	2.56	-0.01	0.03	-3.13**	0.04*	-0.10**	-4.07**	-0.05	5.08*	-0.12**	-1.57	1.64	-0.17
L6XT1	-0.59**	0.03	0.08	0.11	0.86	-0.02	-0.04	-3.19**	-0.08**	0.13**	0.58	0.06*	0.67	0.22**	-1.74	-0.61	0.2
L6XT2	0.59**	-0.03	-0.08	-0.11	-0.86	0.02	0.04	3.19**	0.08**	-0.13**	-0.58	-0.05	-0.67	-0.22**	1.74	0.61	-0.2
L7XT1	-0.86**	0.65*	0.06	0.05	-2.72	-0.01	-0.04	-0.47**	0.03	0.03	-10.67**	0.03	-2.91	-0.03	2.79*	4.21**	0.17
L7XT2	0.86**	-0.65*	-0.06	-0.05	2.72	0.01	0.04	0.47**	-0.03	-0.03	10.67**	-0.02	2.91	0.03	-2.79*	-4.21**	-0.17
L8XT1	-0.81**	1.03**	-0.03	0.02	-3.31*	-0.01	-0.01	-1.93**	-0.04*	-0.03	-10.80**	0.13**	-4.24*	0.06**	2.99**	-0.98	-0.02
L8XT2	0.81**	-1.03**	0.03	-0.02	3.31*	0.01	0.01	1.93**	0.04*	0.03	10.80**	-0.11**	4.24*	-0.06**	-2.99**	0.98	0.02
L9XT1	-0.08	0.09	0.03	-0.07	-2.64	-0.01	-0.03	-0.30**	-0.08**	0.03	1.28**	0.06*	-1.99	-0.19**	1.12	6.29**	0.04
L9XT2	0.08	-0.09	-0.03	0.07	2.64	0.01	0.03	-0.30**	0.08**	-0.03	-1.28**	-0.05	1.99	0.19**	-1.12	-6.29**	-0.04
L10XT1	-0.14	0.65*	-0.06	0.22**	-5.56**	-0.04*	-0.15**	2.16**	0.02	0.10**	-3.58*	-0.03	-7.99**	-0.03	2.91*	-5.35**	0.29
L10XT2	0.14	-0.65*	0.06	-0.22**	5.56**	0.04*	0.15**	-2.16**	-0.02	-0.10**	3.58*	0.04	7.99**	0.03	-2.91*	5.35**	-0.29
L11XT1	-0.05	-0.72*	-0.03	0.15**	-3.47*	-0.03	-0.04	1.92**	0	0.03	7.57**	-0.09**	-3.99	-0.03	-1.17	-2.34**	0.13
L11XT2	0.05	0.72*	0.03	-0.15**	3.47**	0.03	0.04	1.92**	0	-0.03	-7.57**	0.11**	3.99	0.03	1.17	2.34**	-0.13
L12XT1	-0.59**	-0.35	-0.03	0.18**	1.19	-0.03	0.02	1.38**	0.08**	0.03	5.50**	0.13**	8.67**	0.03	1.09	1.37	0.07
L12XT2	0.59**	0.35	0.03	-0.18**	-1.19	0.03	-0.02	-1.38**	-0.08**	-0.03	-5.50**	-0.11**	-8.67**	-0.03	-1.09	-1.37	-0.07
L13XT1	-0.16	0.03	-0.03	0.02	-1.06	-0.01	-0.10**	-0.16	0.05**	-0.03	2.54**	0.03	-2.16	0.09**	-0.79	-1.43	-0.05
L13XT2	0.16	-0.03	0.03	-0.02	1.06	0.01	0.10**	0.16	-0.05**	0.03	-2.54**	-0.02	2.16	-0.09**	0.79	1.43	0.05
L14XT1	0.11	-0.22	-0.01	-0.20**	1.61	0.03	0.04	0.11	0.10**	-0.03	0.5	-0.09**	-0.16	0	-4.07**	-3.45**	0.01

Cross	**GY**	**AD**	**ASI**	**EA**	**EH**	**EPO**	**EPP**	**ER**	**TLB**	**GLS**	**HC**	**PA**	**PH**	**CLR**	**RL**	**SL**	EM
L14XT2	-0.11	0.22	0.01	0.20**	-1.61	-0.03	-0.04	-0.11	-0.10**	0.03	-0.5	0.11**	0.16	0	4.07**	3.45**	-0.01
L15XT1	0.50**	0.59	0.05	-0.04	0.86	0.02	0.16**	0.50**	0.05**	-0.09**	-4.16**	-0.06*	0.17	-0.03	1.19	5.66**	0.2
L15XT2	-0.50**	-0.59	-0.05	0.04	-0.86	-0.02	-0.16**	-0.50**	-0.05**	0.09**	4.16**	0.07*	-0.17	0.03	-1.19	-5.66**	-0.2
L16XT1	-0.18	0.4	0.01	-0.1	4.44**	0.01	0	-0.18	-0.03	-0.03	-4.82**	0.03	3.42	0.15**	1.99	2.47**	0.01
L16XT2	0.18	-0.4	-0.01	0.1	-4.44**	-0.01	0	0.18	0.03	0.03	4.82**	-0.02	-3.42	-0.15**	-1.99	-2.47**	-0.01
L17XT1	0.44*	-0.6	0.06	0.11	-3.97**	0.02	0.02	0.44**	-0.01	0.03	-1.12*	0.06*	-6.24**	-0.07**	-2.44*	-1.13	0.13
L17XT2	-0.44*	0.6	-0.06	-0.11	3.97**	-0.02	-0.02	-0.44**	0.01	-0.03	1.12*	-0.05	6.24**	0.07**	2.44*	1.13	-0.13
L18XT1	0.39*	0.59	0.03	-0.20**	-0.39	0.03	0.05	0.39**	-0.08**	0	-2.23**	-0.09**	1.34	-0.10**	0.22	-0.07	0.13
L18XT2	-0.39*	-0.59	-0.03	0.20**	0.39	-0.03	-0.05	-0.39**	0.08**	0	2.23**	0.11**	-1.34	0.10**	-0.22	0.07	-0.13
L19XT1	0	0.59	0.01	0.21**	-0.06	0	0.12**	0	0.05**	0.13**	0.35	0.03	-1.41	-0.03	2.25*	0.81	-0.05
L19XT2	0	-0.59	-0.01	-0.21**	0.06	0	-0.12**	0	-0.05**	-0.13**	-0.35	-0.02	1.41	0.03	-2.25*	-0.81	0.05
L20XT1	-0.42*	-1.47**	0.12*	0.15**	3.19*	0.02	0.01	-0.42**	0	0.03	4.52**	0.13**	6.26**	0	-2.85*	-0.63	0.17
L20XT2	0.42*	1.47**	-0.12*	-0.15**	-3.19*	-0.02	-0.01	0.42**	0	-0.03	-4.52**	-0.11**	-6.26**	0	2.85*	0.63	-0.17
L21XT1	-0.1	0.46	-0.03	-0.04	-2.14	-0.01	0.04	-0.1	0.05**	0.03	5.38**	-0.03	-5.49**	0.22**	-0.59	0.51	-0.12
L21XT2	0.1	-0.46	0.03	0.04	2.14	0.01	-0.04	0.1	-0.05**	-0.03	-5.38**	0.04	5.49**	-0.22**	0.59	-0.51	0.12
L22XT1	-0.50**	0.4	-0.02	0.05	3.44**	0.02	-0.06*	-0.50**	0.03	-0.09**	-0.24	0.03	4.09*	0.22**	-4.98**	0.96	0.04
L22XT2	0.50**	-0.4	0.02	-0.05	-3.44**	-0.02	0.06*	0.50**	-0.03	0.09**	0.24	-0.02	-4.09*	-0.22**	4.98**	-0.96	-0.04
L23XT1	0.01	1.09**	-0.02	0.11	0.69	-0.01	0.06*	0.01	0.02	0.03	2.89**	0.22**	0.92	-0.16**	-0.95	-0.97	0.04
L23XT2	-0.01	-1.09**	0.02	-0.11	-0.69	0.01	-0.06*	-0.01	-0.02	-0.03	-2.89**	-0.21**	-0.92	0.16**	0.95	0.97	-0.04
L24XT1	-0.08	0.65*	-0.08	-0.01	-2.31	0.01	0.03	-0.08	0.05**	-0.15**	1.86**	0.03	-3.91	-0.07**	-0.02	-1.31	0.07
L24XT2	0.08	-0.65*	0.08	0.01	2.31	-0.01	-0.03	0.08	-0.05**	0.15**	-1.86**	-0.02	3.91	0.07**	0.02	1.31	-0.07
SE	**0.19**	**0.33**	**0.05**	**0.06**	**1.3**	**0.02**	**0.03**	**0.1**	**0.02**	**0.03**	**0.51**	**0.03**	**2.14**	**0.02**	**1.2**	**0.88**	**0.19**
SEd	**0.13**	**0.25**	**0.04**	**0.14**	**2.1**	**0.02**	**0.01**	**0.3**	**0.1**	**0.04**	**0.71**	**0.01**	**3.5**	**0.1**	**1.01**	**1.6**	**0.3**

*= significant at 0.05 probability level, **= significant at 0.01 probability level

GY=grain yield (t ha^{-1}),PH=plant height (cm), EH=ear height (cm), GLS=gray leaf sptot (1-5 scoring), TLB=turcicum leaf blight (1- 5 scoring), CLR=common leaf rust (1-5 scoring), EPP=ears per plant (number), PA=Plant aspect, EA=Ear aspect, AD=anthesis date, ASI= anthesis silking interval, EPO=ear position, SL=stem lodging, HC= bad husk cover, ER=ear rot and EM=endosperm modification.

5.4. Heterosis

Mid parent heterosis (MPH), high parent heterosis (HPH) and standard heterosis were computed and presented in Appendix 7. All crosses showed positive and significant MPH and HPH for GY. However, SH showed positive and significant ($p \leq 0.01$) for all traits except for crosses of L8 x T1, L11 xT1, L12 X T1, L16 xT1 and L22 x T1. Cross of L18xT1 consistently manifested the highest mean percentage of MPH, HPH and SH heterosis for GY. Most crosses attained negative heterosis for EPP. All crosses showed negative and significant MPH for AD except crosses of L4xT1, L4xT2, L9xT1, L9xT2, L10xT1, L10xT2, L24xT1 and L24xT2 whereas all crosses were not significant for HPH except cross of L1xT1, L1xT2, L16xT2, L17xT1, L18xT1, L16xT2, L19xT2, L23xT1 and L23xT2 and L9 x T2.

The positive and significant heterosis observed in most of crosses for PH is an evidence for the increase of plant vigor up on crossing. Ear height also showed positive and significant heterosis over mid parents (MP) and high parents (HP). Plant aspect and EA exhibited positive and significant for both heterosis in all crosses (Appendix 7). The percentage MPH for grain yield ranged from 85.6 to 348.41% with a mean of 222.34% while the HPH ranged from 35.96 to 345.39% with a mean of 204.96% (Table 16).

Crosses L1xT1, L11xT2, L18xT1, L18xT2, L19xT2, L23xT1, L24xT1 and L24xT2 showed higher MPH (above 300%) for grain yield. Generally, about 91.66% and 79.17% of the crosses showed above 100% MPH and HPH values for grain yield, respectively (Appendix 7).

Table 15. Mean, minimum and maximum of mid-parent, high parent and standard heterosis values of grain yield and agronomic-traits of crosses of QPM inbred lines evaluated at APPRC and HARC

Mid-parent heterosis				High-parent heterosis			Standard heterosis		
Traits	mean	minimum	maximum	Mean	minimum	maximum	mean	minimum	maximum
Anthesis date	-6.7	-91.4	-1.09	-3.99	-10	3	-0.54	-4.37	3.75
Pant aspect	4.09	-14.1	7.5	48.9	44.4	88.49	-58.6	-59.9	-51.44
Ear height	115	74.9	166.29	99.4	57.2	179.7	-12.5	-33.7	13.6
Plant height	4.09	-58.6	48.87	97.4	-97	177.86	-6.68	-21.7	6.73
Number of ears per plant	37.1	-1.41	103.58	29.7	-58	286.55	-5.07	-35	28.28
Ear aspect	-15	-39.3	17.34	-3.39	-37	40.71	-9.75	-34.7	13.78
Ear position	23	2.14	48.35	31.7	1.32	61.62	-19.9	-29.6	-7.63
Grain yield	222	85.7	348.41	187	36	345.37	5.27	-33.9	47.11

5.4.1 Association of phenotypic distance with heterosis

The relationship between morphological distances with MPH, HPH and SH of grain yield and agronomic traits are presented in table 16. Correlations between heterosis and phenotypic traits based distances were all positive but only few had statistical significance. The MPH for grain yield showed positive association with morphological distances. The correlation of morphological distance to heterosis is non- significant for most of traits. MPH for EPP and EH is correlated to morphological distance. The correlation of morphological distance to SH is significant for GY and EH. In all cases of heterosis, Anthesis date and plant height are not correlated to the morphological distance whereas, heterosis for anthesis date is not correlated to both distances.

5.4.2 Association of molecular distance with heterosis

A diversity measure based on SNP markers has showed significant association with HPH and SH for grain yield and plant height , ($r = 0.32$, $p \leq 0.05$) and with SH for number of ears per plant ($r = 0.32$, $p \leq 0.05$). Correlation coefficient between molecular distance and MPH were non-significant for all traits.

Table 16. Pearson correlation coefficients of SNP based distance and morphological distances with heterosis for grain yield and agronomic traits

	Molecular distance			Morphological distance		
Traits	Mid- parent	High-parent	Standard	Mid- parent	High-parent	Standard
Grain yield	0.23	0.32*	0.32*	0.28	0.30	0.30
Anthesis date	0.2	0.22	0.24	0.22	0.23	0.26
Plant height	0.31	0.32*	0.32*	0.28	0.3	0.31
Ear height	0.24	0.27	0.28	0.33**	0.31	0.32*
number of ears per plant	0.23	0.29	0.32*	0.33**	0.32*	0.31

5.5. Discussion

5.5.1.Trait variation and distribution among inbreed lines and implications for improvements

The existence of significant differences observed among inbred lines indicate the presence of a high level of variation for various characteristics which makes selection possible for improved grain yield and agronomic traits. In this study, 23 QPM inbred lines were characterized using 26 morpho-agronomic traits and SNP markers. Both morpho-agronomic and molecular characterizations were effective in distinguishing the germplasm. More variations were observed for the agronomical characteristics (TKWT, PH, EH, NKR and GY) than were obtained for the morphological traits (Table 7).

The broad range of means of the inbred lines from morpho-agronomic data showed that there is great potential for the development of hybrids or OPVs using these materials. Similar result was reported by several investigators on maize (Hadji, 2004; Akande; Lamidi, 2006; Dagne, 2008; Cleopatra 2012). The existence of a broad morpho-agronomic diversity was further substantiated by principal component analysis, which indicated that the total variation was fairly distributed across the traits followed by molecular characterization using SNP marker. Genetic diversity was high and therefore being useful in improving cultivars for yield.

The present study showed that variation existed between the inbred lines. The low variation shown by some of the qualitative traits indicated a low phenotypic plasticity of these characters, and thus a low environmental effect over the traits. This suggested that characterization of qualitative traits may be done in one natural environment (Gustavo *et al.*, 2011).

Leaf orientation is important trait in relation to ground cover and the distribution of light in the canopy. Some of the lines were able to achieve well ground coverage as well as intercept more light. Lines such as L20, L21 and L23 showed erect and narrow leaves and would be ideal for intercropping. The inbred lines with erect leaves could be planted at higher plant populations, where moisture and fertility are adequate for production of high yields. The majority of inbred lines showed pendant leaf orientation, which is an advantage to control weeds. Inbred lines with pendant leaves obstruct sunlight from reaching the bottom part of the plant hence smoother weeds and would be desirable for farmers who have very limited capacity to deal with weed infestation (Fanadzo *et al.*, 2010). Husk covering is particularly important for farmers as poor husk covering exposes the ear to bird and insect damage. As reported by Warfield and Davis (1996) cob rots may also occur when husk covering is poor, due to trickling of moisture through the tip of the cob.

The existence of broad range of morphological variation among genotypes was further substantiated by PC analysis which indicated that the overall diversity observed could not be explained by a few eigenvectors. The variations in all traits were dissected into 26 PCs which accounted for 100% of the variability existing among the lines. The percentage contribution of the first nine PCs to gross genetic variation obtained in the current study was 85.4%. The separation of inbred lines was mainly due to yield and other agronomic traits such as, plant height, ear height, ears per plant, thousand kernel weight, number of kernel row per ear, number of kernels per row and ear diameter. Principal component 1 represented variables reflecting yield and its components, while PC3 reflected morphological traits that contributed well to yield that is leaf length, leaf area, tassel length, tassel peduncle length and number of primary branch on tassel. Traits such as, leaf length, number of leaves, anthesis date, grain yield, plant aspect, number of kernels per row, number of primary branch on tassel, leaf width and stem color that had high values in the first nine components indicated their importance as maize descriptors and would be useful for differentiation of maize genotypes.

Overall, principal component analysis was able to identify the most important traits for classifying the variation among the inbred lines. Kamara (2003) similarly used PCA to identify traits that accounted for most of the variance among different maize genotypes. The present finding is higher than Yoseph *et al*., (2005) who obtained 71.8%. These authors reported that ear height, number of kernels per row, thousand kernel weight, tassel size, leaf length and width are the traits predominantly contributing to the total variation. Cleopatra (2012) also reported 77.54% contribution to the entire variation.

Genetic distances estimated based on morpho-agronomic data revealed the existence of considerable variability among the inbred lines. Cluster analysis also confirmed the presence of variation among the lines. The dendrogram showed the resolution power of morphological and agronomic traits for grouping maize inbred lines. In some cases, lines closely clustered together are related by pedigree. The high yielding inbred line in cluster IV could be used for the development of high yielding cultivars. Whereas inbred lines in cluster II can be sought to the improvement of reduced ear height, plant height and high grain yield. The outcome of cluster analysis was consistent with the results of PCA, whereby the major differences between clusters were attributed to the same traits that contributed most to the first and second principal components. Previous reports by, Lucchin *et al*., (2003) clustered 20 Italian flint maize landraces into groups using morphological and agronomic traits. Yoseph *et al*., (2006) classified 62 traditional highland maize accessions into three groups using 15 morphological traits. In diversity analysis of 45 maize inbreds, Gerdes and Tracy (1994) successfully grouped closely related inbred lines by morphological data in agreement with pedigree data. However, reports consistently indicate that morphological markers have shortcomings in that they are highly influenced by prevailing environmental conditions (Gerdes and Tracy, 1994; Yoseph *et al*., 2005).

Several research reports revealed grouping using the UPGMA clustering methods follows pedigree data (Warburton *et al*., 2002; Betran *et al.,* 2003a; Cleopatra 2012). Cluster analysis revealed four clusters. According to the pedigree data, lines L1, L2, L3 and L4 showed a close relationship having a common parent TUXCML159 hence, grouped in same cluster. However, L22 was also clustered with this group. All of SNP based clustering was the same with pedigree data and this is in agreement with earlier studies (Vaz Patto *et al*., 2004; Legesse *et al*., 2007 and Dagne 2008). Inbred lines L5, L6, L7, L8, L10, L11 and L13 were grouped in the same cluster and have line SADVLACML176 in common, just as lines L15, L17, L18, L19, L20, L21, L23 and L24 were close due to the common parent P502 SRCML 384X176 in their pedigree. This, therefore, suggests that these lines share the same ancestral parents . A group of closely related lines tend to bring redundant values to a breeding program, and hence a set of genetically unique lines can be chosen based on marker information as suggested by Kassa *et al*., (2012).

5.5.2. Genetic diversity and patterns of distribution and implication for hybrid variety development

Information on genetic diversity and relationship among breeding materials is important in choosing parents for hybrid variety development. Significant differences observed among hybrids for individual and across environments indicate the existence of a high level of variation for various characteristics which makes selection possible for improved grain yield and agronomic trait. Similar results were reported for QPM diallel entries evaluated under (Bhatnagar *et al*., 2004; Xingming *et al*., 2004; Hadji, 2004; Dagne 2008). Hybrid that showed high GY accompanied with other desirable agronomic traits and resistance reaction to major foliar diseases could be included in national varietal trails for possible release. Lower yield observed in QPM hybrids compared to the normal maize hybrid check indicated the need for more breeding effort for the development of competent QPM germplasm. Bhatnagar *et al.*, (2004) also reported that QPM hybrids yield less than the commercial non-QPM hybrid checks.

5.5.3. GXE interaction and gene action in maize lines

Line by tester interaction were not significant for all traits except for EA and HC, indicated that additive gene effects were involved in the control of most of the traits (Vasal *et al*., (1993a; b). The proportion of GCA sum of squares was higher than that of SCA for all traits except RL. This showed the greater contribution of the additive gene effects to genetic variability of the traits than the non-additive one in the crosses. Significant mean squares due to line GCA were observed for most of the studied traits except for ASI, RL, EPO and GY. In line with this finding, Teshale (2001) and Yoseph *et al*., (2011) reported highly significant mean squares due to GCA for almost all traits they studied.

Significant tester GCA mean square was observed in most traits except in ASI, RL, SL, PA, EPO and GLS. Similarly, Aminum and Izge (2013) reported significant mean squares for the testers they used for most of the studied traits. Both line and tester GCA mean squares were greater than the values of their interaction mean squares with environment for most of the studied traits showing that the main effect was important than their interaction effects (Dagne, 2008). Additive genetic variances were more important in controlling the traits in this experiment. The result showed that the lines and testers were significantly different from the crosses in their characteristics as it was reported by El-Gazzar and Khalil (2012). This suggests that effective selection or systematic hybridization could be employed in improving these traits as it was suggested by Dagne (2008). The lines by tester (SCA) mean square in most cases were found to be non-significant, indicating that non-additive genetic variance across the locations was not important.

The highly significant GCA of tester indicated the higher share of additive gene effects to the total variation observed in crosses for GY in this particular set of cross combinations. Similar to the finding of the present study, several researchers have indicated the importance of additive gene effect in the inheritance of different agronomic traits in maize populations (Leta *et al*., 1998; Vacaro *et al*., 2002).

In the current study the main effect of lines and testers contributed most to the total genetic variance in all the studied traits. Contribution of the lines for the total genetic variability observed among the genotypes was 11.5% while that of the tester was 79.53%. The LxT interaction contributed only 8.97% for grain yield (Table 13). Similarly, several authors reported the importance of additive genetic variance in controlling different traits (El-Gazzar and Khalil, 2012; Divan *et al*., 2013).

Absence of the interaction effects might be attributed to the similarity in the experimental site used for the study. On the contrary significant environment by line or tester interaction effect was reported by Bayissa (2004). The contradiction might be attributed to the difference in the genetic background of the materials used and the environmental conditions in which the experiments were conducted.

These inbred lines are desirable parents for hybrid development as well as for inclusion in the breeding programs, as the lines may contribute favorable alleles in the synthesis of new varieties. Similarly, Hadji (2004) and Dagne *et al*., (2010) found significant positive and negative GCA effects for plant height. The proportional contribution of GCA for the total variation was higher indicating the importance of additive gene effects than the non-additive one. Similarly Mosisa *et al.*, (2008) observed higher proportion of GCA sum of squares than SCA sum squares for secondary traits (EH and PH) in 635 CIMMYT tropical mid altitude inbred lines evaluated under contrasting nitrogen (both low and high nitrogen) environments.

The highly significant ($p<0.01$) GCA effect observed for PH and EH in the present study was in accordance with the findings of Vasal *et al.* (1993b) for PH in CIMMYT's subtropical quality protein maize germplasm and Leta *et al.*, (1998) for both PH and EH in Ethiopian maize composites observed a highly significant GCA effects while the SCA effect was not significant in both studies. The higher magnitude and the highly significant GCA of Vasal *et al.*, (1993b) and Leta *et al.*, (1998) might be attributed to the genetic composition of the materials used .

5.5.4.Trait variation and implication for early maturing maize

The presence of high and negative GCA effect for AD indicated the desirability of these lines for early maturity. These lines had a tendency to enhance lateness. The lines used in the current study showed inconsistent performance for GCA effects of anthesis date across environments. However, some lines showed acceptable GCA effects in most of the test environments, indicating the possibility of selection for earliness. Similar results were reported by El-Gazzar and Khalil (2012) on yellow maize inbred lines. Testers used in the current study are late and medium maturing. According to Pswarayi and Vivek (2008) report, late maturing testers would resulted in late testcrosses. Crosses with low anthesis date and anthesissilking intervals, that could potentially escape short duration of rain season are good source materials for the breeding programs. Tester 1 contributed towards lateness while Tester 2 contributed towards earliness.

Anthesis-silking interval is a very important secondary trait in maize breeding for drought tolerance (Pswarayi and Vivek, 2008). So, the inbred lines with shorter anthesis-silking interval identified in the present study are highly desirable for future breeding work in order to fit with climatic change. In conformity, Duraes *et al.*, (2002) reported that lines with negative GCA effects for anthesis-silking interval depicted good yield showing they were more tolerant to drought stress.

5.5.5.Variation in Plant architecture and varietal development

Inbred lines that were significantly GCA for PH contributed to taller plant stature. Similarly, L9 had positive and highly significant GCA effect for PH. These show the tendency to increase ear and plant height in their crosses. Significant and negative GCA effects were exhibited by inbred lines L14 and L16 for ear and plant height, similarly inbred line L6 had negative and significant GCA indicating these lines showed desirable plant stature in their crosses (Table 15). Reasonably shorter stature maize varieties are desirable for lodging tolerance (Gudeta, 2007; Dagne, 2008). In addition, shorter plants are highly preferable for mechanized agriculture over the taller ones because of their suitability for machine harvesting.

The inbred lines that were significant and positive GCA for PA are in the desirable direction for high contribution of good plant conditions such as, uniform, free of diseases and ear placement. Regarding the EPP inbred lines L1 and L23 had significant and positive GCA effects this indicates that this characteristics is vital since it has high contribution for grain yield. While inbred lines L12 and L24 have showed significant and negative GCA effects for this trait.

Inbred line (L18 and T1) could be nominated as potential testers in the breeding program for studying combining ability, classifying newly developed inbred lines into heterotic groups and identification of best single crosses. Moreover, the single cross hybrid can be used for the purpose of identification of best three way crosses for direct variety release.

Regarding GLS, inbred line (L18) was the best parent for tolerance to foliar disease GLS, which was also the best parent for grain yield as well. The finding of this study is in agreement with Menkir (2005), Asefa *et al.,* (2008) and Legesse *et al*., (2009).

Inbred lines that showed positive and significant to lodging are tend to the undesirable and vulnerable effect. On the other hand, inbred lines L18 and L24 revealed negative and significant GCA effects which is to the desirable direction and could be used in breeding program for the development of stalk lodging tolerant improved maize varieties. Similarly, Bhatnagar *et al*., (2004) found significant differences among GCA effects for the same trait.

The negative GCA effects across the test conditions of lines showed their tendency towards increased EA (yield, healthiness, flintiness, row arrangements etc) in their crosses. Parental lines with positive and significant GCA effects are in the direction of undesirable which contributed to open husks. Whereas negative significant GCA is to the desirable direction and contributes plants with good ear character that could be exploited in breeding programs for unopened husk cover Girma *et al.*, (2015) reported similar finding.

SCA is used to designate deviations of certain crosses from expectations on the basis of the average performance (GCA effects) of the lines involved (Sprague and Tatum, 1942). High SCA value indicates the increased concentration of favorable alleles, particularly the significance of non-additive gene action and thus it is generally manifested between crosses of two genetically divergent materials mainly due to the prevalence difference allelic frequency (Legesse *et al*., 2009).

In the current study, good correspondence between SCA effects and mean performance were attained indicating, crosses with the higher value of SCA effect also showed higher values of mean grain yield performance grain yield. Hence, such cross combinations could effectively be exploited in hybrid breeding program in maize research. On the other hand, ten cross combinations L1xT2, L3xT2, L6xT1, L7xT1, L8xT1, L12xT1, L15xT2, L17xT2, L18 xT1, L20 xT1 and L22xT1 expressed negative and significant SCA effects for grain yield which is undesirable as these crosses showed a tendency to reduce grain yield performance. On the contrary, Vasal *et al*., (1992c) argued that positive SCA effects indicate that lines are in opposite heterotic groups while negative SCA effects indicate that lines are in the same heterotic group.

The findings of the current study are in line with that of Nigussie (1999) who reported significant positive and negative SCA effects for grain yield in 8 x 8 diallel study of drought tolerant maize populations at Melkasa . However, Pswarayi and Vivek (2008) and Jumbo and Carena (2008) reported non-significant positive and negative SCA effects for grain yield.

5.5.6 Variation among maize lines and implications for disease resistance

Several crosses were good specific combiners for resistance to ER. Hence, such cross combinations could effectively be exploited in hybrid breeding program in maize research for developing ear rot free genotypes. This finding agrees with Mosisa *et al*., (2008) and Girma *et al*., (2015) who reported significant GCA effect for the same trait.

Most of the crosses were good specific combinations for resistance to TLB. Hence such cross combinations could effectively be exploited in hybrid breeding program in maize research for developing tolerant maize genotypes to TLB. On the other hand, crosses L3xT2, L4xT2, L5xT2, L6xT2, L8xT2, L9xT2, L12xT1, L13xT2, L14xT1, L15xT1, L18xT2, L19xT1, L21xT1 and L24xT1 revealed positive and significant SCA effects, which are undesirable as these crosses showed higher percent incidence in their hybrid combinations. On contrary to this finding, Legesse *et al*., (2009) reported non-significant SCA effects for the same trait. In general, the preponderance of GCA effects for expression of GY and yield related traits considered in this study indicated the possibility for improvement of these traits through simple selection procedures. However, the chance of success could be hampered in the presence of substantial amount of epistatic component. In such cases selection procedure would not be fruitful in immediate progenies and process has to be delayed to later generations when appreciable homozygosity is achieved (Sofi *et al*., 2006). For complex traits like GY and similar traits, recurrent selection procedure that exploits both the additive and non additive component of genetic variation are more appropriate in bringing about a better improvement. Similar suggestion was made by Mosisa *et al*., (2008) for secondary traits.

5.5.7. Selection potential for endosperm modification

The significant differences in mean squares indicated the presence of adequate variation for endosperm modification among genotypes. Additive gene action was more important than non-additive gene action for this trait. This is in compliance with reports by Derera *et al*., (2008). However, in a study by Hossain *et al*., (2008) additive and non-additive gene action had equal contribution to endosperm modification. Inbred line (L1) showed the desirable GCA effect for endosperm modification was also involved in the best specific combiner (L1 x T2) for this trait as would have been expected. Line 16 had the highest general combining ability for endosperm modification (EM) (1.88) (high levels of opaqueness) not desirable. The implications of endosperm modification being under the control of additive genes means that the inheritance cannot be explained only by Mendelian models, but also through quantitative genetic models (Hallauer and Miranda, 1988).

In order to come up with a QPM cultivar, three genetic systems are developed which involve the recessive allele of the *opaque -2* gene, modifiers that confer high lysine and tryptophan and endosperm modifier genes to make the kernels hard. Since all the genotypes had a score for endosperm modification, this implies that they all inherited the *o2* allele in the homozygous state. According to Ignjatovi-micic *et al*., (2008), lysine and tryptophan levels should be monitored when developing new cultivars because the levels of these two amino acids are variable and one could end up with a variety with the *opaque 2 gene,* but low levels of the two amino acids.

The different scores of endosperm modification observed in germplasm used in this study also indicated that there was segregation of genes for kernel hardness. The high endosperm modification scores implied that the kernels had high levels of opaqueness and a soft endosperm. The less opaque kernels indicated the presence of modifier genes as was seen in genotypes with scores 1 to 3. In this study, score of 2 and 3 was considered desirable and guarantees the presence of the *opaque-2* gene and good modification (Vivek *et al.,* 2008).

The present study was aimed to determine superior QPM parental lines in tryptophan and lysine content. These two essential amino acids are highly correlated. The presence of differences among lines indicated the presence of inherent genetic differences among traits studied. The tryptophan content ranged from 0.034 to 0.091 is different from previous reports of Vassal *et al., (*1993): Villegas *et al*., (1999) and Sentayehu (2008). This is may be due to differences in the genotypes studied.

Normal maize which is deficient in tryptophan and lysine is the main sources of calorie in developing countries. The nutritional quality of the diet is usually considered unsatisfactory for adults and children when more calories is derived from cereals like normal maize (Martin *et al.*, 1976) and (Sintayehu 2008). The high tryptophan contents of the studied lines could raise the quality of protein and improves consumer's dietary intake.

5.5.8. Heterosis and its implication for hybrid development

The high level of heterosis observed in the present study was mainly due to the use of inbred parents which on crossing recovered their vigor and yield ability lost during inbreeding. The extent of heterotic response of the F1 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).

Several hybrids showed superiority over their parents for various traits indicating the existence of substantial heterosis in the hybrids and the potential of these inbred lines for hybrid development. For GY, many authors reported the occurrence of significant positive heterosis over mid parent (MP) and high parent (HP) (Ojo *et al.*, 2007; Abdel-Moneam *et al.*, 2009). Abdel *et al.*, (2009) reported high magnitude of heterosis (400%) over MP. However, Vasal *et al.*, (1993a & b) reported a lower level of heterosis which ranged from -10.80 to 15.60% for lowland tropical materials and -14.80 to 11.50% for subtropical materials. The heterosis observed in decreasing direction for days to anthesis is desirable as it indicates the earlier flowering of hybrids compared to the parental inbred lines.

The difference in percentage of heterosis in different reports involving inbred parents, is mainly attributed to the stage of inbreeding of the materials used, the environmental conditions in which they were exposed and the performance of the parental inbred lines. The positive and significant heterosis observed for PH is an evidence for the increase of plant vigor up on crossing. The positive significant showed in this finding is in agreement with previous report by Bayisa *et al.*, (2005), in highland crosses evaluated at Ambo and Kulumsa, they observed positive heterosis except that few crosses showed negative heterosis. Dagne *et al.*, (2007) also reported positive MPH for PH and EH in all crosses they studied. Most QPM hybrids performed better than commercial checks except crosses of L8xT1, L11xT1, L12xT1, L16xT1 and L24xT1, indicating the need for more effort to develop variety using superior crosses identified in the present study.

In maize, breeding for hybrid varieties is a well suitable approach for yield increment through the exploitation of heterosis. The role of genetically divergent germplasm is of primary importance for the phenomenon of heterosis to occur. Such phenomenon has been reported repeatedly in maize where genetically unrelated parents will have better cross performance (Hallauer and Miranda, 1988; Saleh *et al.*, 2002).

5.5.9. Use of Morphological and SNP markers for diversity study

Developing a guideline helpful to select the most heterotic parents is important in hybrid variety development. Theoretical (Falconer and Mackay 1996) and practical (Moll *et al.*, 1965) considerations of heterosis suggest the dependence of heterosis on genetic diversity. Strong association of heterosis to phenotypic distance were observed for few agronomic traits. The ability to provide distance measures between the inbred lines that reflect pedigree relationship ensures a more stringent evaluation of the adequacy of a marker profile data. The fact that maximum distance revealed by both SNP markers and morphological distance between inbred lines with unrelated pedigree is a good indication conferring the ability of the two distance measures to distinguish between maize inbred lines. The efficiency of molecular markers in genetic diversity analysis of maize inbred lines has been proven valuable (Pejic *et al*., 1998; Warburton *et al*., 2002; Bantte and Prasanna, 2003; Pinto *et al*., 2003; Legesse *et al*., 2007). Morphological traits are also useful for preliminary evaluation because it is fast, simple, and can be used as a general approach for assessing genetic diversity among morphologically distinguishable accessions (Yoseph *et al*., 2005). However, compared with morphological traits, SSR markers were more reliable and efficient in grouping the inbred lines according to their pedigree relationships. Morphological markers have shortcomings to detect differences among closely related genotypes and are influenced by prevailing environmental conditions (Smith and Smith, 1992).

In the current study, highly significant positive correlations manifested between SNP marker-based genetic distance and heterosis for grain yield, indicating the effectiveness of molecular markers. Such significant correlations between genetic distance and grain yield, and suitability of molecular distance for predicting the maize single cross performance were reported by several investigators (Barbosa *et al*., 2003; Betran *et al*., 2003d; Xu *et al*., 2004; Kiula *et al*., 2008; Legesse *et al*., 2008;). Highly significant positive correlation of SNP marker distance with plant height is observed. Consistent with the current study, Legesse *et al*., (2008) reported positive and significant correlation between plant height and molecular genetic distance.

The magnitude of correlation coefficients of SNP marker and morphological distances with MPH, HPH and SH of grain yield and agronomic traits were mostly low. This indicates that the two distance measures may not be suitable predictors of heterosis in the set of materials studied. Similar results were previously reported (Parentoni *et al*., 2001; Makumbi, 2005; Legesse *et al*., 2008). Contrary to the current finding, Betran *et al*., (2003d) reported a highly significant correlation (r = 0.80) between genetic distance and SCA effects in tropical maize inbred lines. Melchinger (1999) and Betran *et al.*, (2003d) suggested that hybrid performance and heterosis can better be predicted when genetic distance is smaller than a certain threshold, depending on the germplasm under consideration. In this study, the range of genetic distance estimates of the inbred lines were as high as the genetic distances among unrelated inbred lines in which the correlation between marker-estimated genetic distance and MPH is expected to be weak (Melchinger, 1999). Saghai-Maroof *et al.*, (1997) noted that the level of correlations between genetic distance and heterosis depend on the germplasm used. Several suggestions have been given concerning the low correlation of genetic distance with heterosis. These include lack of linkage between genes controlling the traits measured, unequal genome coverage, random marker distribution and diversified effect of dominance (Charcosset *et al*., 1991; Bernardo, 1992; Kwon *et al*., 2002).

5. CONCLUSIONS AND RECOMMENDATION

5.1. Conclusion

The result of this study showed that the existence of high genetic distance among most pairs of lines, clearly indicating the uniqueness of the majority of the inbred lines used in this study. The results from this study will be useful to breeders in selecting best parental combinations for new breeding crosses.

Combined analysis of variance across four locations showed highly significant ($P< 0.01$) mean squares due to crosses for several parameters studied except ASI. Similarly, GCA mean square due to interaction of lines by location were significant ($P<0.01$ or $P<0.05$) across location for EA, CLR, PA, AD, EH and HC. In the same way, GCA mean square of testers were also significant for AD, PA, CLR, EA, GY, HC, EH ,EPP and SL in the contrary, SCA were non-significant for most traits except, EA and HC, this shows that additive genetic variance was the most important genetic components in controlling the traits than the non-additive genetic variance. The significant mean square due to crosses indicates the existence of genetic variability, which could be exploited for the improvement of traits of interest. In most of the cases, the proportion of GCA sum of squares was higher than that of SCA indicating the greater contribution of the additive gene effects to the genetic variability observed in the crosses.

The GCA effect analysis showed that inbred lines L14, L16, L17, L1, L6 and L18 were found good general combiners for grain yield. Inbred lines L2, L6, L7, L8, L11, L12, L14 and L15 were found good general combiners for earliness. Inbred line L1 was found persistent combiner for GY, EPP and PA while L6 was for GY, AD, PA, PH similarly, L12 was consistent for AD, EA, EPO and EPP whereas L16 for GY, EH, PA, PH. Inbred lines L17, L18 and L23 were good general combiners for (GY, AD, EH, PA, PH), (GY,) and (GY, EH, EPO, EPP and PH), respectively.

Estimation of SCA effects for all the traits studied showed that some combinations of inbred lines had effects that were significantly higher or lower than what had been predicted based on their parental performances. This deviation is usually attributed to genetic variation caused by non-additive gene effects such as dominance and different types of epistasis. The promising crosses observed in this study can be used for future breeding. This study:-

- Confirmed the importance of GCA, indicative of additive gene effects for most traits;
- demonstrated the existence of genetic variability for GY;
- Identified inbred lines that combine well for GY;
- Identified the genetic relationship among inbred lines. In general, the information from this study will be useful for researchers who intend to develop varieties with high GY.

The desirable heterosis observed in this study for grain yield and earliness indicates the potential of the inbred lines for hybrid development. Single nucleotide polymorphism markers and morphological distances estimated between all possible pair-wise comparisons indicated the presence of considerable variations among the inbred lines studied, which can systematically be exploited in the breeding program. Between the two distance measures, however, SNP markers more reliably and efficiently grouped the inbred lines in accordance with pedigree relationship. Molecular marker-based genetic distances showed correlation values with grain yield.

5.2. Recommendation

Combining ability studies containing more inbred lines should be conducted in multi-locational field trials in the highland areas, to determine the best performing inbred lines and single cross hybrids.

This investigation revealed that genetic factors influence the protein tryptophan and lysine contents of the QPM lines. The finding of this study is in agreement with the earlier reports that QPM varieties contain two- fold lysine and tryptophan. However, the magnitude of the tryptophan content observed in parental lines is not adequate amount; hence attention to improvement of this essential amino acid is needed. Therefore, the QPM genotypes could be incorporated in the breeding program targeted in developing cultivars with high content of essential amino acids such as lysine and tryptophan in developing countries.

Even though the correlations of SNP marker and morphological distances with heterosis were significant in some cases, the magnitudes were low to be of predictive value. In general, the two distance measures can be useful for the identification of genetically similar/different genotypes; but of limited importance for predicting heterosis. However, this conclusion calls for further investigation.

Further study is needed in QPM breeding to develop a variety with enhanced levels of essential amino acids through accelerated marker assisted selection.

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7. APPENDICES

Appendix 1. data of yield and yield related parameters of line trial evaluated at APPRC in 2014

Code	GY	AD	ASI	PH	EH	EPO	RL	SL	EPP	HC	ER	MOI	NP	EA	PA
L1	1.97	108.50	2.50	125.00	69.00	0.55	1.80	-4.14	1.10	13.70	2.40	10.15	24.50	3.32	2.48
L2	2.22	102.00	7.00	126.00	58.00	0.46	0.00	-1.79	1.17	2.50	2.97	10.70	23.00	2.32	2.66
L3	1.59	106.50	5.50	126.00	61.00	0.49	0.00	-3.94	1.19	0.00	4.30	12.75	23.00	3.07	1.98
L4	1.73	101.50	2.50	115.50	58.50	0.51	0.00	3.01	1.35	0.00	9.80	11.20	26.00	3.57	2.23
L5	2.25	103.50	3.50	133.50	50.50	0.38	5.15	-2.51	1.30	0.00	9.06	10.90	23.50	3.02	2.47
L6	1.63	100.50	1.50	114.00	45.00	0.40	12.50	4.24	0.94	8.35	12.57	11.75	19.50	3.02	2.40
L7	2.02	100.00	2.00	143.50	53.00	0.37	8.35	3.09	1.42	5.55	21.31	10.10	18.00	3.02	2.97
L8	2.17	101.00	2.00	142.50	56.00	0.39	9.40	-0.01	1.03	15.95	29.26	13.55	23.00	2.77	2.47
L9	2.59	101.00	1.50	136.50	65.50	0.48	10.70	-2.51	1.36	21.45	22.16	13.00	24.50	3.02	2.97
L10	2.32	103.00	2.00	137.00	48.00	0.35	7.15	-3.54	1.26	20.35	15.55	10.00	27.00	3.07	3.23
L11	1.30	102.00	4.50	137.00	54.00	0.40	2.10	-2.01	0.98	0.00	1.67	15.75	21.50	2.77	1.90
L12	2.22	102.00	7.00	141.50	70.50	0.50	0.00	0.56	1.17	0.00	4.57	16.10	24.50	2.32	2.66
L13	1.22	102.50	1.50	135.00	55.50	0.41	12.50	6.56	1.00	25.00	2.40	12.35	16.00	3.07	3.23
L14	0.85	101.00	17.00	121.00	45.50	0.38	1.80	-0.59	0.93	38.10	8.15	11.90	20.00	4.07	2.23
L15	3.51	100.00	2.00	124.50	57.50	0.47	0.00	-0.86	1.81	20.25	1.56	12.15	25.50	3.02	2.22
L16	2.15	101.00	2.00	106.00	39.00	0.37	0.00	-2.51	1.38	32.00	0.36	10.45	24.00	3.27	2.97
L17	1.89	110.00	0.00	124.50	50.00	0.40	0.00	-4.11	0.90	37.00	10.27	14.35	22.00	3.27	2.65
L18	1.50	105.50	4.00	102.50	37.00	0.37	2.40	-4.11	0.93	2.40	4.32	13.80	20.50	2.52	2.15
L19	0.81	109.50	1.50	109.50	44.00	0.40	0.00	-4.11	1.07	0.00	8.42	14.30	11.50	3.77	2.15
L20	1.73	106.50	4.50	118.00	52.00	0.44	3.35	-7.54	1.29	5.00	6.22	11.75	25.00	3.57	2.16
L21	2.68	105.00	2.00	105.00	54.50	0.52	4.75	-7.54	1.11	9.50	6.57	10.45	25.50	3.57	2.41
L22	2.10	106.50	3.50	148.00	67.50	0.46	6.00	-0.54	1.16	0.00	7.97	10.35	27.50	2.32	1.91
L23	1.48	110.00	3.00	125.50	56.50	0.45	3.15	-4.11	1.08	9.40	-0.98	16.60	13.50	2.27	2.15
L24	0.20	103.00	4.00	118.50	43.50	0.37	3.35	1.56	0.35	0.00	2.40	11.20	17.50	3.57	2.98
T1	2.17	111.50	1.00	119.00	55.50	0.47	2.80	-7.54	1.29	0.00	4.77	15.95	23.00	3.07	2.66
T2	0.94	109.00	1.50	114.00	41.00	0.36	0.00	-4.11	0.87	5.90	53.42	14.70	19.50	4.52	2.40
Mean	**1.82**	**104.33**	**3.42**	**124.96**	**53.38**	**0.43**	**3.74**	**-1.89**	**1.13**	**10.48**	**9.67**	**12.55**	**21.88**	**3.13**	**2.49**
LSD (0.05)	**1.05**	**3.00**	**9.21**	**18.40**	**12.04**	**0.07**	**13.13**	**10.27**	**0.36**	**20.82**	**14.15**	**4.22**	**6.35**	**0.88**	**0.72**
MSe	**0.25**	**2.13**	**20.00**	**79.83**	**34.17**	**0.00**	**40.66**	**23.31**	**0.03**	**102.1**	**45.07**	**4.20**	**9.52**	**0.18**	**0.12**
CV	**27.53**	**1.40**	**130.66**	**7.15**	**10.95**	**7.95**	**170.4**	**-255.5**	**15.4**	**96.48**	**69.42**	**16.33**	**14.10**	**13.50**	**13.74**
P	**	***	ns	***	***	***	ns	ns	***	**	***	*	**	**	*
Min	**0.20**	**100.00**	**0.00**	**102.50**	**37.00**	**0.35**	**0.00**	**-7.54**	**0.35**	**0.00**	**-0.98**	**10.00**	**11.50**	**2.27**	**1.90**
Max	**3.51**	**111.50**	**17.00**	**148.00**	**70.50**	**0.55**	**12.50**	**6.56**	**1.81**	**38.10**	**53.42**	**16.60**	**27.50**	**4.52**	**3.23**

Appendix 2. data of yield and yield related parameters of line trial evaluated at HARC in 2014

Code	GY	AD	ASI	PH	EH	EPO	RL	SL	EPP	HC	ER	GLS	CLR	TLB	MOI	NP	EA	PA
L1	2.15	120.02	-12.00	119.50	59.82	0.50	2.33	1.25	0.93	0.00	1.85	1.50	1.61	2.19	13.18	32.25	3.00	1.99
L2	3.72	109.48	3.00	114.80	59.01	0.51	15.56	0.66	1.53	0.00	0.00	1.00	2.17	1.38	11.42	32.13	2.50	2.51
L3	2.91	109.98	3.00	111.00	48.01	0.43	4.56	1.56	1.53	0.00	0.00	1.00	2.17	0.88	14.82	20.63	3.00	3.01
L4	2.60	110.55	3.00	104.50	47.97	0.45	15.68	9.96	1.19	0.00	0.90	1.00	2.32	1.08	18.44	41.24	3.50	2.97
L5	5.83	106.05	3.00	156.50	61.97	0.38	-2.17	4.41	1.57	0.00	0.00	1.50	4.32	2.08	17.49	38.24	2.25	2.47
L6	3.93	103.05	3.00	126.50	43.47	0.33	9.48	10.86	0.91	0.00	0.00	1.00	3.82	1.08	14.54	38.24	2.00	2.97
L7	4.42	104.59	-6.50	136.00	44.77	0.32	1.49	9.66	1.18	6.40	0.00	1.00	2.76	1.88	13.50	32.36	1.75	2.45
L8	5.58	105.98	3.00	138.90	50.51	0.35	1.36	0.21	1.28	3.95	0.00	1.00	3.17	1.88	12.52	35.13	1.75	2.01
L9	4.71	102.98	3.00	137.00	65.01	0.46	-0.04	1.56	1.28	16.65	3.40	1.00	4.17	1.38	11.02	34.13	2.50	2.01
L10	3.92	109.05	3.00	120.00	49.97	0.40	27.78	20.71	1.02	1.45	1.60	1.00	3.82	1.58	12.29	32.74	4.00	3.47
L11	1.11	103.52	1.50	121.50	44.32	0.36	25.13	-0.25	1.10	0.00	0.00	1.00	4.11	2.19	9.88	16.75	2.75	3.99
L12	2.10	111.05	3.00	124.00	53.97	0.41	4.13	2.36	0.66	0.00	8.35	1.00	3.32	1.08	12.84	31.24	3.00	2.97
L13	2.04	107.09	3.00	132.00	60.77	0.45	26.14	15.21	0.76	0.00	0.00	1.00	4.26	2.38	13.15	30.36	2.50	3.45
L14	1.75	102.52	3.00	119.00	37.82	0.32	22.18	-0.25	0.79	32.85	4.75	1.00	4.11	2.19	9.68	27.75	3.25	2.99
L15	3.24	107.52	3.00	147.00	66.82	0.46	2.63	-0.25	1.13	0.00	1.80	1.00	2.11	1.69	13.18	29.75	3.00	2.49
L16	2.52	109.09	3.00	87.50	27.77	0.31	8.04	0.26	1.00	1.60	5.00	1.00	4.26	1.38	9.90	31.86	3.25	2.95
L17	0.38	129.09	3.00	135.50	44.27	0.31	-0.06	0.26	0.31	0.00	0.00	1.00	1.76	0.38	11.68	24.36	3.50	2.45
L18	2.93	111.05	3.00	122.50	37.97	0.30	-2.17	-0.74	1.24	0.00	2.45	1.00	1.32	1.58	17.14	34.74	2.75	1.97
L19	2.13	112.48	3.50	117.50	48.01	0.40	5.81	2.76	1.12	0.00	2.80	1.50	3.67	0.88	16.67	20.13	2.50	2.51
L20	3.61	111.59	3.00	113.50	52.77	0.46	-0.06	0.26	1.94	0.00	0.65	1.00	3.26	1.88	14.60	41.36	2.25	1.95
L21	4.26	-2.41	-0.50	97.00	42.27	0.43	-0.06	0.26	1.67	0.00	0.00	1.00	3.26	1.38	14.90	42.36	3.00	1.95
L22	2.34	113.98	4.00	146.50	64.01	0.42	-1.54	1.31	0.93	0.00	0.00	1.00	4.17	1.88	15.42	34.13	3.00	2.01
L23	2.20	115.59	-11.50	134.00	56.27	0.42	-0.06	0.26	0.64	0.00	0.00	1.00	2.76	1.38	8.40	16.86	3.00	2.95
L24	3.66	111.52	4.00	137.50	65.32	0.47	-0.72	-0.25	1.61	0.00	0.00	1.00	2.61	0.69	18.58	36.75	3.00	1.99
T1	2.19	112.52	4.00	124.50	54.32	0.43	1.93	-0.25	1.24	0.00	0.00	1.50	4.11	2.19	14.18	29.25	3.25	1.99
T2	2.20	114.48	4.00	114.00	41.51	0.36	1.51	-1.24	0.91	0.00	0.00	1.00	3.67	1.38	18.08	26.63	3.25	3.51
Mean	**3.05**	**105.86**	**1.48**	**124.55**	**51.11**	**0.40**	**6.50**	**3.10**	**1.13**	**2.42**	**1.29**	**1.08**	**3.19**	**1.54**	**13.97**	**31.21**	**2.83**	**2.61**
LSD (0.05)	**1.74**	**65.95**	**13.78**	**20.80**	**18.43**	**0.11**	**10.23**	**15.30**	**0.32**	**11.77**	**5.93**	**0.57**	**0.99**	**1.06**	**4.70**	**10.33**	**0.75**	**1.45**
MSe	**0.58**	**1020.6**	**44.78**	**102.09**	**76.93**	**0.00**	**23.41**	**53.82**	**0.02**	**32.65**	**8.30**	**0.08**	**0.22**	**0.25**	**4.11**	**23.94**	**0.13**	**0.49**
CV	**25.05**	**30.18**	**451.92**	**8.11**	**17.16**	**13.03**	**74.49**	**236.77**	**13.77**	**236.20**	**223.28**	**25.75**	**14.75**	**32.45**	**14.51**	**15.68**	**12.87**	**26.70**
p	**	ns	ns	**	*	**	**	ns	**	**	ns	ns	**	*	**	**	**	ns
Min	**0.38**	**-2.41**	**-12.00**	**87.50**	**27.77**	**0.30**	**-2.17**	**-1.24**	**0.31**	**0.00**	**0.00**	**1.00**	**1.32**	**0.38**	**9.68**	**16.75**	**1.75**	**1.95**
Max	**5.83**	**129.09**	**4.00**	**156.50**	**66.82**	**0.51**	**27.78**	**20.71**	**1.94**	**32.85**	**8.35**	**1.50**	**4.32**	**2.38**	**18.58**	**42.36**	**4.00**	**3.99**

Appendix 3. data of yield and yield related parameters of hybrid trial evaluated at APPRC in 2014

Code	GY	AD	ASI	PH	EH	EPO	RL	SL	EPP	HC	ER	GLS	CLR	TLB	MOI	NP	EA	PA
L1 x T1	8.89	92.8	1.3	247.1	148.5	0.6	6.9	4.6	1.95	12.0	2.8	1.2	1.2	1.6	11.2	20.8	2.7	2.5
L1 x T2	5.61	94.2	1.3	224	129.8	0.57	4.0	3.6	1.3	15.7	4.8	1.4	1.5	1.5	15.2	19.8	3	2.5
L2 x T1	7.97	94.6	1.2	247	145	0.58	4.5	3.5	1.48	10.2	4.0	1.5	1.5	1.7	11.2	21	2.3	2.5
L2 x T2	6.33	91.8	1.3	218.2	113.9	0.52	6.0	3.5	1	14.1	5.0	1.3	1.5	1.7	11.1	21.2	2.9	2.5
L3 x T1	8.07	96.6	1.2	240.5	131.4	0.55	6.4	4.8	1.78	11.1	4.3	1.1	1.5	1.3	14	18.7	2.2	2.4
L3 x T2	4.72	94.2	1.2	226.6	122.7	0.54	3.5	5.7	1.22	16.9	7.5	1.3	1.5	1.8	14.2	20.2	3.5	2.5
L4 x T1	8.1	94.9	1.3	237.9	132.1	0.55	4.4	4.9	1.62	9.3	3.1	1.2	1.3	2	11.3	19.9	2.2	2.6
L4 x T2	6.08	95.4	1.2	218.7	125.3	0.57	7.1	6.7	1.45	13.4	6.3	1.5	1.5	2.6	14.4	18.9	2.8	2.8
L5 x T1	6.81	97.1	1.2	225.8	118	0.52	9.5	4.0	1.57	6.8	3.7	1.5	1.4	1.6	13.5	18.4	2.8	2.5
L5 x T2	5.6	93.1	1.3	220.5	113.6	0.5	4.2	3.3	1.14	9.9	4.5	1.5	1.5	2	12.9	20.8	2.4	2.2
L6 x T1	5.8	93.8	1.2	223.5	121.4	0.54	3.4	3.6	1.42	8.2	2.4	1.3	1.5	1.9	11.9	21	3.3	2.5
L6 x T2	6.89	90.4	1.2	207.9	109.1	0.52	2.8	3.6	1.36	12.9	7.5	1.4	1.5	1.9	12	19.5	2.8	2.2
L7 x T1	7.55	94.9	1.2	247.2	133.4	0.54	7.4	7.8	1.81	8.5	1.9	1.5	1.5	1.6	15.1	19.9	2.8	2.5
L7 x T2	8.93	93.2	1.2	219.7	127.3	0.58	5.0	3.6	1.55	18.5	2.2	1.2	1.7	1.5	11.5	21	2.6	2.5
L8 x T1	8.78	95.7	1.2	230.4	124.6	0.54	5.7	3.2	1.84	7.5	2.1	1.5	1.5	1.9	13.8	20.9	3.2	2.8
L8 x T2	7.49	94.2	1.1	229.9	112	0.48	3.5	4.7	1.38	18.6	4.8	1.4	1.5	2.4	12.7	19.2	3	2.5
L9 x T1	7.27	95.2	1.2	230	125.7	0.55	4.8	4.4	1.77	8.9	4.7	1.4	1.4	1.7	12.4	20.8	2.9	2.3
L9 x T2	6.39	93.2	1.3	238.8	128.4	0.53	3.5	4.8	1.47	11.9	3.9	1.2	1.5	1.5	14.2	20	2.3	2.5
L10 x T1	6.55	98.8	1.2	232.8	118.2	0.51	5.6	4.8	1.41	7.7	5.6	1.8	2	1.8	11.4	19.3	2.9	2.6
L10 x T2	6.87	95.9	1.1	237.9	119.8	0.5	3.9	3.5	1.21	16.8	4.3	1.6	1.6	2	15.1	20.8	3	2.5
L11 x T1	6.27	91.2	1.3	238.2	122.8	0.52	4.0	3.7	1.58	10.4	2.5	1.5	1.5	1.9	16.3	18.5	2.4	2.5
L11 x T2	7.37	89.5	1.3	234.4	114.5	0.48	6.4	3.7	1.14	10.7	4.2	1.4	1.5	1.5	15.8	20.1	2.1	2.7
L12 x T1	7.58	93.9	1.3	234.9	132	0.56	3.1	3.3	1.47	3.1	4.9	2	2	1.6	15.9	20.6	2.1	2.6
L12 x T2	5.5	91.8	1.2	228.9	125.9	0.55	2.8	4.6	1.02	7.2	4.9	1.5	1.5	1.8	14.8	20.8	2	2.5
L13 x T1	6.18	95.1	1.2	233	135.3	0.57	3.5	3.4	1.21	8.7	4.2	1.5	1.8	1.6	13.3	20.3	3.4	2.5
L13 x T2	5.91	95.4	1.2	224.8	133.1	0.58	4.5	3.7	1.5	10.6	4.1	1.5	1.8	1.7	11.3	19.8	3.2	2.5
L14 x T1	5.57	94.3	1.2	225.1	120.1	0.53	8.2	3.7	1.58	13.9	3.8	1.6	1.5	2.3	12.7	19.4	2.8	2.5

Appendix 3. continued

L14 x T2	6.91	89.7	1.3	205.6	98.9	0.48	6.7	6.6	1.25	18.2	4.3	1.5	1.5	1.9	14	17.9	3.1	2.5
L15 x T1	9.08	92.7	1.1	233.4	123.7	0.52	5.6	6.3	1.96	10.9	2.0	1.6	1.8	1.8	13.5	20.3	2.4	2.3
L15 x T2	6.28	90.5	1.2	217.1	115.8	0.52	5.7	3.3	1.33	15.5	6.0	1.7	1.7	1.2	12.6	19.1	3.3	2.5
L16 x T1	7.57	94.9	1.3	212.9	104.3	0.5	5.3	3.6	1.88	5.0	2.7	1.5	1.8	2.1	12.3	20	2.4	2.7
L16 x T2	6.37	91.9	1.3	192.8	89.9	0.46	4.7	3.6	1.26	17.4	8.6	1.2	1.4	1.8	11.1	19.3	3.4	2.8
L17 x T1	9.46	97.9	1.2	239.7	135.3	0.56	2.3	3.6	2.08	10.7	2.6	1.3	1.5	1.8	15.1	20.4	2	2.3
L17 x T2	8.03	94	1.3	247.1	123.6	0.49	6.1	5.9	1.23	18.9	4.9	1.5	1.5	1.4	13.3	19.2	2.8	2.1
L18 x T1	8.74	95.8	1.2	231.2	128.4	0.55	7.0	3.4	1.74	17.7	2.8	1.2	1.3	1.3	13.8	20.9	2.4	2.5
L18 x T2	7.47	93.1	1.3	226.6	115.8	0.5	3.3	3.5	1.34	19.1	4.5	1.3	1.5	1.8	13.4	20.2	3	2.5
L19 x T1	6.33	99.7	1.3	226.8	124.3	0.55	6.6	3.5	1.65	5.7	3.7	1.7	2	1.4	13.5	20.3	2.5	2.5
L19 x T2	5.84	97.3	1.3	209.7	108.8	0.52	3.7	3.7	1.1	12.9	5.4	1.7	1.7	1.7	15.6	19.5	2.9	2.3
L20 x T1	7.07	95.6	1.2	228	124.1	0.54	4.4	3.6	1.71	7.9	3.8	1.2	1.8	1.3	11.7	19.4	2.7	2.5
L20 x T2	7.7	95.8	1.2	210.6	115.6	0.54	6.9	4.7	1.35	12.1	4.5	1.5	1.7	1.4	13.3	20.2	2.5	2.3
L21 x T1	7.68	96.2	1.2	236.5	126.2	0.52	5.3	3.7	1.85	15.1	4.7	1.5	1.5	1.7	13.1	19.9	2.4	2.6
L21 x T2	6.27	95.4	1.2	229.2	110.3	0.48	6.1	3.6	1.34	11.7	5.7	1	1.2	1.6	10.8	19.3	3.4	2.6
L22 x T1	7.35	95.2	1.3	242.5	131.8	0.54	3.8	3.6	1.47	7.9	5.7	1.2	1.5	2.1	11.3	20.9	3.1	2.5
L22 x T2	6.53	94.7	1.3	235.4	111.6	0.48	8.2	3.5	1.2	14.0	5.0	1.5	1.5	1.8	14.7	19.5	3.1	2.4
L23 x T1	8.36	95.3	1.3	262.9	153.7	0.58	3.6	4.7	1.93	9.3	1.9	1.5	1.5	2	12.2	19.9	2.3	2.5
L23 x T2	8.99	95.9	1.2	247.8	147.1	0.58	6.8	3.6	1.49	13.7	2.7	1.5	1.7	2.1	12.8	19.8	2.5	2.5
L24 x T1	8.18	97.7	1.2	235.9	121.8	0.52	3.7	4.8	1.53	9.3	3.8	1.5	1.5	1.4	12.5	20.5	2.7	2.3
L24 x T2	6.42	96.9	1.2	236.3	106.6	0.45	5.1	3.9	1.13	14.3	8.6	1.2	1.4	1.3	14.1	18.5	3.1	2.2
Check 1	5.22	94.8	1.2	246.3	131.2	0.53	5.0	3.9	1.48	7.2	5.3	1.3	1.6	1	14.3	17.4	3	2.4
Check 2	6.31	89.7	1.2	248.8	138	0.54	4.3	3.6	1.13	8.3	6.5	1.2	1.5	1.1	11.7	19.9	2.9	2.5
Mean	**7.07**	**94.4**	**1.2**	**230.5**	**123.4**	**0.53**	**5.1**	**4.2**	**1.47**	**11.7**	**4.4**	**1.4**	**1.6**	**1.7**	**13.2**	**19.9**	**2.8**	**2.5**
LSD (0.05)	**2.04**	**2.8**	**0.1**	**18.6**	**13.9**	**0.06**	**3.8**	**3.0**	**0.28**	**4.6**	**2.8**	**0.4**	**0.4**	**0.2**	**3**	**2.2**	**0.7**	**0.4**
MSe	**1.09**	**2.1**	**0.0**	**90.4**	**47**	**0**	**4.2**	**2.2**	**0.02**	**6.7**	**2.1**	**0.1**	**0**	**0.1**	**2.5**	**1.2**	**0.1**	**0**
CV	**14.8**	**1.5**	**6.3**	**4.1**	**5.6**	**5.62**	**40.2**	**35.4**	**9.44**	**22.0**	**32.8**	**16**	**13.5**	**20.7**	**12**	**5.6**	**12.4**	**8.3**
P	**	**	ns	**	**	**	ns	ns	**	**	**	*	*	ns	**	ns	**	ns
Min	**4.72**	**89.5**	**1.1**	**192.8**	**89.9**	**0.45**	**2.3**	**3.2**	**1**	**3.1**	**1.9**	**1**	**1.2**	**1**	**10.8**	**17.4**	**2**	**2.1**
Max	**9.46**	**99.7**	**1.3**	**262.9**	**153.7**	**0.6**	**9.5**	**7.8**	**2.08**	**19.1**	**8.6**	**2**	**2**	**2.6**	**16.3**	**21.2**	**3.5**	**2.8**

Appendix 4. data of yield and yield related parameters of hybrid trial evaluated at HARC in 2014

Code	GY	AD	ASI	PH	EH	EPO	RL	SL	EPP	HC	ER	GLS	CLR	TLB	MOI	NP	EA	PA
L1 x T1	9.98	112.6	4	216.8	102.7	0.51	4.7	4.1	1.93	18.6	0.2	0.9	2.2	2.4	15.6	19.7	3	1.6
L1 x T2	5.55	111.8	2.8	215.1	93	0.47	13.9	1	1.64	25.5	0.4	0.9	2.1	2.6	18.8	14.5	3.3	2
L2 x T1	7.7	105.6	2.8	210.4	95.1	0.46	15.7	11.5	1.81	8.7	3.7	1.7	4.1	2.2	14.7	16	2.5	2.2
L2 x T2	6.38	105.4	2.8	180.2	86.4	0.46	0.1	5.3	1.37	43.2	0.3	1.3	3	3.3	15.6	16.9	3.1	3.3
L3 x T1	9.63	108.1	3	211.5	115.8	0.58	11.7	8.2	1.91	7.9	1.4	1	2.1	1.9	22.1	18.3	2	1.7
L3 x T2	4.13	109.2	2.9	184.9	82.5	0.47	12.7	0.7	1.62	20.8	3.6	0.8	2.5	1.7	15.7	7.3	2.9	3.1
L4 x T1	6.98	109.6	3	209.7	100.3	0.48	19.7	11.3	1.78	5.8	0.2	1.8	3.6	2.9	17.8	16.3	2.5	2.5
L4 x T2	5.59	108.5	3.5	187.7	98.5	0.51	2.9	22.4	1.25	4.8	7.1	1.2	3.8	2.4	15.9	13.9	3.2	2.8
L5 x T1	9.84	108.4	2.8	192	99.8	0.48	8.3	25.1	1.84	13.3	1.2	2	4.5	2.5	17	21.8	3	2.5
L5 x T2	6.88	108.9	2.9	183.3	92.9	0.51	6.1	16.5	1.25	9.1	0.2	0.9	3.2	2.4	14	21	2.2	1.8
L6 x T1	8.91	106	2.7	195	84	0.46	6.2	2.1	1.79	1	0.4	2.2	4.1	2.7	21.1	19	3	2
L6 x T2	9.23	106.2	2.7	203	95.1	0.49	3.7	3.4	1.46	43.3	0.2	0.9	2.9	2.8	20.7	21.1	2.6	1.8
L7 x T1	6.24	109.2	2.9	189.4	75.1	0.44	14.3	10.1	1.69	10	0.2	1.3	4.1	2.5	17.7	13.1	2.6	2.7
L7 x T2	9.33	105.9	2.5	220.2	97.2	0.45	6.7	0.5	1.67	58.5	0.2	1.1	3.9	2.6	16.4	19.9	2.5	3.1
L8 x T1	4.33	103.4	2.7	199	82.1	0.38	14.8	18.2	1.56	11.9	0.1	1.9	3.9	2.5	15.7	12.3	3.3	2.3
L8 x T2	6.82	104.9	2.8	192.3	91.4	0.48	4.7	1.3	1.36	64.3	0.1	1.8	3.5	2.4	16.4	17.4	2.9	2.2
L9 x T1	6.57	111.9	2.8	226.3	84.5	0.39	8.8	17.9	1.47	9.6	0.2	1	3.2	1.9	15.7	15.5	2.7	2.2
L9 x T2	4.04	110.5	4.4	201.4	93.3	0.48	0.4	1.9	1.19	45.3	0	1.6	3.5	2.2	20.4	14.3	3.8	2.9
L10 x T1	3.62	113.4	-0.2	177.9	66.6	0.39	28.5	2	0.96	8.6	2.9	2.1	4.3	2.6	17.2	16.8	3.5	3.8
L10 x T2	5.21	109	2.3	199.6	88.9	0.44	29.8	5	1.12	22.3	0.2	2	4.5	2.6	15.7	13.8	2.8	4.2
L11 x T1	6.98	106.8	2.9	195.1	91	0.44	10.3	2	1.41	6.1	0.6	1.8	4.2	3	17.3	18.3	2.5	2.7
L11 x T2	4.97	106.4	2.9	187.2	92.5	0.46	12.9	8.9	1.01	3.3	0	1.3	4.2	2	14.7	13.5	2.7	2.2
L12 x T1	5.6	110.5	3	208.8	97.4	0.46	18.7	2.6	1.49	8.1	0.3	1.8	3.9	3.2	19.6	13.9	2.2	2.6
L12 x T2	8.56	107.8	3.3	168.8	91.3	0.55	10.4	6.1	1.03	3.2	0.1	2.3	4	2.2	17.9	17.8	2.1	1.9
L13 x T1	7.93	108.3	2.8	197.1	106	0.56	0.7	10	1.49	17.4	2.3	1.5	4	2.4	16.9	19.4	2.4	2.5
L13 x T2	5.86	108.9	2.8	203.6	91.4	0.46	1.6	2.6	1.24	19.1	0.1	1.7	3.5	2.6	19.6	19.1	2.9	1.2
L14 x T1	6.7	103.4	2.9	186.3	942	0.51	52.5	6.6	1.84	3.4	1.9	2.1	3.9	3	14.3	14.4	3.3	3.2

Appendix 4. continued

L14 x T2	4.15	109	2.8	183	82.5	0.48	26.7	6.6	1.26	15.9	0.5	2.1	4.1	2.6	14.4	10.8	4.1	3.4
L15 x T1	5.77	108.8	1.4	204	109.9	0.53	30.6	5.5	1.83	4.1	0.6	2.3	4.1	2.7	20	14.8	3.2	2.9
L15 x T2	2.91	105.1	3	194	95	0.49	19.8	4.9	1.25	35.4	0.1	2.6	4	2.4	17.1	9.8	3.2	3.1
L16 x T1	6.12	108.8	2.8	189.5	72.7	0.42	35	11.9	1.41	13.2	0.6	2	5	3	17.4	19.4	3.6	3.7
L16 x T2	5.46	107.7	2.9	166.7	72.8	0.43	4	6.3	1.22	20.1	1.6	2.2	4.6	3	13.1	19.1	3.2	3.2
L17 x T1	8.7	112.5	3	219.4	89.8	0.41	2.7	1.6	1.73	11.4	0.3	1.2	2.3	2.3	15.3	16.6	2.8	1.8
L17 x T2	10.09	114	3.3	214.4	100	0.55	6.4	6.9	1.66	13.6	0.1	0.8	2.1	2.3	20.3	17.4	2.2	1.4
L18 x T1	10.94	110	3.2	198	80.7	0.33	13.3	1.4	2.01	12.3	0.2	1.3	2.2	2.1	17.9	20.7	2.7	1.9
L18 x T2	9.36	107	2.7	192.3	93.9	0.52	10.5	2.7	1.45	32.1	0.2	1.2	2.2	2.4	13.9	20.4	3	1.8
L19 x T1	5.83	112.1	3.3	200.6	101.7	0.52	11.5	35.5	1.75	9.5	0.4	2	4.1	3.1	16.5	16.7	3.2	2.3
L19 x T2	6.71	107.6	3	189	92.5	0.48	3.6	2	1.45	15.3	0.2	0.7	4	2.3	19.8	16.8	2.8	2.9
L20 x T1	8.29	108.8	2.4	204.6	95.4	0.49	2.9	1.2	1.66	3.9	0	2.1	4.1	2.8	17.4	21.3	2.9	2.6
L20 x T2	8.42	107.6	2.9	175.6	81.4	0.46	5.1	0.8	1.43	10	0.1	1.2	4	2.4	14.1	21.4	2.3	2.5
L21 x T1	7.54	110.6	1.7	187.9	91.8	0.51	0.9	17.7	1.67	10.2	0.6	1.2	3.9	3.2	11.1	16.6	3.6	2.6
L21 x T2	6.22	109.6	2.9	206.8	77.7	0.41	3.6	2	1.33	13.6	0.4	1.2	3.2	1.8	14.4	16	3	2.1
L22 x T1	6.16	114.4	2.8	213	96.6	0.5	7.6	0.9	1.5	12.9	0.2	1.4	4.7	3.5	19.9	17.8	3	3.6
L22 x T2	5.44	113.4	2.9	186.4	95.2	0.49	21.6	2.2	1.23	32.5	0.5	1.7	3.7	3.5	15.2	17	3.3	2.7
L23 x T1	7.9	114.7	3.1	234	125.4	0.58	8.3	3.3	2.13	16.4	0.2	1.8	2.8	2.6	14.1	14.8	2.8	2.4
L23 x T2	6.59	112.2	2.8	212.5	104	0.48	4.1	0.4	2.04	16.5	0	1.6	2.5	3.1	15.4	11.7	2.2	1.6
L24 x T1	9.21	109.3	2.9	197.8	87.8	0.4	1.4	1.6	1.68	9.5	0.8	0.9	3.9	2.5	17	20.5	1.7	2.3
L24 x T2	9.01	109.3	2.6	190.1	79.7	0.44	2.1	6.3	1.19	10	0.1	1.4	4.2	2.9	20.1	21.4	1.7	2
Check 1	8.17	109.7	2.8	170.2	114.4	0.72	5.2	8	1.69	14.9	0	1.6	2.5	2.4	20.3	20.3	2.4	2.9
Check 2	8.15	108.1	-2.2	188.1	104.7	0.55	1.4	3.1	1.61	3.8	0.1	0.9	2	1.9	16.2	17.2	2.5	2.3
Mean	7.01	109	2.7	197.2	92.7	0.48	10.982	6.802	1.53	16.604	0.718	1.5	3.5	2.6	16.9	16.9	2.8	2.5
LSD (0.05)	2.81	5.5	2.6	37.3	22.6	0.12	2.6	14.9	0.54	23.9	2.6	0.8	0.8	0.9	5.6	6	0.91	0.9
MSe	1.98	7.4	1.6	85.6	39.3	0.01	23.9	77.4	0.07	13.8	1.8	0.2	0.2	0.3	8.7	9.5	0.1	0.2
CV	20.05	2.5	4.7	9.4	14.3	14.83	18.6	162.2	17.67	37	21	30.8	11	20.2	17.4	18.2	11.9	19.8
p	**	**	ns	ns	**	**	ns	**	**	**	**	**	**	**	ns	**	ns	**
Min	2.91	103.4	-2.2	166.7	66.6	0.33	0.1	0.4	0.96	1	0	0.7	2	1.7	11.1	7.3	1.7	1.2
Max	10.94	114.7	4.4	234	125.4	0.72	52.5	35.5	2.13	64.3	7.1	2.6	5	3.5	22.1	21.8	4.1	4.2

Appendix 5. data of yield and yield related parameters of hybrid trial evaluated at KARC in 2014

Code	GY	AD	SAI	PH	EH	EPO	RL	SL	EPP	HC	ER	CLR	TLB	MOI	NP	EA	PA
L1 x T1	10.83	101.5	1.2	195.4	98.6	0.51	3.6	9.2	2	4.9	2.6	0.9	1	17.5	19.5	2.5	2.3
L1 x T2	8.84	96	1.5	177.1	81.5	0.47	4.0	5.4	1.64	11.0	3.9	1.4	1.3	15.1	20	3	2.6
L2 x T1	8.7	99	1.3	194.5	86.5	0.45	4.8	2.5	2.03	4.5	7.2	1.7	1.5	17.1	17.5	2.5	2.3
L2 x T2	6.76	102	1.1	184.1	87.2	0.48	5.5	5.5	1.61	13.9	9.3	1.7	1	15.7	15.5	3	2.9
L3 x T1	9.62	103.5	1.3	199.2	100	0.52	3.2	9.7	1.94	4.2	3.1	1.3	1	19.1	18	2.5	2.6
L3 x T2	6.12	101.5	1.2	182.8	86.6	0.48	4.5	10.6	1.55	12.4	7.5	1.4	1	17.1	17	3.3	3
L4 x T1	10.46	98.5	1.3	197.7	99.5	0.52	3.2	3.1	1.91	3.3	3.8	2.3	1.5	17.4	21	2.5	2.1
L4 x T2	7.13	99.5	0.6	178	91.9	0.53	3.9	7.1	1.34	6.3	8.6	1.7	1.3	18.4	19	3.3	2.6
L5 x T1	9.12	100	1.3	191.6	97.4	0.52	5.3	8.6	1.53	3.5	3.7	2.3	1.3	19.8	20.5	2.5	2.6
L5 x T2	8.02	99	1.3	199.9	97.6	0.51	6.6	8.2	1.33	5.7	5.8	2.3	1.5	16.3	20	2.5	2.9
L6 x T1	8.39	95	1.2	185.2	93.8	0.52	3.6	10.8	1.71	4.9	2.5	2.9	1.5	18.5	19	3	2.4
L6 x T2	8.87	94	1.2	175.8	82.5	0.48	5.9	5.2	1.27	6.4	6.1	1.6	1.5	15	19	3	2.6
L7 x T1	9.6	97.5	1.2	190.9	96.4	0.52	4.6	17.7	1.93	6.3	2.3	2.7	1.5	16	20	3	2.9
L7 x T2	7.09	94.5	1.3	190.4	85.1	0.46	5.0	8.7	1.13	15.0	3.9	1.9	1.3	14.6	19	3.3	3.1
L8 x T1	8.95	99.5	1.3	183.3	92.6	0.51	3.6	5.6	1.88	3.6	3.1	2.2	1.5	17.9	20	2.8	2.3
L8 x T2	8.1	93	1.3	183.3	88.8	0.5	4.0	11.2	1.41	10.8	4.5	2.1	1.3	15.7	18.5	3	2.8
L9 x T1	6.39	98.5	1.3	205.5	94.4	0.48	7.1	15.2	1.53	7.2	6.4	1.4	1.3	17.6	18	3	2.9
L9 x T2	5.81	95	1.3	201.5	88.2	0.45	4.1	3.5	1.12	11.7	5.7	1.8	1.3	16.4	17	3.3	2.6
L10 x T1	9.91	101	1.3	203.6	97.9	0.5	3.6	8.5	1.95	3.2	2.0	2.5	1.3	16.3	21	2.8	2.7
L10 x T2	6.69	100	1.3	181.8	91.3	0.52	7.0	17.6	1.67	11.7	5.3	2.1	1.3	15.8	16	3.3	3.1
L11 x T1	8.35	93	1.3	184	82.9	0.47	3.5	4.6	1.58	4.1	4.1	2.3	1.5	17.8	18	2.8	2.4
L11 x T2	5.61	95	1.4	191.1	96	0.51	3.5	8.1	1.15	4.5	5.0	1.9	1.5	17.3	17.5	2.8	3.1
L12 x T1	7.46	95	1.3	195.7	105.7	0.56	3.9	12.3	1.68	5.2	2.6	2.5	1.5	18.2	18.5	2.8	2.6
L12 x T2	6.51	95	1.1	177.7	91.8	0.53	6.6	5.0	1.28	4.3	3.3	2.4	1.3	17	16	2.8	3.2
L13 x T1	8.11	105	1.2	196.4	83.9	0.43	4.5	5.2	1.94	4.7	2.0	2.6	1.8	16.9	19	3	2.3
L13 x T2	7.06	98	1.3	188.1	80.9	0.44	4.0	10.9	1.34	8.2	4.2	2.1	1.5	16.4	20.5	3	3.1
L14 x T1	6.57	99.5	1.3	191.9	93.9	0.49	3.6	12.3	1.75	8.9	3.7	2.9	1.5	16.5	19.5	2.8	2.8

Appendix 5. continued

L14 x T2	5.33	94.5	1.3	197.8	84.6	0.44	6.2	17.6	1.28	14.5	5.1	2.7	1.5	16.4	18.5	3.3	3.1
L15 x T1	8.2	102	0.9	176.6	79.3	0.45	5.9	17.5	1.98	4.1	3.4	2.7	1.5	17.3	17.5	3	2.8
L15 x T2	5.75	97.5	1.2	175.2	81.3	0.47	5.1	11.0	1.18	12.2	8.4	1.9	1.5	15.6	16.5	3.3	3.1
L16 x T1	6.67	99.5	1.2	181.8	97.3	0.55	2.7	6.9	1.68	3.7	3.4	3.1	1.5	16.3	20	3	2.8
L16 x T2	4.95	100.5	0.9	175.5	81.5	0.47	2.9	5.0	1.22	11.0	5.8	2.5	1.5	16.2	18	3.3	3.5
L17 x T1	12.46	102	1.3	201.9	105.6	0.54	3.4	9.3	1.7	3.6	3.0	1.3	1.3	17.2	20.5	2.8	2.4
L17 x T2	10.12	101.5	1.3	214.1	106.4	0.52	3.7	6.9	1.36	11.9	2.6	1	1.5	16.8	18	2.5	2.2
L18 x T1	10.41	104	1.4	202	96.2	0.48	3.2	5.2	2	3.2	1.9	1.5	1	16.3	21	2.5	2.1
L18 x T2	8.46	99.5	1.5	183	92.7	0.51	3.3	5.8	1.51	16.0	3.6	1.5	1	16.4	20.5	3	2.6
L19 x T1	10.19	100.5	1.3	188.8	93.5	0.51	3.3	6.2	1.77	3.3	2.3	2.4	1.5	17.9	21	3	2.4
L19 x T2	6.12	99	1.2	189	93.9	0.52	4.9	7.8	1.11	11.4	3.8	2.1	1	16.9	16	3	2.6
L20 x T1	7.99	97	1.4	193.6	88.2	0.47	3.5	7.1	1.72	3.4	4.1	2.4	1.5	16.2	21	3	2.4
L20 x T2	6.61	102	1.3	181.6	80.2	0.45	6.7	5.7	1.13	6.0	7.6	2.1	1	17.3	18	2.8	2.5
L21 x T1	7.95	105.5	1.3	190	79.7	0.43	3.6	5.2	1.84	4.5	2.7	2.3	1.5	17.3	20.5	3	2.4
L21 x T2	7.29	99.5	1.3	179.3	89.3	0.51	3.2	9.4	1.22	4.8	3.3	1.6	1	14.7	20.5	3	2.8
L22 x T1	7.43	101	1.3	206.2	101.7	0.51	3.7	8.4	1.76	6.2	4.4	3	1.5	15.5	19	3.3	2.1
L22 x T2	8.8	99.5	1.3	190.6	93.7	0.5	4.3	1.1	1.42	11.7	6.5	2.1	1.5	15.3	19	2.8	2.5
L23 x T1	8.93	104	1.3	179.2	99.3	0.56	6.2	5.0	2.13	3.1	2.2	1.4	1.3	16.3	19.5	3	2.8
L23 x T2	6.24	103.5	1.3	197.8	103.1	0.53	3.7	8.6	1.22	6.8	2.5	1.4	1.3	17.1	19.5	2.8	2.4
L24 x T1	8.83	104.5	1.2	198.4	95.9	0.49	4.1	4.5	1.57	4.0	3.8	2.2	1.5	16.8	19	2.5	2.4
L24 x T2	8.02	100	1.3	204.3	99	0.5	3.6	3.9	0.97	8.3	3.7	1.9	1.3	16.2	19	3	2.4
Check 1	7.02	102.5	1.2	213.1	105.3	0.49	3.7	7.9	1.38	8.0	7.5	1.5	1	16.2	18	3	2.8
Check 2	6.94	96.5	1.4	198.3	104	0.54	3.0	3.9	1.35	7.8	4.8	1.3	1	14.9	18.5	3	2.8
Mean	**7.92**	**99.3**	**1.3**	**190.9**	**92.5**	**0.5**	4.3	8.0	**1.55**	**7.2**	**4.4**	**2**	**1.3**	**16.7**	**18.9**	**2.9**	**2.6**
LSD (0.05)	**1.78**	**6.9**		**22.6**	**19.2**	**0.1**	**2.8**	**6.9**	**0.49**	**4.7**	**3.3**	**0.5**	**0.4**	**2.4**	**3.2**	**0.5**	**0.6**
MSe	**0.78**	**11.7**	**0.0**	**124.7**	**88.6**	**0**	**2.4**	**13.7**	**0.06**	**5.5**	**3.1**	**0.1**	**0**	**1.4**	**2.6**	**0.1**	**0.1**
CV	**11.15**	**3.4**	**16.6**	**5.8**	**10.2**	**9.51**	**35.8**	**45.9**	**15.83**	**32.4**	**40.2**	**12.8**	**13.5**	**7.1**	**8.5**	**9.2**	**10.5**
p	**	*	ns	*	ns	ns	*	**	**	**	**	**	**	*	*	*	**
Min	**4.95**	**93**	**0.6**	**175.2**	**79.3**	**0.43**	2.7	1.1	**0.97**	**3.1**	**1.9**	**0.9**	**1**	**14.6**	**15.5**	**2.5**	**2.1**
Max	**12.86**	**105.5**	**1.5**	**214.1**	**106.4**	**0.56**	7.1	17.7	**2.13**	**16.0**	**9.3**	**3.1**	**1.8**	**19.8**	**21**	**3.3**	**3.5**

Appendix 6. data of yield and yield related parameters of hybrid trial evaluated at AARC in 2014

Code	GY	AD	ASI	EPP	HC	ER	GLS	CLR	TLB	MOI	NP	EA	PA
L1 x T1	5.45	97.8	3.7	0.62	6	42	1.6	1.2	2.1	13.1	21.5	3.1	2.6
L1 x T2	3.19	99.6	3.3	0.94	0.2	89.2	1.5	1.2	1.8	11.4	20.7	3.9	2.9
L2 x T1	4.63	93.8	2.3	0.97	1.4	46.3	1.5	1.6	1.4	11.9	21.4	1.9	2.8
L2 x T2	3.6	92.7	3.4	0.95	17.9	73.6	2.3	2	1.9	12.3	18.2	3.4	3.3
L3 x T1	4.37	97.8	3.5	0.78	2.3	48.7	1.6	1.2	1.3	12.2	20.8	2.5	3.4
L3 x T2	3.79	94.1	4.7	0.89	1.2	59	2.7	1.4	2	13.9	19.2	3.1	3.6
L4 x T1	3	94.1	3.1	1.03	0.6	68	1.7	2	2	12.7	15.9	3.4	4.1
L4 x T2	3.64	94.2	3.6	0.9	2.7	42.8	1.5	2.3	2	11.8	19.9	2.7	3.9
L5 x T1	4.6	94.8	1.7	0.88	1	56.4	1.8	2.5	2.2	14.2	20.7	3.4	3.2
L5 x T2	4.1	94.1	3.5	0.74	4.4	75	2.1	1.5	2	13.8	19.8	3.9	3.8
L6 x T1	5.74	94.4	2.4	0.85	1.7	31.9	1.7	1.8	1.5	13.5	21.5	2.4	3.1
L6 x T2	5.61	91.4	3.4	1	0.1	54.5	1.4	1.8	2	11.8	20.1	3.1	3.2
L7 x T1	3.39	98.2	3.9	0.89	0.9	67.1	1.5	1.8	1.7	13.7	20.4	3	4.1
L7 x T2	3.99	93.9	4.2	0.84	23.5	97.7	2.1	2	1.7	11.8	19.5	3.4	4
L8 x T1	3.31	101	3.1	0.81	2.9	54.9	1.5	2.4	1.9	13.8	19	3.3	3.7
L8 x T2	5.13	91.8	3.9	0.88	29.4	78.4	1.7	1.8	1.9	12.6	20.7	4.1	3.1
L9 x T1	3.81	96.6	2.2	0.71	1.7	55.9	1.7	1.2	1.5	13.9	17.8	3.3	3.7
L9 x T2	3.33	94.6	3.1	1.02	7.4	66.1	1.5	1.1	1.9	11.5	18.5	4.1	3.8
L10 x T1	4.23	98.9	2.8	1	0.1	39.4	2	1.7	1.5	12.2	19.6	3.4	3.9
L10 x T2	3.79	93.4	4.7	1.07	19	59.2	1.7	1.9	1.4	11.6	16.3	3.1	3.6
L11 x T1	3.33	94.7	4.3	0.98	4.8	53.2	1.9	2.1	1.5	13	18.5	3.3	3.7
L11 x T2	4.46	90.5	2.6	1.04	2	64.4	2.3	2.3	1.6	12.8	17.8	3.3	4
L12 x T1	4.02	92.8	2	0.91	0.3	31.5	2	2.3	2.1	12.8	18.5	2.8	3.7
L12 x T2	5.18	91.4	3.5	1.05	0.4	42.8	2.3	2.3	2.2	12.9	19.3	2.5	3.4
L13 x T1	3.28	101.1	4	1.13	2.7	55.2	1.5	1.7	2.1	13.9	17.9	3.1	3.4
L13 x T2	4.24	97.1	4.1	1.1	12.7	76.7	1.7	1.5	1.7	13.5	18.8	3	3.9
L14 x T1	3.95	96.8	3.9	1.03	1	35.1	1.5	2.2	2.1	12.8	15.5	2.6	4.2

Appendix 6. continued

L14 x T2	3.37	91.1	3.9	0.97	14.4	52.8	1.8	2	1.8	12.3	20.8	3.9	3.7
L15 x T1	4.81	97.2	3.2	0.93	2.6	70	1.5	1.5	1.9	11.5	19.7	2.6	3.5
L15 x T2	5.03	90.6	2.5	0.69	10.9	64.4	2.1	2	1.8	13.1	17.9	2.2	3.5
L16 x T1	3.42	101.2	4.7	1	1.5	48.5	1.6	2.5	2	14.2	19.2	2.6	3.4
L16 x T2	3.99	91.4	3.9	1.13	8.2	80.5	2.4	2.3	1.9	11.3	18.2	3.4	3.5
L17 x T1	4.11	100.6	2.9	1.15	7	51.3	2	1.3	1.6	13.6	18.9	2.9	2.8
L17 x T2	3.94	98.5	4.1	0.9	6.9	73	1.5	1.4	1.7	11.7	19.1	3.3	3
L18 x T1	5.32	99.1	0.6	1.22	4.2	57.8	1.5	1.2	1.3	12.7	20.4	3.2	2.9
L18 x T2	3.33	95.2	2.8	0.91	12.6	70.5	1.6	1.2	1.6	11.1	20.3	3.9	3.8
L19 x T1	3.87	101.5	3.2	1.04	3.8	77.2	1.4	1.5	1.8	13.3	19.9	3.4	3.7
L19 x T2	4.56	96.9	2.7	0.74	0.3	68.9	1.6	1.8	1.7	8.6	21.3	3.2	3.1
L20 x T1	4.2	98.6	4.2	1.1	0.8	25.6	2.2	1.7	1.5	13.7	20.6	2.8	3.7
L20 x T2	4.42	98	3.3	0.83	2.1	73.9	1.9	1.5	1.7	13.9	22	3.5	3.6
L21 x T1	3.89	105.6	2.7	1.06	4.1	65.4	1.9	1.7	1.2	12.7	19.8	2.8	3.1
L21 x T2	4.48	98.9	3.1	1.05	5.5	79.5	1.6	1.5	1.7	12	20.7	3.5	3.4
L22 x T1	3.7	99.5	4.3	0.95	3.1	51.2	1.6	2.6	2.3	11.4	20.5	2.9	3.3
L22 x T2	3.74	92.9	4	0.8	13.8	62.5	2.1	2.2	2.1	9.9	19.8	4.2	3.2
L23 x T1	3.41	105.9	3.1	0.94	1	66.6	1.7	1.3	1.7	10.7	20.5	2.8	3.3
L23 x T2	4.83	96.5	3.3	0.99	2.5	89.9	1.6	1.7	1.5	12.2	19.2	3.5	3.1
L24 x T1	3.25	103.5	2.8	0.7	3.4	57.9	1.6	1.4	1.7	13.9	19.9	3	3
L24 x T2	4.12	93.9	3.3	0.67	0.3	50.3	2.6	1.7	1.9	11.8	22.1	3	3.1
Check 1	3.03	97.4	4	0.9	5.1	89.9	1.7	1.7	1.7	10.9	18	4.4	3.6
Check 2	**4.42**	**91.7**	**2.9**	**1.11**	**16.4**	**75.2**	**1.4**	**1.7**	**1.7**	**13**	**19.2**	**3.4**	**3.4**
Mean	**4.09**	**96.3**	**3.3**	**0.94**	**5.576**	**61.356**	**1.8**	**1.8**	**1.8**	**12.5**	**19.5**	**3.2**	**3.5**
LSD (0.05)	**1.52**	**3.8**	**1.7**	**0.28**	**12.4**	**22**	**0.5**	**0.7**	**0.5**	**2.9**		**1.2**	**0.8**
MSe	**0.61**	**4.3**	**1**	**0.03**	**4**	**17.5**	**0.1**	**0.1**	**0.1**	**2.1**	**3.3**	**0.3**	**0.2**
CV	**19.07**	**2.2**	**30.2**	**18.92**	**15.3**	**21.6**	**14.9**	**21.4**	**18.5**	**11.6**	**9.3**	**18.1**	**12**
p	*****	*******	******	******	*******	*******	*******	******	*******	**ns**	**ns**	*****	******
Min	**3**	**90.5**	**0.6**	**0.62**	**0.1**	**25.6**	**1.4**	**1.1**	**1.2**	**8.6**	**15.5**	**1.9**	**2.6**
Max	**5.74**	**105.9**	**4.7**	**1.22**	**29.4**	**97.7**	**2.7**	**2.6**	**2.3**	**14.2**	**22.1**	**4.4**	**4.2**

Appendix 7. Standard, mid and better parent heterosis values evaluated at APPRC and HARC in 2014

	AD			PA			EH			PH			EPP		
Code	**SH**	**MP**	**BP**	**SH**	**MP**	**BP**	**SH**	**MP**	**BP**	**SH**	**MP**	**BP**	**SH**	**MP**	**BP**
L1XT1	0.45	-91.42**	-5.34**	-44.23**	-37.00**	-39.13**	2.23	110.48**	94.90**	0.32	-37.00**	102.92**	22.83**	71.13**	54.15**
L1XT2	0.71	-5.31**	-5.09**	-31.59**	-18.61**	-17.25**	-9.30	110.85**	72.90**	-9.06	-18.61	90.33**	-7.01**	54.78**	45.59**
L2XT1	-2.14	-6.27**	-1.90	-24.23**	-17.31**	-17.31**	-2.27	111.68**	105.10**	0.30	-17.31	105.15**	3.80**	25.78**	21.59**
L2XT2	-3.53**	-6.51**	-3.30*	12.71**	29.32**	36.33**	-18.46*	100.76**	71.10**	-11.40	29.32**	91.41**	-25.03**	5.84**	-12.19**
L3XT1	0.10	-6.10**	-3.90**	-41.10**	-26.30**	-13.65**	0.60	125.87**	125.00**	-2.33	-26.30**	102.99**	16.58**	40.73**	35.55**
L3XT2	-0.56	-5.64**	-4.53**	7.65**	42.69**	57.83**	-16.48*	114.23**	88.20**	-8.00	42.69**	98.75**	-10.40**	25.93**	4.17**
L4XT1	-0.01	-4.00**	0.73	-13.61**	2.58**	12.47**	-5.36	114.99**	111.70**	-3.39	2.58	116.29**	7.60**	34.49**	33.96**
L4XT2	-0.32	-3.16	0.42	-2.09**	22.78**	27.46**	-8.87	136.91**	110.20**	-11.20	22.78	98.81**	-14.56**	25.09**	6.37**
L5XT1	0.50	-4.41**	-0.71	-13.41**	-2.00**	1.77**	-11.34	95.97**	93.70**	-8.33	-2.00	85.44**	7.82**	26.76**	19.22**
L5XT2	-1.20	-4.92**	-2.39	-38.41**	-26.57**	-25.50**	-15.92*	111.86**	83.70**	-10.49	-26.57*	93.38**	-24.19**	3.34**	-16.17**
L6XT1	-2.30	-5.76**	-0.60	-29.65**	-19.28**	-14.90**	-16.38*	107.21**	87.00**	-9.23	-19.28	85.89**	1.60**	47.39**	27.50**
L6XT2	-3.84**	-6.14**	-2.17	-38.63**	-25.77**	-25.77**	-16.87*	138.88**	130.80**	-15.56	-25.77*	82.41**	-10.79**	55.87**	53.33**
L7XT1	-0.16	-3.47	2.08	-6.28**	-3.35**	2.29**	-15.14	100.83**	89.80**	0.36	-3.35	103.00**	10.68**	36.73**	34.63**
L7XT2	-2.63	-4.73**	-0.44	7.13**	15.83**	29.59**	-8.59	149.09**	129.60**	-10.78	15.83	92.74**	2.01**	47.31**	24.08**
L8XT1	-2.63	-6.30**	-1.43	-19.77**	-9.19**	-5.70**	-15.84*	91.13**	88.20**	-6.43	-9.19	89.27**	7.44**	40.99**	38.17**
L8XT2	-2.66	-5.21**	-1.46	-25.20**	-10.82**	-9.52**	-17.22*	115.15**	90.90**	-6.67	-10.82	101.63**	-13.18**	34.59**	-58.46**
L9XT1	1.30	-2.52	2.55	-25.64**	-23.31**	-18.84**	-14.44	74.91**	61.00**	-6.59	-23.31	88.95**	2.53**	25.67**	22.82**
L9XT2	-0.38	-2.99	0.86	1.33**	9.55**	22.56**	-9.72	108.22**	69.90**	-3.04	9.55	109.46**	-15.98**	20.23**	0.65**
L10XT1	3.75**	-1.09	3.00*	29.75**	27.90**	41.60**	-24.78**	77.86**	68.20**	-5.48	27.90**	-96.91**	-25.18**	-1.41**	-6.75**
L10XT2	0.17	-3.38	-0.56	44.36**	48.87**	74.62**	-15.06	131.23**	113.00**	-3.39	48.87**	108.71**	-26.39**	14.67**	2.10**
L11XT1	-3.17*	-7.25**	-2.93*	-5.72**	20.04**	44.05**	-12.98	105.41**	94.60**	-3.27	20.04	95.65**	-5.51**	29.92**	18.58**
L11XT2	-4.17**	-7.13**	-3.94**	-23.06**	3.89**	17.56**	-15.75*	128.91**	110.50**	-4.83	3.89	105.60**	-32.02**	11.40**	3.37**
L12XT1	-0.07	-4.29**	0.17	-11.74**	-3.68**	-3.68**	-6.63	95.82**	108.90**	-4.61	-3.68	171.60**	-6.57**	36.16**	23.88**
L12XT2	-2.41	-5.42**	-2.17	-33.48**	-23.67**	-19.53**	-11.58	109.88**	74.50**	-7.05	-23.67*	100.79**	-34.98**	14.24**	12.99**
L13XT1	-0.52	-4.94**	-0.77	-14.83**	-16.05**	-7.05**	-1.76	113.48**	107.60**	-5.38	-16.05	91.40**	-14.78**	25.94**	286.55**
L13XT2	-0.12	-3.43	-0.37	-59.86**	-58.60**	-51.44**	-8.61	125.87**	93.10**	-8.72	-58.60**	97.19**	-13.27**	54.97**	54.10**
L14XT1	-3.29*	-6.93**	-2.10	9.29**	29.76**	42.27**	-12.76	121.92**	95.10**	-8.59	29.76**	84.90**	8.26**	61.50**	35.87**

Appendix 7. continued

L14XT2	-2.84	-5.38**	-1.63	15.65**	45.02**	50.55**	-26.17**	118.73**	117.70**	-16.52	45.02**	80.34**	-20.64**	43.41**	40.99**
L15XT1	-1.48	-4.74**	0.73	-1.02**	17.76**	29.43**	-4.91	99.53**	87.90**	-5.22	17.76	71.95**	19.72**	38.68**	28.78**
L15XT2	-4.37**	-6.43**	-2.22	6.96**	34.42**	39.87**	-14.18	103.85**	69.60**	-11.86	34.42**	59.91**	-18.58**	9.11**	-12.41**
L16XT1	-0.37	-4.12**	0.87	26.60**	30.56**	38.17**	-27.95**	100.46**	61.20**	-13.56	30.56**	120.04**	3.80**	33.98**	30.26**
L16XT2	-2.39	-4.95**	-9.27**	8.65**	17.47**	31.42**	-33.74**	118.04**	97.20**	-21.73*	17.47	99.24**	-21.65**	19.13**	4.11**
L17XT1	2.86	-5.04**	-3.51**	-37.72**	-31.91**	-32.03**	-8.38	120.55**	104.90**	-2.65	-31.91**	96.92**	20.38**	103.58**	51.07**
L17XT2	1.74	-5.00**	-1.39	-51.98**	-44.79**	-41.92**	-9.00	152.88**	137.10**	0.34	-44.79**	116.76**	-8.57**	92.76**	62.44**
L18XT1	0.68	-5.13**	-5.99**	-35.27**	-21.87**	-12.60**	-14.89	126.29**	178.90**	-6.12	-21.87	105.52**	18.70**	59.74**	48.96**
L18XT2	-2.16	-6.72**	-8.22**	-38.90**	33.74**	-17.51**	-14.65	166.29**	179.70**	-8.01	33.74**	101.38**	-11.91**	40.69**	27.78**
L19XT1	3.55**	-4.18**	-0.58	-22.24**	3.69**	5.00**	-8.01	123.93**	105.70**	-7.90	3.69	99.84**	7.39**	44.52**	34.77**
L19XT2	0.18	-6.24**	-3.82**	0.78**	27.21**	35.45**	-18.06*	130.67**	118.70**	-14.84	27.21*	84.78**	-19.45**	28.65**	16.85**
L20XT1	-0.05	-6.24**	-2.67	-11.56**	11.94**	18.86**	-10.65	104.57**	99.80**	-7.41	11.94	97.00**	6.36**	17.20**	4.46**
L20XT2	-0.53	-5.61**	-3.14*	-13.19**	7.64**	-5.26**	-19.80**	110.38**	88.00**	-14.50	7.64	84.71**	-12.01**	11.30**	-13.58**
L21XT1	1.16	-4.45**	-2.88	-10.55**	-4.64**	7.75**	-11.25	111.06**	98.50**	-3.97	-4.64	134.16**	11.47**	33.03**	26.81**
L21XT2	0.28	-4.17**	-2.35	-27.71**	42.89**	9.88**	-23.44**	109.79**	94.30**	-6.95	42.89**	126.88**	-15.70**	16.93**	5.80**
L22XT1	2.52	-3.83*	-1.58	24.01**	34.63**	88.49**	-7.03	89.26**	73.60**	-1.52	34.63**	177.86**	-6.11**	29.10**	42.75**
L22XT2	1.80	-3.40	-2.27	-5.39**	43.08**	43.80**	-15.82*	93.22**	57.20**	-4.43	43.08**	106.46**	-23.16**	25.92**	-3.56**
L23XT1	2.70	-5.18**	-3.99**	-18.49**	-3.79**	10.05**	13.60	150.74**	147.40**	6.73	-3.79	115.90**	28.28**	91.36**	127.92**
L23XT2	1.74	-4.99**	-5.43**	-44.74**	11.36**	-25.39**	2.21	157.13**	122.60**	0.63	11.36	117.40**	11.60**	101.69**	40.06**
L24XT1	1.23	-3.49	0.49	-20.74**	-5.89**	-22.79**	-14.67	91.74**	90.90**	-4.22	-5.89	93.75**	1.76**	43.67**	64.19**
L24XT2	0.83	-2.74	0.09	-30.53**	16.21**	-32.33**	-24.17**	94.72**	71.20**	-4.04	16.21	84.63**	-26.57**	24.18**	30.46**
min	**-4.37**	**-91.42**	**-3.99**	**-59.86**	**-58.60**	**-51.44**	**-33.74****	**74.91**	**57.20**	**-21.73**	**-58.60**	**-96.91**	**-34.98**	**-1.41**	**-58.46**
mean	**-0.54**	**-6.68**	**-10.21**	**-14.12**	**4.09**	**7.50**	**-12.51**	**114.65**	**99.40**	**-6.68**	**4.09**	**97.36**	**-5.07**	**37.06**	**29.68**
max	**3.75**	**-1.09**	**3.00**	**44.36**	**48.87**	**88.49**	**13.60**	**166.29**	**179.70**	**6.73**	**48.87**	**177.86**	**28.28**	**103.58**	**286.55**
CD α ≤ 0.01	**5.71**	**4.95**	**5.71**	**1.06**	**0.92**	**1.06**	**30.77**	**26.64**	**30.77**	**37.87**	**32.80**	**37.87**	**0.70**	**0.61**	**0.70**
CD α ≤ 0.05	**4.08**	**3.71**	**4.08**	**0.76**	**0.69**	**0.76**	**21.98**	**19.98**	**21.98**	**27.05**	**24.60**	**27.05**	**0.50**	**0.46**	**0.50**

Appendix 7. continued

	EA			EPO			GY		
Code	**SH**	**MP**	**BP**	**SH**	**MP**	**BP**	**SH**	**MP**	**BP**
L1XT1	-10.53**	-13.91**	-13.91**	-11.83**	12.54**	22.54**	41.05**	345.17**	338.42**
L1XT2	-2.66**	-16.04**	-6.34**	-16.35**	17.57**	45.33**	-16.58**	207.53**	170.95**
L2XT1	-24.56**	-17.64**	-4.83**	-16.80**	11.90**	15.63**	17.03**	204.08**	163.64**
L2XT2	-3.25**	-6.61**	22.06**	-21.90**	16.30**	35.68**	-4.98**	180.07**	114.06**
L3XT1	-26.03**	-27.45**	-26.01**	-9.67**	24.18**	25.56**	32.28**	299.57**	293.36**
L3XT2	13.78**	-0.16	13.80**	-19.54**	22.74**	39.79**	-33.87**	131.65**	96.65**
L4XT1	-28.36**	-34.98**	-31.07**	-17.59**	10.85**	14.54**	12.67**	246.60**	245.80**
L4XT2	-6.36**	-23.36**	7.85**	-13.77**	28.42**	49.82**	-12.74**	212.20**	169.04**
L5XT1	-7.29**	-2.80**	6.78**	-20.25**	20.19**	31.26**	24.41**	167.66**	106.04**
L5XT2	-19.65**	-25.17**	-7.45**	-18.93**	37.04**	33.43**	-6.75**	122.43**	54.44**
L6XT1	7.18**	14.95**	29.83**	-20.19**	23.25**	38.65**	9.91**	196.53**	164.53**
L6XT2	-7.10**	-11.73**	12.54**	-19.96**	39.06**	39.06**	20.50**	270.69**	190.02**
L7XT1	-6.28**	2.68**	19.23**	-21.68**	22.46**	39.96**	3.09*	155.47**	114.22**
L7XT2	-14.31**	-17.02**	9.02**	-17.69**	45.01**	47.09**	36.45**	281.20**	183.53**
L8XT1	4.58**	17.34**	40.71**	-26.52**	12.10**	24.22**	-2.06	116.28**	68.90**
L8XT2	-2.85**	-3.94**	30.70**	-23.62**	30.88**	29.11**	7.00**	162.73**	84.52**
L9XT1	-4.98**	-2.40**	4.68**	-24.88**	2.14**	4.41**	3.40**	137.34**	89.54**
L9XT2	-23.40**	-29.95**	-15.62**	-19.45**	21.40**	39.94**	-22.04**	99.86**	42.92**
L10XT1	-5.03**	-13.80**	-8.62**	-28.03**	8.47**	18.46**	-23.99**	91.92**	63.01**
L10XT2	-2.38**	-20.10**	-16.16**	-24.72**	27.26**	30.79**	-9.69**	157.68**	93.67**
L11XT1	-20.80**	-18.65**	-12.75**	-22.81**	16.34**	27.05**	-0.99	96.00**	203.89**
L11XT2	-31.00**	-36.90**	-23.99**	-25.06**	26.68**	30.19**	-7.77**	345.56**	293.06**
L12XT1	-29.36**	-26.19**	-19.25**	-18.79**	11.64**	12.88**	-1.49	203.74**	202.34**
L12XT2	-34.05**	-38.77**	-24.62**	-12.38**	48.11**	52.22**	5.06**	276.92**	225.44**
L13XT1	11.48**	13.94**	21.49**	-9.51**	28.63**	23.03**	5.44**	270.35**	223.63**
L13XT2	5.20**	-4.23**	14.65**	-16.74**	31.84**	44.65**	-12.02**	267.93**	261.16**
L14XT1	-8.92**	-18.79**	-12.36**	-16.93**	29.89**	20.83**	-8.28**	252.70**	181.51**
L14XT2	2.66**	-17.31**	-14.71**	-23.03**	35.61**	33.72**	-17.39**	285.17**	252.05**

Appendix 7. continued

L15XT1	-21.91**	-23.03**	-21.11**	-16.40**	14.92**	49.40**	10.93**	166.98**	119.59**
L15XT2	8.30**	-4.55**	9.41**	-19.91**	22.18**	43.13**	-31.32**	85.66**	35.95**
L16XT1	-19.76**	-23.99**	-22.79**	-27.10**	15.43**	1.32**	2.31	202.89**	192.53**
L16XT2	12.34**	-4.45**	4.78**	-29.55**	25.89**	22.39**	-11.65**	202.38**	152.63**
L17XT1	-34.65**	-39.33**	-37.12**	-22.62**	19.49**	42.34**	35.71**	242.00**	216.52**
L17XT2	-7.22**	-22.49**	-16.78**	-17.08**	44.06**	52.53**	35.35**	285.37**	276.83**
L18XT1	-22.30**	-18.54**	-10.52**	-29.33**	13.33**	22.78**	47.11**	348.41**	345.37**
L18XT2	0.06**	-6.81**	15.25**	-18.17**	48.35**	42.17**	25.73**	345.08**	280.64**
L19XT1	-17.98**	-20.83**	-20.58**	-14.73**	25.49**	61.62**	-9.12**	233.19**	178.93**
L19XT2	-4.82**	-17.66**	-7.83**	-20.17**	31.39**	51.30**	-6.17**	313.02**	299.87**
L20XT1	-11.28**	-11.11**	-7.30**	-17.80**	14.24**	28.53**	14.80**	216.75**	252.35**
L20XT2	-19.29**	-27.82**	-15.67**	-20.39**	22.94**	38.31**	20.50**	280.32**	213.55**
L21XT1	-20.50**	-25.04**	-26.52**	-17.68**	11.92**	14.41**	13.78**	169.48**	119.39**
L21XT2	13.02**	-4.28**	4.45**	-29.16**	6.77**	23.08**	-6.62**	155.38**	80.06**
L22XT1	1.17**	5.71**	15.65**	-17.12**	16.48**	15.19**	0.97	207.09**	204.32**
L22XT2	1.41**	-5.85**	15.92**	-22.54**	21.13**	34.58**	-10.52**	215.95**	169.70**
L23XT1	-23.90**	-20.21**	-12.35**	-7.63**	31.31**	28.39**	21.55**	344.41**	273.06**
L23XT2	-16.68**	-22.41**	-4.04**	-14.94**	34.68**	47.78**	16.45**	208.58**	196.28**
L24XT1	-12.72**	-17.71**	-16.02**	-26.73**	5.34**	9.11**	29.96**	323.13**	298.87**
L24XT2	2.81**	-12.92**	-4.98**	-29.19**	13.55**	23.01**	15.37**	341.09**	299.95**
min	**-34.65**	**-39.33**	**-37.12**	**-29.55**	**2.14**	**1.32**	**-33.87**	**85.66**	**35.95**
mean	**-9.75**	**-14.63**	**-3.39**	**-19.93**	**22.95**	**31.69**	**5.27**	**222.34**	**187.43**
max	**13.78**	**17.34**	**40.71**	**-7.63**	**48.35**	**61.62**	**47.11**	**348.41**	**345.37**
CD ɑ ≤ 0.01	**1.00**	**0.86**	**1.00**	**0.14**	**0.12**	**0.14**	**3.96**	**3.43**	**3.96**
CD ɑ ≤ 0.05	**0.71**	**0.65**	**0.71**	**0.10**	**0.09**	**0.10**	**2.83**	**2.57**	**2.83**

AD=Anthesis date, PA=plant aspect, EH=ear height (cm), PH=plant height (cm), EPP=number of ears per plant, EA=ear aspect (1-5 score), EPO=ear position, GY=grain yield (t ha^{-1})

Addis Ababa University

School of Graduate Studies

This is to certify that a thesis prepared by, **Worknesh Terefe Gebre**, entitled a "**Genetic variability and combining ability of Highland Quality Protien Maize (*Zea mays* L.) Inbred lines**" submitted in fulfillment of the requirement of for the Degree of Master of Science in Biology (Applied Genetics) complies with the regulations of the university with respect to originality and quality.

Singed by the examining committee

Examiner____________________________Signature_____________Date_______________

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Advisor______________________________Signature____________Date________________

Advisor______________________________Signature____________Date________________

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Student ____________________________Signature_____________Date_______________