COMBINING ABILITY OF KERNEL QUALITY AND SOME AGRONOMIC CHARACTERISTICS IN QUALITY PROTEIN MAIZE (QPM) AND NON-QPM INBRED LINES

BY

CHARLES MUTIMAAMBA

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Department of Crop Science

School of Agricultural Sciences

University of Zambia

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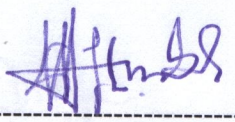
Abstract

Maize is the principal staple food and source of protein and calories for millions of people in Southern Africa. Common maize is deficient in lysine and tryptophan and thus predisposes mothers and children to the risk of malnutrition. The consumption of quality protein maize (QPM), which has twice the lysine and tryptophan levels, and same appearance and taste as common maize can potentially avert this risk. The national program in Zimbabwe is currently using QPM inbred lines sourced from CIMMYT as donors for converting its elite non-QPM inbred lines to QPM as well as developing new ones. The donor capability of these QPM lines and their compatibility with the national program elite inbred lines has never been systematically tested and validated. The current study was conducted to identify appropriate QPM donor inbred lines for use in the development of new inbred lines and the conversion of existing elite non-QPM inbred lines to QPM, through endosperm phenotyping and testing for agronomic adaptability to stress and non-stress environments. Five white QPM inbred lines were crossed to twelve non-QPM yellow inbred lines following a modified North Carolina Design II in which each line was not used strictly as male or female. The thirty-five hybrids were evaluated in an alpha lattice design with three replications at four sites in winter 2009. The study involved the assessment of the relative importance of general (GCA) and specific combining ability (SCA) effects, additive and dominance gene action, and mid-parent heterosis in the phenotypic expression of endosperm modification (MOD), levels of tryptophan (TRP), lysine (LYS) and protein (PROT); grain yield (GY), anthesis date (AD) and anthesis-silking interval (ASI) among the inbred lines. On the basis of GCA effects, line CML511 was the best donor for MOD, LYS and PROT, and gave good GCA effects for the highest number of agronomic traits and environments. Line CZL082 was the second best donor for MOD, TRP and LYS. Line HX482P was the best general combiner in terms of desirable GCA effects for kernel quality and agronomic traits under more environments, followed by line L7 which outperformed the other lines in terms of number of traits with desirable GCA effects. In terms of SCA effects, line CZL082 was the best donors because it was involved in more cross combinations with desirable SCA effects for kernel quality and agronomic traits under more environments. Line CML511 was the second best donor, given that it was involved in cross combinations that had the best SCA effects for MOD and LYS. Line L7 was the most outstanding line for giving the highest number of traits with desirable SCA effects for agronomic traits in addition to MOD. Line CML511 was a constituent parent in separate cross combinations with the highest means for MOD and PROT. On the basis of mid-parent heterosis, line CML511 was the best donor for featuring in crosses with the highest positive mid-parent heterosis for MOD. Lines EL77P and L7 had the best mid-parent heterosis by virtue of being constituent parents in the best five endosperm-modified hybrids. The mid-parent heterosis of two of the five least modified crosses was higher for TRP, LYS and PROT than that for the best modified hybrids, with line CML511 featuring in one of these crosses. Additive genetic effects were preponderant in the control of all kernel quality traits. Dominance gene action was predominant in influencing GY under MSV disease conditions and across all sites, while additive genetic effects were more important in controlling this trait under optimum conditions and drought stress. Dominance gene action was predominant in controlling AD under optimum conditions, whereas additive genetic effects were preponderant in governing the same trait under MSV disease. Both additive and dominance gene action were dominant in the control of ASI under all environments except MSV disease. Maternal additive gene action was predominant in the genetic control of ASI under all environments besides MSV disease. Narrow sense and broad sense heritability were low for all kernel quality traits and GY, while narrow sense heritability was medium for AD and low for ASI under drought stress. Broad sense heritability was high for both AD and ASI, but medium for GY under drought stress. Lines CZL082 and CML511 were therefore identified on the basis of GCA and SCA effects, mid-parent heterosis and mean performance as the most appropriate QPM donors for line conversion and recycling of non-QPM inbred lines in the national program.

Declaration

This dissertation represents the author's original work and has never been previously submitted nor will it be submitted to any university for any award of degree, diploma or certificate. Where sources of information from work by other authors have been cited, due acknowledgement has been made in the text.



Charles Mutimaamba (Candidate)

Approval

The University of Zambia approves this dissertation for Charles Mutimaamba as fulfilling the requirements for the award of the degree of Master of Science in Plant Breeding and Seed Systems

Examiner's Name	Signature	Date
Dr. D.M. Lungu	----------	29/07/10
Dr. M. Mwala	----------	29/07/2010
Dr. M. Chisi	----------	29/07/10

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Dedication

To three wonderful and special people in my life, my dear wife Sharlotte, loving daughter Anesu Vanessa and son Tinotenda, for their cherished and loyal encouragement. Special dedication is also extended to my father Ernest, late mother Gladys and grandmother whose nuggets of life are my source of inspiration.

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CHAPTER 1

INTRODUCTION

Maize (*Zea mays* L.) grain, in its different stages of maturity and processed forms, is the principal staple food for millions of people in the developing world, particularly Southern Africa (SnA), supplying the majority of their protein and calorie requirements (Bressani, 1995). Although maize grain protein is nutritionally unbalanced, maize still remains the staple crop because of its advantages, such as high productivity, good palatability, low labour demands, and ease of growing, transport, storage, and processing (DeVries and Toenniessen, 2001). In SnA maize is grown mostly on a subsistence level by almost every farmer both as a staple and source of livelihood. According to Mashingaidze (2004), 99 % of Zimbabwe's 15 million inhabitants are dependent on maize as a staple food for their daily calories and proteins. Zimbabwe is ranked fourth among selected Eastern and Southern African countries in terms of the importance of maize in the diet of individuals, due to its high dependence on maize as source of calories (38 %) and proteins (46 %) in the total diet (Krivanek *et al.,* 2006). The national annual requirement of maize grain is 1.8 million tonnes, 64 % of which is for human consumption in the country (Mashingaidze, 2006).

All the maize produced and consumed by rural and urban dwellers in SnA is white-grained, whilst yellow maize is predominantly grown by large-scale commercial farmers for use as stockfeed. Seed sales figures for Seed Co Ltd, whose maize seed market share exceeds 70 %, indicate that yellow varieties constituted 1-3 % of annual sales for the period 2002 to 2008 (J. MacRobert, personal communication, 2008). Consumers generally perceive yellow maize as inferior to white. However nutritional education can potentially change such perceptions, thereby promoting the consumption of the relatively more nutritious yellow maize. Findings by Muzhingi, *et al.,* 2008, indicate that 94 % of households are willing to consume yellow maize meal if they know it is more nutritious (e.g., rich in provitamin A) than white maize. The potential for stimulating yellow maize production and consumption in SnA is relatively high given that maize is the region's staple food crop. Therefore, the promotion of the production and consumption of yellow quality protein maize (QPM), rich in both provitamin A carotenoids, and the essential amino acids lysine and tryptophan, may be a cost effective biofortification strategy for preventing and alleviating vitamin A and protein deficiency among children of school going age and pregnant/lactating mothers in Zimbabwe. Common maize is a poor dietary protein source when fed to monogastric animals and humans since they lack the capacity to internally synthesise lysine, tryptophan and threonine. In countries where maize is grown as a staple food and often a significant source of protein as is the case in SnA, high dependence on maize puts people, especially, young children, pregnant and lactating mothers, and the ill (notably people living with HIV and AIDS) at risk of dietary protein deficiency (Pixley and Bjarnason, 2002).

Semagn *et al.,* (2006), describe QPM as a genotype in which the *opaque-2* gene has been incorporated along with associated endosperm and amino acid modifiers and contains twice the amount of lysine (4.2 %) and tryptophan (0.9 %) compared to that of normal maize endosperm. Preformed vitamin A is present only in foods of animal origin, which are expensive and beyond the reach of a large proportion of the Zimbabwean populace. Use of QPM grain in place of common maize in feed formulation or supplements, has the potential to significantly reduce the use of expensive legume or animal-based protein sources in livestock production. This intervention has the potential of enhancing the affordability of animal-derived foods, which consequently alleviates vitamin A and protein deficiency among vulnerable groups. Yellow QPM is a relatively inexpensive plant food source of vitamin A (provitamin A) and proteins. Therefore yellow QPM has the potential to partially meet the protein and vitamin A requirements of children and women of child-bearing age.

Human malnutrition due to dietary protein deficiency in maize-based diets may be ameliorated by the substitution of common maize with quality protein maize. QPM has been developed to help reduce human malnutrition in areas where protein deficiency is prevalent and where maize is the major protein source in the diet, as is the case in various parts of Sub-Saharan Africa (SSA) (Alan *et al.*, 2006). The nutritional benefits of QPM for people who depend on maize for their energy and protein intake and other nutrients are indeed quite significant. QPM contains the *opaque-2* (o2) mutation which confers higher lysine and tryptophan in maize kernel endosperm, but is phenotypically indistinguishable from conventional maize. Of the natural maize mutants (*floury-2, floury-3, opaque-2, opaque-6, opaque-7)* conferring higher lysine and tryptophan, *opaque-2* was found to be the most suitable for genetic manipulation in QPM breeding programs (Vivek *et al.,* 2008).

The breeding of QPM involves the manipulation of three distinct genetic systems, namely, the recessive mutant allele of the *opaque-2*, alleles of endosperm hardness modifier genes, and a distinct set of amino acid modifier genes. The *opaque-2* gene, through the suppression of the production of zein proteins, enhances the synthesis of non-zein proteins which are characteristically higher in endosperm lysine and tryptophan levels (Gibbon *et al.*, 2005). Endosperm hardness modifier genes are responsible for the conversion of the soft/opaque mutant endosperm to a hard/vitreous endosperm with little loss of protein quality. Amino acid modifier genes, on the other hand ensure that the relatively high levels of lysine and tryptophan synthesised by the *opaque-2* gene are maintained in the grain endosperm.

In Zimbabwe, maize produced and consumed by both rural and urban folk, and used as livestock feed is presently entirely non-QPM, despite common maize being less nutritionally balanced than QPM.

This is partly attributable to lack of clear national policies aimed at stimulating QPM development, production and utilisation in Zimbabwe. QPM variety development, testing, deployment and adoption is, however, beset with numerous operational challenges, a scenario that has curtailed QPM breeding by the national program in Zimbabwe. The lack of production and utilisation of QPM in the country can be partly attributed to absence of necessary national awareness policies on the benefits of QPM. Despite the commercial release of the first QPM hybrid ZS261 by the country's national program in 2006, lack of QPM awareness is still prevalent in the country. There is therefore, a need to ensure that promotional strategies necessary for stimulating production and consumption of QPM are developed and implemented if nutritional benefits of QPM are to be fully exploited in the country. A possible strategy would be capacitating and strengthening the national program's QPM breeding to enable it to embark on conversion of conventional maize elite inbred lines to QPM. The national program in Zimbabwe is currently using QPM inbred lines sourced from CIMMYT as donors for converting its elite non-QPM inbred lines to QPM as well as developing new ones. The donor capability of these QPM lines and their compatibility with the national program elite inbred lines has never been systematically tested and validated. The current study was conducted to identify appropriate QPM donor inbred lines for use in line conversion and recycling of non-QPM inbred lines to QPM through endosperm phenotyping and testing for agronomic adaptability to stress and non-stress environments.

1.1 Objectives

The overall objective of this study was to identify appropriate QPM donor inbred lines for use in line conversion of common maize inbred lines to QPM through endosperm phenotyping and testing for agronomic adaptability to stress and non-stress environments. The specific objectives were:

i. To estimate the general (GCA) and specific combining ability (SCA) effects of the inbred lines for kernel quality and some agronomic characteristics in stress and non-stress environments.

ii. To determine gene action in the phenotypic expression of kernel quality and some agronomic traits.

CHAPTER 2

LITERATURE REVIEW

2.1 Maize production and consumption in Zimbabwe

Maize (*Zea mays* L.) ranks first in cereal production in Zimbabwe in terms of the number of hectares grown. Maize production varies annually according to amount and distribution of rainfall and management. The contribution to the overall national maize production by total communal, resettlement and small scale commercial production increased from 76 % during the 1979/80 season to approximately 80 % in the mid 1990s (Mashingaidze, 2004). This increase in the post independence maize production can be attributed to increases in the area planted to maize, better yields and improved support services, notably, credit facilities, research and extension (Mashingaidze, 2006).

Zimbabwe maize breeding has been a success story over the years with hybrids being developed for both small scale and commercial production. According to Mashingaidze (2006), commercial adoption of hybrids in terms of area planted increased from 22 % (1949/50) to 88 % (1960/61) and 93 % (1966/67). Adoption was however slower in the communal lands before independence. The Mangwende communal area, for instance, had a hybrid adoption of 42 % in 1975 but by 1985 it stood at 99 % (Mashingaidze, 2006). Currently 90 % of the total production area is planted to hybrids with the remainder being planted to other products such as open pollinated varieties (OPVs), synthetics and recycled seed.

The nature of consumer preferences, in terms of grain colour, is a complex and controversial issue, where for example, yellow maize is commonly perceived as a "poor man's" grain because food aid is mostly of yellow maize (Tshirley and Santos, 1995). This attitude has been a strong contributing factor to the types of maize production and marketing systems that have evolved in the region. In Zimbabwe, yellow grain and meals were presumed to have very little or no human consumption demand, and have typically been unavailable to consumers. This assumption resulted in government officials and industrial millers discouraging the availability of yellow maize and maize meal for people to buy during normal years, contending that consumers would strongly resist eating meals from yellow maize (Takavarasha and Jayne, 1994). However, consumer surveys conducted by Rubey (1993) indicated that about 10 % of urban consumers actually prefer the taste of yellow maize meal, and would buy it if available at the same price as white maize meal. Rubey, in the same study, also concluded that at a 26 % price discount, 62 % of urban households in the lowest-income quintile stated that they would switch to yellow maize, compared with 39 % in the highest income quintile. Furthermore, findings by Muzhingi, *et al.,* 2008, indicate that 94 % of rural households are willing to consume yellow maize meal if they know it is more nutritious (e.g., rich in provitamin A) than white maize. In Zimbabwe,

white maize is preferred for human consumption as maize meal (or "sadza"), while yellow maize is used for livestock feeds as well as consumed by humans when either roasted or boiled.

2.1.1 Maize production constraints

The yield potential of maize for Sub-Saharan Africa is 5 t/ha in tropical highlands, 7 t/ha in subtropical and mid-altitude zones and 4.5 t/ha in tropical lowlands, compared to the current yields of 0.6, 2.5 and 0.7 t/ha, respectively (Pingali, 2001). This large yield gap is attributable to both biotic and abiotic constraints (Wambugu and Wafula, 2000). The major abiotic constraint is drought that causes an annual yield loss of about 15 % (Kamara *et al.,* 2003), while the second most important constraint is nitrogen and phosphorus deficiency (Nziguheba *et al.,* 2002; Whitbread *et al.,* 2004). Biotic factors that reduce maize yields in Africa are stemborers, the parasitic weed Striga and maize streak virus (MSV). The latter reportedly causes yield losses that range from a trace to almost 100 % (Kyetere *et al.,* 1999; Alegbejo *et al.,* 2002), and affects an estimated 60 % of the area planted to maize. This ranks MSV as the most widespread biotic constraint to maize production in Africa (De Vries *et al.,* 2001).The other diseases that affect maize include leaf blight, rusts, stalk and ear rots, and systemic foliar diseases (Alegbejo *et al.,* 2002). Annual maize production losses due to diseases and pests were estimated at 13 % of the total production in East and Southern Africa.

2.1.2 Maize as a source of provitamin A

In sub-Saharan Africa a significant number of people suffer from vitamin A deficiency, especially children of school going age and pregnant/lactating mothers (Muzhingi *et al.,* 2008). Vitamin A deficiency is of great concern to policy makers because of its health related problems such as compromised immune response and hence increased risk of death from HIV/AIDS, and mortality from measles (Muzhingi *et al.,* 2008). A recent study in Zimbabwe by the Ministry of Health and Child Welfare showed that 34% of women of child-bearing age, 35% of children under five years of age and 18 % of those of school going age (between 6 and 14 years of age) were vitamin A deficient (MOHCW, 1999). Yellow maize contains both pro-vitamin A and nonprovitamin A carotenoids with potential health benefits to humans (Menkir *et al.,* 2008). Dietary intake of either vitamin preformed in animal products (e.g. eggs, and dairy products) or as provitamin A carotenoids, mainly β-carotene in plant products such as vegetables, fruits and cereals can prevent or control vitamin A deficiency (Blumhoff, 1994). Unlike white maize, yellow-kernelled maize contains provitamin A carotenoids such as α-carotene, β-carotene, and β-cryptoxanthin (Muzhingi *et al.,* 2008). The provitamin A carotenoids in yellow maize in combination with essential amino acids (lysine and tryptophan) represents one of the best-balanced nutritional packages available among the most productive staple crops in developing countries (Muzhingi *et al.,* 2008). Therefore yellow QPM has the potential to provide a low cost option for preventing or controlling both vitamin A and protein deficiency among

vulnerable groups in Zimbabwe if effective promotional strategies are developed and implemented to stimulate human consumption demand, given that yellow maize is unpopular among consumers in southern Africa.

2.2 Genetic systems in QPM breeding

Poor understanding of modifier gene action, slight decreases in nutritional quality upon seed modification, and instability of endosperm phenotype are among the major factors delaying more vigorous efforts in the development of QPM inbred lines and commercial cultivars (Vasal *et al.*, 1980; Ortega and Bates, 1983; Belousov, 1984; Bjarnason and Vasal, 1992). Efficient strategies for solving these problems cannot be devised without a better understanding of the genetic and biochemical processes that direct seed modification (Lopes and Larkins, 1994). The breeding of QPM involves the manipulation of three distinct genetic systems (Krivanek *et al.*, 2006), namely, the recessive allele of the *opaque-2* gene, endosperm hardness modifier genes, and amino acid modifier genes.

2.2.1 Recessive mutant allele of the *opaque-2* gene

The *o2* mutation is a defect in the gene that encodes a regulatory protein controlling transcription of a subset of α zein genes (Schmidt, 1993). Zeins and particularly α zeins are the most abundant proteins in the grain endosperm (Gibbon *et al.*, 2005) but are also characteristically poor in the amino acids lysine and tryptophan. The homozygous *o2* mutant suppresses the production of these zeins, and hence consequently enhances the synthesis of non-zein proteins, which are characteristically higher in endosperm lysine and tryptophan levels (Gibbon *et al.*, 2005). The *o2* transcription factor also controls the production of the enzyme involved in free lysine degradation, and thus in grains with the *o2* mutation, a dramatic reduction in this enzyme leads to a corresponding increase in free lysine in the grain endosperm (Brochettobraga *et al.*, 1992). The presence of the *o2* allele in the recessive condition only predisposes, but not necessarily guarantees, maize to the synthesis of higher levels of lysine and tryptophan in endosperm protein. This, therefore, necessitates the need for the presence of another genetic system that ensures that the occurrence of the recessive *o2* allele confers higher levels of these amino acids in the grain endosperm.

2.2.2 Amino acid modifier genes

The second genetic system critical to QPM breeding consists of amino acid modifiers. Amino acid modifier genes are responsible for the control of the relative levels of lysine and tryptophan content in the grain endosperm. Lysine levels, as a percentage of total protein in whole grain flour, range across genetic backgrounds from 1.6 – 2.6 % (average 2.0 %) in normal maize and 2.7 – 4.4 % (average 4.0 %) in their *o2* converted counterparts (Moro *et al.*, 1996). The range of tryptophan levels, as a

percentage of total protein in whole grain flour across genetic backgrounds is 0.2 – 0.5 % for normal maize, and 0.5 – 1.1 % for QPM (CIMMYT, 2005). According to FAO (1985), the lysine and tryptophan requirements for a pre-school child (2 – 5 years) are 5.8 % and 1.1 % respectively, and QPM is therefore better placed to meet these guideline nutritional requirements than non-QPM. Multiple genes have been identified that are involved in controlling amino acid content in maize (Krivanek *et al.*, 2006). At least three gene loci have been implicated in controlling the levels of a protein synthesis factor correlated with lysine levels and these have been mapped to locations on chromosomes 2, 4, and 7 (Wang *et al.*, 2001; Wu *et al.*, 2002). If lysine or tryptophan levels are not continuously measured during the breeding process, the additional gains in protein quality may be lost even though the *o2o2* genotype is maintained (Krivanek *et al.*, 2006).

2.2.3 Endosperm hardness modifier genes

Kernel endosperm hardness modifiers are comprised of alleles whose function is to convert the soft/opaque mutant endosperm to a hard/vitreous endosperm with little loss of protein quality. According to Glover and Mertz (1987), modified endosperm texture is polygenically controlled with additive type of genetic variation playing an important role, although in some materials a few genes may contribute significantly to kernel modification. Increased levels of gamma zein likely contribute to the recovery of a hard endosperm phenotype as the *o2*-modified (QPM) grains have approximately double the amount of gamma zein in the endosperm relative to the *o2*-only mutants (Wallace *et al.*, 1990). These endosperm modifiers along with the *o2* mutant allele can be selected for using a light table, which is a rapid and low cost method of selection, whereby light is projected through the vitreous grains or blocked by the opaque grains respectively. Grain endosperm opaqueness/vitreousness is rated on a scale of 1 – 5 where all grains with a score of 2 – 5 are homozygous for the *o2* allele, but only grains with score 2 – 3 have sufficiently modified hard endosperm to be selected as QPM grains. This semi-quantitative measure has been used to map two genetic loci which affect the modification of the endosperm hardness in *o2o2* backgrounds to the long arm of chromosome 7 (Lopes *et al.*, 1995).

2.3 Selection for *opaque-2* endosperm modification background phenotypes

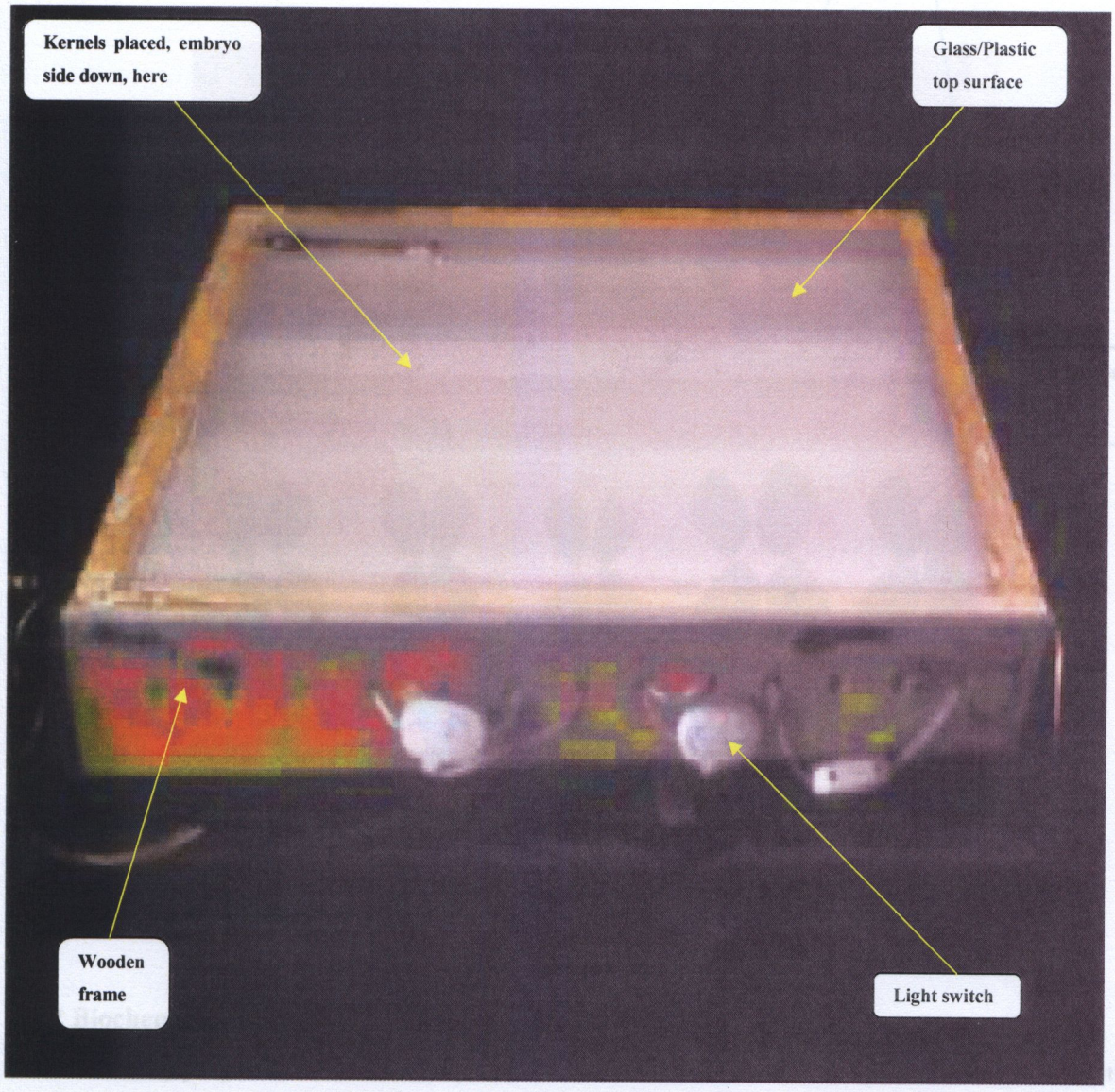
The process of selecting for endosperm modifier genes along with the *o2* mutant allele involves use of a light table, and determination of endosperm lysine and tryptophan levels using calorimetric analysis (Villegas *et al.*, 1984; Tsai *et al.*, 1972., Guiragossian *et al.*, 1979) and/or enzyme-linked immunosorbent assay (ELISA) (Habben *et al.*, 1995). Microsatellite or simple sequence repeat (SSR) markers are available for use in marker-assisted selection (MAS) for the *opaque-2* allele. Markers for endosperm modification are currently unavailable, and thus selection for this phenotype is done using

the light table. However, Danson *et al.,* (2006) have demonstrated the possibility of identifying fully modified QPM kernels using molecular markers. Therefore MAS for the *opaque-2* gene can effectively compliment calorimetric analysis and ELISA, as well as light table since there is a very high correlation between marker data and phenotypic expression due to co-segregation of the marker and the *opaque-2* gene (Danson *et al.,* 2006).

2.3.1 Light-table analysis

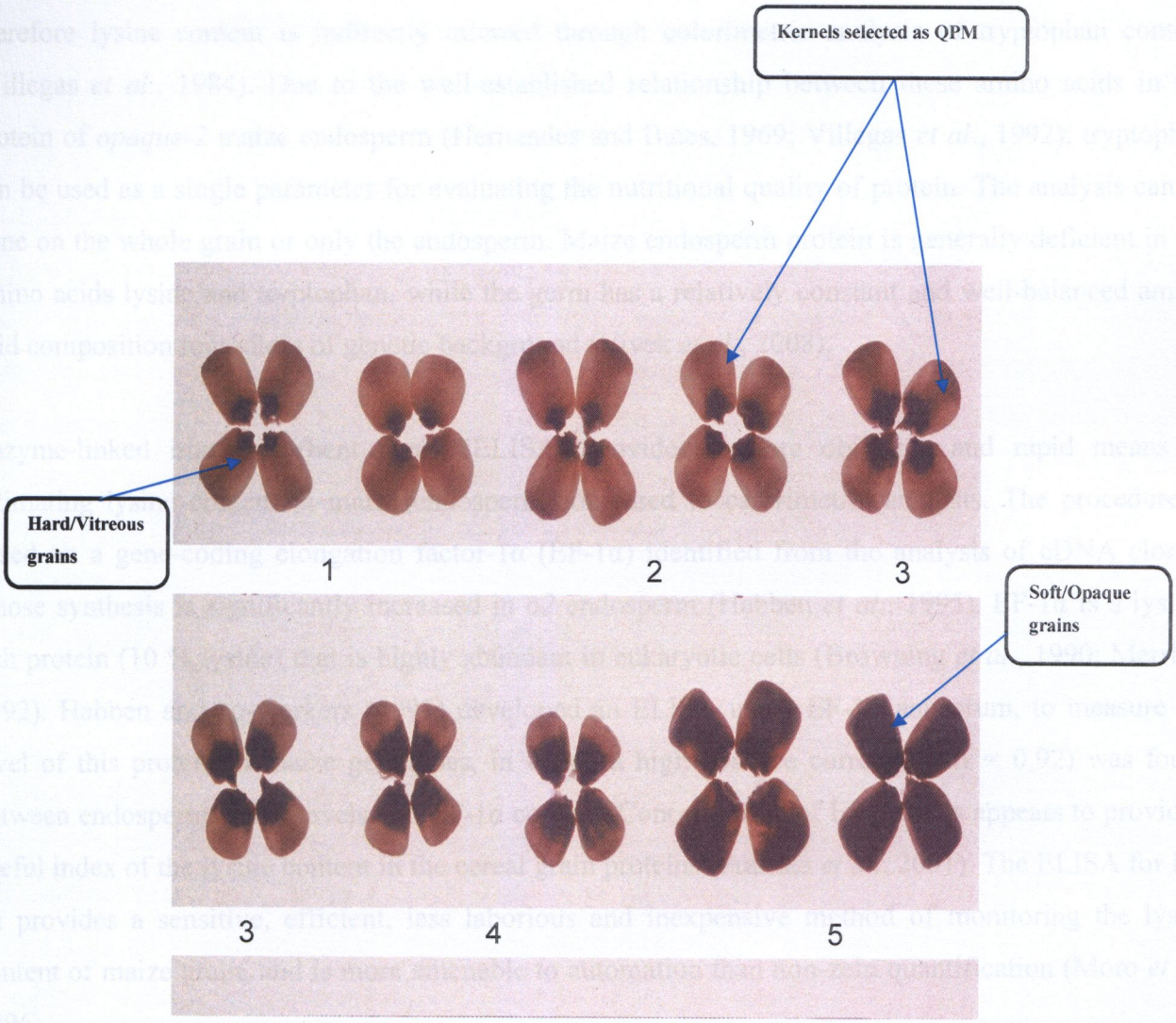
An increased level of gamma zein likely contributes to the recovery of a hard endosperm phenotype, given that the *o2*-modified (hard endosperm) grains have approximately double the amount of gamma zein in the endosperm relative to the *o2*-only mutants (Wallace *et al.,* 1990). The light table is a rapid, low-cost method of selecting for the beneficial alleles of the modifying loci that control gamma zein production.

A light-table consists of a wooden box (minimum size 27.5 cm long X 15 cm wide X 7.5 cm high) with semi-transparent glass or plastic top surface and fluorescent bulbs inside (Figure 2.1). Hard endosperm maize is differentiated from soft *o2o2* genotypes by placing maize grain on the glass/plastic surface and switching on the light. Light table selection is based on the principle that *o2o2* genotypes carry an undesirable characteristic kernel softness, which, on a light table, is seen as complete opaqueness (Vivek *et al.*, 2008). Gradation in the opaqueness is scored on a 1 to 5 scale (Figure 2.2) under a light table, where 1 = completely modified (i.e., translucent, normal phenotype); 2 = 75% modified; 3 = 50% modified; 4 = 25% modified; and 5 = completely opaque (Pixley and Bjarnason, 2002).Due to segregation of genes for endosperm hardness (softness), varying degrees of softness/hardness are expressed in the endosperm of segregating generations (Vivek *et al.*, 2008).



Adapted from Vivek *et al.*, 2008

Figure 2.1 Light table



Adapted from Vivek *et al.*, 2008

Figure 2.2 Endosperm modification scale

2.3.2 Biochemical analysis

Most breeding programmes traditionally rely on indirect measurement of lysine based on calorimetric analysis (Villegas *et al.*, 1984; Tsai *et al.*, 1972., Guiragossian *et al.*, 1979) or by indirectly inferring lysine content through colorimetric analysis of tryptophan content (Villegas *et al.*, 1984). This procedure has been used effectively to improve QPM germplasm at CIMMYT and seems justifiable in light of the cost savings relative to separation and analysis of endosperm tissue (Pixley and Bjarnason,

2002). Lysine measurements made by conventional amino acid analysis of maize kernels (Tsai *et al.*, 1972) are not only expensive and slow, but their reproducibility is affected by many factors, thus making them prohibitively more expensive for most breeding programmes. Tryptophan and lysine values are highly correlated (r = 0.99) (Cordova and Krivanek, 2006, cited by Vivek *et al.*, 2008), and therefore lysine content is indirectly inferred through colorimetric analysis of tryptophan content (Villegas *et al.*, 1984). Due to the well-established relationship between these amino acids in the protein of *opaque-2* maize endosperm (Hernandes and Bates, 1969; Villegas *et al.*, 1992), tryptophan can be used as a single parameter for evaluating the nutritional quality of protein. The analysis can be done on the whole grain or only the endosperm. Maize endosperm protein is generally deficient in the amino acids lysine and tryptophan, while the germ has a relatively constant and well-balanced amino acid composition regardless of genetic background (Vivek *et al.*, 2008).

Enzyme-linked immunosorbent assay (ELISA) provides a more objective and rapid means of estimating lysine content in maize endosperm compared to calorimetric analysis. The procedure is based on a gene-coding elongation factor-1α (EF-1α) identified from the analysis of cDNA clones, whose synthesis is significantly increased in *o2* endosperm (Habben *et al.*, 1995). EF-1α is a lysine-rich protein (10 % lysine) that is highly abundant in eukaryotic cells (Browning *et al.*, 1990; Merrick, 1992). Habben and co-workers (1995) developed an ELISA using EF-1α antiserum, to measure the level of this protein in maize genotypes, in which a high positive correlation (r = 0.92) was found between endosperm lysine levels and EF-1α content. Concentration of EF-1α thus appears to provide a useful index of the lysine content in the cereal grain proteins (Prasana *et al.*, 2001). The ELISA for EF-1α provides a sensitive, efficient, less laborious and inexpensive method of monitoring the lysine content of maize grain, and is more amenable to automation than non-zein quantification (Moro *et al.*, 1996).

2.4 Nutritional superiority and biological value of QPM

Biological value refers to the amount of absorbed nitrogen needed to provide the necessary amino acids for different metabolic functions. The biological value (BV) of conventional maize protein is 45 %, while that of *o2* maize is 80 % (Prasana *et al.*, 2001). Only 37 % of conventional maize protein intake is utilized compared to 74 % of the same amount of *o2* maize protein (Prasana *et al.*, 2001). Therefore, in terms of BV and utilisable maize protein, nutritional requirements for metabolic functions can be met from less amounts of QPM compared to common maize. This is critical in times of famine or food shortages, which is a common occurrence in disadvantaged communities. The nitrogen balance index for skim milk and *o2* maize protein is 0.80 and 0.72, respectively, which indicates that the protein quality of QPM is 90% of that of milk (Prasana *et al.*, 2001). This makes *o2* maize an ideal supplement for skim milk for children in poor communities. Besides, around 24 g of

common maize per kg of body weight is required for nitrogen equilibrium, compared to only around 8 g for QPM (Bressani, 1995; Graham *et al.*, 1980), which translates to a consumption of conventional maize of three times that of *o2* maize per kg of body weight. The other nutritional benefits of QPM include higher niacin availability due to a higher tryptophan and lower leucine content, higher calcium and carbohydrate (Graham *et al.*, 1980), and carotene utilization (De Bosque *et al.*, 1988). Further, high quality protein maize can be transformed into edible products without deterioration of its quality or acceptability, and can be used in conventional and new food products (Prasana *et al.*, 2001).

2.4.1 QPM in human nutrition and utilization

In infant feeding trials conducted in Ghana (Akuamoa-Boateng, 2002), children fed on QPM thin maize gruel/porridge (Koko), had fewer sick days and better chances of escaping death due to Diarrhoea and other infections, than children fed on common maize Koko. In the same studies, QPM had better growth-enhancing capabilities and less stunting than did normal maize, when fed to weaning children (Akuamoa-Boateng, 2002). In Guatemala, it was demonstrated that *o2* maize has 90 % of the nutritive value of milk protein in young children (Prasana *et al.*, 2001). Children in Colombia suffering from Kwashiorkar, a severe protein deficiency disease, were brought back to normalcy on a diet containing only *o2* maize as the source of protein (Prasana *et al.*, 2001). QPM would have equally beneficial effects on adults, as in case of infants and children (Bressani, 1990).

QPM can also be used as an ingredient in the preparation of composite flours to supplement wheat flour for bread and biscuit preparation. Composite flours (10 % maize flour) are used commercially in sub-Saharan countries such as Zambia, Zimbabwe and Ghana. Brazil uses composite maize-wheat flours, with QPM substitution of up to 20 % for commercial bread-making (Magnavaca, 1990). For biscuit preparation, the substitution could be up to 50 % (Villegas, 1995).

2.4.2 QPM and animal nutrition

Besides its obvious significance in human health, QPM could play an increasingly important role in reducing the protein supplement in animal feed, if used as an ingredient. According to Gevers and Lake (1992), in pig feeding trials conducted at the University of Kwa-Zulu Natal, 22 % less fishmeal could be used in pig diet without influencing performance if QPM replaced common maize. In an experiment with finisher pigs to compare QPM and common maize, Burgoon *et al.* (1992) found that less soybean meal was needed in QPM than common maize-based diets to maximize performance. When QPM is substituted for regular yellow maize at a rate of 60 % in broiler diets, the use of soybean meal and the cost of the ration are reduced (Subsuban, 1990). In Kenya, Nyanamba *et al.* (2003) found that substituting QPM for regular maize in the production of broiler feed reduces the amount of expensive protein sources used. Substitution of common maize with QPM in feed formulation and/or

supplements in monogastric animals (pigs, poultry and fish) has the potential to significantly reduce the use of expensive legume or animal-based protein sources, thereby reducing the price and enhancing the affordability of protein of animal origin (e.g., eggs, meat and dairy products) to vulnerable groups.

2.5 Development of QPM donor stocks

The development of QPM stocks with well modified kernel phenotype and good protein quality is indeed important for accelerating rapid development of QPM germplasm. Selection for kernel modification has to be practised at all stages, while simultaneously maintaining protein quality. Two approaches are effectively exploited in developing QPM donor stocks. The first is intra-population selection for genetic modifiers in *o2* backgrounds exhibiting a higher frequency of modified *o2* kernels. Controlled full-sib pollination is employed in the initial cycle, followed by modified ear-to-row system suggested by Lonnquist (1964). Selection is practised for modified ears and modified kernels at all stages (Bjarnason and Vasal, 1992).

The second approach involves recombination of superior hard endosperm (*o2)* families. Genetic mixing coupled with selection of modified ears, showing high frequencies of modified kernels with good protein quality, is practised for 3–4 cycles. Once it is determined that a high degree of endosperm modification has been achieved in these materials, the genotypes are ready for utilization as QPM donor stocks, as well as populations for further improvement using appropriate schemes.

2.6 Conversion of non-QPM materials into QPM versions

QPM conversion involves transferring the mutant *opaque2* allele and the associated endosperm and amino acid modifier genes into other elite lines that lack the QPM phenotype. Typically this is done using some type of backcross breeding scheme. The QPM donor parent is crossed with the recurrent non-QPM recipient parent, usually an elite material that exhibits many beneficial characteristics but lacks the desirable allele. Of the resulting progeny, those containing the desirable allele that most resemble the recurrent recipient parent are then "backcrossed" to the recurrent recipient parent. Heterozygous plants containing one copy of the mutant o2 allele are identified by self-fertilisation of all plants in a population and planting the seed from the self-fertilised plants that exhibit QPM phenotype in the next season. This process is normally repeated several times. After repeated cycles of backcrossing and self-pollination, the breeder ends up with plants that are nearly identical to the recurrent parent and that also contain the desirable allele introduced from the donor parent. QPM plants are identified by analysing maize grain samples in the laboratory for lysine and tryptophan.

Each conventional backcross generation needs to be self-pollinated to identify the *opaque-2* recessive gene and a minimum of six backcross generations are required to recover satisfactory levels of recurrent parent genome. With marker-assisted backcrossing, self-fertilisation accompanying each backcross to identify the *opaque-2* recessive gene is eliminated, thus reducing the number of backcross generations to one-half.

2.7 Breeding for tolerance to drought stress

Maize is relatively more sensitive to moisture and nutrient stress compared to other graminaceas crops such as sorghum and millets. Therefore response to selection is maximized when selection is conducted in the same target environments where future varieties will be grown (Ceccarelli *et al* 1996). Findings by Bänziger *et al.,* 2004, also suggest that simultaneous selection for tolerance and resistance to drought stress, while also monitoring performance under high potential conditions, can result in significant breeding progress in target environments where combinations of those stresses occur. Good selection progress is achievable by having useful variation in the germplasm in characteristics that confer drought tolerance. The ability of a plant to yield well in dry environments may be due to drought avoidance, drought tolerance or both mechanisms. Maize is most vulnerable to moisture deficit stress that occurs two weeks bracketing flowering (Bänziger *et al*., 2000). Moisture stress during flowering lengthens the anthesis-silking interval (ASI) and reduces the number of silks that are viable for pollen germination to fertilize the embryos. Grain yield (GY) and secondary traits are simultaneously employed when screening for drought tolerance (Bänziger *et al*., 2000). This is largely because the heritability of suitable secondary traits is less or not affected by stress (Bänziger and Lafitte, 1997). Anthesis-silking interval is one of the secondary traits recommended by Bänziger *et al*. (2000) for use in a drought-breeding program Therefore, under managed drought stress, a drought tolerant genotype is one that produces high grain yield and number of ears per plant, low or negative ASI, delayed leaf senescence, small tassel size, and reduced leaf rolling. Vivek *et al.,* (2008) suggest that several studies have shown that the quality of protein (lysine and tryptophan levels) is unaffected when maize is grown under both low soil nitrogen and drought conditions, though protein quantity is lower under the former. It has however, been noted that some QPM genotypes under severe drought stress can significantly increase the frequency of soft or poorly modified grains relative to the same genotypes under optimal moisture growing conditions (Ngaboyisonga *et al.,* 2006).

2.8 Incidence and breeding for maize streak virus resistance

Maize streak virus epidemics are noted to be frequent in the tropics due to alternate and successive cropping of maize plant hosts and the presence of other hosts such as wild grasses (Mesfin *et al.,* 1995). Often infection of the crop by the MSV disease at seedling stage results in no ear formation, but later infection leads to undersized and poorly filled ears (Kaitisha, 2001). Infection of a maize crop in

the first three weeks of planting often results in 100 % yield loss (Bosque-Perez and Buddenhugan, 1999). Similarly, a maize crop that is planted at the end of the rainy season seems to be most severely affected. Symptoms of MSV tend to appear quicker in younger plants: 3 to 5 days in a one-week old plant, and 7 to 9 days in a 9-week-old plant (Mesfin *et al.,* 1995). MSV severity is measured on a scale of 1 – 5 as described by Martin *et al.* (1999).

Maize varieties with resistance to MSV were developed at the Institute of Tropical Agriculture (IITA) and at the Harare station of the International Maize and Wheat Improvement Centre (CIMMYT) (Efron *et al.,* 1989). According to Kim *et al.* (1981), it has been noted that resistance to MSV by the IITA maize germplasm is controlled by two or three major gene pairs, with the possible involvement of minor genes. Welz *et al.* (1998) mapped out the quantitative trait loci (QTL) for resistance to MSV; but Kyetere *et al.* (1999) went further and demonstrated the presence of a single major gene (designated as *msv 1*) that controls MSV tolerance.

2.9 Combining ability

The combining ability estimate is a measure of the value of genotypes based on performance of their offsprings, produced in a definite mating system (Allard 1960). The combining ability enables the prediction of performance, but the genotype performance cannot be predicted for traits that are polygenic. The performance of a hybrid is related to the general (GCA) and specific (SCA) combining abilities of the inbred lines involved in the cross. The concept of GCA and SCA was introduced by Sprague and Tatum (1942) and its mathematical modelling was set about by Griffing (1956). Combining ability studies provide information on the genetic mechanism controlling quantitative traits and enable plant breeders to select suitable parents. Estimate of general (GCA) to specific combining ability (SCA) variance ratio is useful to evaluate the variability either due to additive or non-additive or both types of gene actions (Farshadfar *et al.,* 2002). For plant height, Choukan (1999) and Griffing (1956) concluded that control of this trait was influenced by non-additive gene action, whereas according to Shreenivasa and Singh (2001), control of plant height is due to additive gene action. On the other hand, Sain *et al.,* (2001) and Zelleke (2000) observed that both additive and non-additive gene action are responsible for controlling plant height. Where additive gene action is observed to be operative in a trait, selection in early generations may be more appropriate in improving the character. On the other hand, for a trait that is influenced by non-additive gene action, improvement of such a character is more achievable through selection in advanced generations.

The GCA is the mean performance of a line in all its combinations expressed as a deviation from the overall population mean. GCA is associated with additive effects of the genes. The deviation can either be positive or negative and is trait specific. Specific combining ability is the deviation of a particular

cross performance from that predicted on the basis of GCA. The term specific combining ability is used to designate those cases in which certain combinations do relatively better or worse than would be expected on the basis of average performance of the lines involved. SCA is related to dominance and epistatic effects (non-additive effects) of the genes. In maize yield, GCA was found to be more important than SCA in unselected populations, whereas SCA was found to be more important for previously selected lines. The GCA test is used in the early screening of segregating populations in a breeding program. General combining ability is also used to identify types of gene interactions governing traits of interest. Plant breeders have used measures of GCA and SCA effects to establish heterotic patterns among populations and pools.

A high SCA indicates a non-additive gene action. In addition SCA estimates can be used to determine heterotic relations among different genotypes. However it should be noted that yield only is used to determine heterotic relations among the different genotypes. The value of any population depends on its potential *per se* and combining ability in crosses (Malik *et al* 2004). In hybrid development, specific combinations are desired and therefore SCA effects would help in selection of parental material for hybridisation. With open pollinated variety (OPV) development, a line with higher GCA effects can be used in synthetic development.

2.10 Heritability

Heritability is a ratio that describes the amount of phenotypic variation that can be attributed to the differences in the additive genetic merit of individuals in a population (Singh 2003). It is a quantitative measure which provides information about the correspondence between genotypic variance and phenotypic variance expressed as a percentage (Dabholkar, 1999). If this percentage is large, the character concerned is regarded as highly heritable, while if small, environmental agency is considered as mostly responsible for the phenotypic manifestation of the character. Narrow sense heritability is a measure of the contribution of fixable genetic variation to total or phenotypic variation and is therefore of more interest to the breeder than broad sense heritability. In addition heritability does not indicate the degree to which a trait is genetic, but measures the proportion of the phenotypic variance that is the result of genetic factors. The magnitude of heritability in the broad sense depends on a number of factors, which include (i) developments that may have occurred in the original material (e.g. intensity of selection), (ii) type of gene action involved in the expression of a character (e.g. characters controlled largely by genes acting in an additive fashion have higher values of heritability than ones governed by genes with large non-additive effects), and (iii) populations that are more uniform genetically are expected to show lower heritability than genetically variable populations. Heritability is also dependent on conditions of management, whereby more variable environmental conditions reduce the magnitude of heritability while more uniform conditions increase it (Dabholkar, 1999).

According to Robinson (1966), heritability estimates in cultivated plants can be placed in the following categories: (i) low heritability (5 to 10 %), e.g. yield, (ii) medium heritability (10 to 30 %), e.g. components of grain yield and plant height, and (iii) high heritability (30 to 60 %), e.g. maturity and chemical composition characters. This classification is approximate and represents averages of heritability estimates over various crop plants, procedures of determination and several environments comprising of locations and years.

2.11 North Carolina Design II (NCDII)

The design was developed by Comstock and Robinson in 1952. This mating design is used for the purpose of obtaining genetic information from experimental populations. In developing the experimental progenies different sets of parents are used as males and females. Each male (m) is crossed with each female (f) and the total number of crosses will be an (m x f) product. In this design both maternal and paternal half sibs are produced. The design is cross classified in terms of analysis. In this design the genetic expectations for males and females are equivalent to general combining ability (GCA), while the male x female interaction is equal to specific combining ability (SCA).This design separates the variance of progenies into three fractions, namely, variance due to males, variance due to females and variance due to the male and female interaction. Appropriate F tests can thereafter be made to test for the differences among males, females and for interaction.

Although Design II has not been used extensively in maize compared to the diallel, Hallauer and Miranda (1989) found some merits of this design over the diallel designs which include: (i)more parents can be included for a given level of resources, (ii) two independent estimates of additive genetic variance are available, (iii) an estimate of dominance variance is determined directly from the mean square, and (iv) a greater number of parents can be included by subdividing parents into sets.

CHAPTER 3

MATERIALS AND METHODS

3.1 Germplasm and mating design

The study involved crossing five white QPM inbred lines from CIMMYT-Zimbabwe to 12 non-QPM yellow inbred lines, of which six were from the Department of Research & Specialist Services (DR&SS) and six from CIMMYT-Zimbabwe. The 12 inbred lines whose crosses provided sufficient seed for conducting trials are presented in Table 3.1. The six non-QPM yellow inbred lines from CIMMYT-Zimbabwe were beta-carotene conversions developed in Zimbabwe and Mexico from white lines. Inbred lines from DR&SS are intermediate to late maturing with dent to semi-dent grain texture. RL17p and EL77p are yellow derivatives of the white inbred line N3.2.3.3. The QPM inbred lines VL06375, CZL0613, CZL082, VL0523, and CML511 are QPM conversions of the non-QPM inbred lines CML312, CML144, CML141, CML202, and CML176, respectively. VL06375 and CML511 are resistant to MSV, whilst CZL0613 is tolerant to both MSV and drought. VL06375 is in type A heterotic group, while CZL082, VL0523, and CML511 belong to type B heterotic group.

The mating among lines in each set was a modified North Carolina Design II (Comstock and Robinson, 1952) in which each line was not used strictly as male or female (i.e., reciprocal crosses were bulked). Of the 54 progenies successfully produced, 35 F_1 genotypes (Table 3.2) provided sufficient seed for multi-location evaluation at four sites in winter 2009.

Table 3.1. Parental inbred lines

Code	Name	Pedigree	% Tryptophan [b]	% Lysine	% Protein
L1 [a]	RL17P	RL17P	0.070	0.362	13.36
L2	EL77P	EL77P	0.049	0.286	10.98
L3	HX482P	HX482P	0.055	0.282	10.73
L4	YCOBY7P	YCOBY7P	0.040	0.272	11.19
L5	NA[#]	[[EV7992]C1F2-430-3-3-3-X-7-B-B/CML202]-6-2-2-3-B*3/[BETASYN]BC1-12-4-1-1-1-B	0.053	0.259	12.23
L6	NA	[Ent320:92SEW2-77/[DMRESR-W]EarlySel-#I-2-4-B/CML390]-B-13-2-B-4-#/[BETASYN]BC1-9-1-1-1-2-B	0.060	0.299	11.86
L7	NA	CML445/[BETASYN]BC1-2-1-1-1-1-B	0.061	0.302	13.21
L8	VL06375	(CLQRCWQ50/CML312SR)-2-2-1-BBB	0.078	0.402	11.90
L9	CZL082	[CML141/[CML141/CML395]F2-1sx]-4-2-1-B*4-1-B	0.093	0.432	11.36
L10	VL0523	[CML202/CML144]F2-1-1-3-B-1-B*5-1-B	0.098	0.425	12.29
L11	CZL0613	[CML144/[CML144/CML395]F2-8sx]-1-2-3-2-B*4-1-B	0.078	0.375	12.54
L12	CML511	[CML389/CML176]-B-29-2-2-B*5	0.078	0.339	10.56

[a] Lines L2 to L4 are from the Department of Research and Specialist Services, L5 to L12 are from CIMMYT Zimbabwe, L1 to L7 are non-QPM and L8 to L12 are QPM.

[b] Percentage in the grain

[#] Name not available

Table 3.2 F_1 genotypes

Entry	Code	Pedigree
1	H1	L5/L8 [A]
2	H2	L5/L9
3	H3	L5/L11
4	H4	L5/L10
5	H5	L5/L12
6	H6	L6/L8
7	H7	L6/L9
8	H8	L6/L11
9	H9	L6/L10
10	H10	L6/L12
11	H11	L7/L8
12	H12	L7/L9
13	H13	L7/L11
14	H14	L7/L10
15	H15	L7/L12
16	H16	L2/L8
17	H17	L2/L9
18	H18	L2/L11
19	H19	L2/L10
20	H20	L2/L12
21	H21	L3/L8
22	H22	L3/L9
23	H23	L3/L11
24	H24	L3/L10
25	H25	L3/L12
26	H26	L1/L8
27	H27	L1/L9
28	H28	L1/L11
29	H29	L1/L10
30	H30	L1/L12
31	H31	L4/L8
32	H32	L4/L9
33	H33	L4/L11
34	H34	L4/L10
35	H35	L4/L12

[A] The first line is the female and the second line is the male or donor.

[β] The single cross hybrid was used as a check variety

3.2 Evaluation sites

The F_1crosses were generated at CIMMYT-Zimbabwe (31° 50′E, 17° 80′S, 1468 masl) in Harare during 2008/09 summer for evaluation under four environments in winter 2009. The F_1 progeny families were evaluated following an alpha (0, 1) lattice experimental design (Patterson *et al.,* 1978) with three replications and six plots per incomplete block. Off-season (winter) evaluation was conducted at Muzarabani (16.0°S, 480 masl) under artificially inoculated MSV disease and optimum conditions, whilst that for managed severe drought stress was done at Chiredzi Research Station (32° 14′E, 20° 48′S, 429 masl) for two planting dates in 2009. $F_{1:2}$ grain for light table, protein and SSR marker analyses was obtained from the Muzarabani optimum trial by shoot-bagging and self-pollinating two random ears per F_1 plot in winter 2009.

3.3 Field management

The materials for the first crossing block were established in the second week of November 2008, followed by the second block a week later. Trials at Muzarabani were planted on 17 April 2009 and 22 April 2009, respectively, whilst those at Chiredzi were planted on 7 May 2009 and 11 May 2009, respectively. Each plot in the crossing blocks and evaluation trials consisted of a 4 m single-row plot at each site. The inter-row and intra-row spacing was 0.75 m and 0.25 m, respectively, for both evaluation trials and crossing blocks. Two seeds were hand-planted per station for both crosses and trials and thinned to 53 300 plants per hectare at three weeks of crop emergence.

Site-specific fertilizer recommendations, manual and chemical weed and chemical pest control were used at each site. At CIMMYT Harare, the crossing blocks were only given supplementary irrigation whenever necessary whilst at Muzarabani 5 - 7 day cycles of 8 hours were used until physiological maturity. At Chiredzi irrigation was applied as per recommendation by Bänziger *et al.,* (2000).

3.4 Managing drought stress at Chiredzi

A total of 250 mm of irrigation water was applied during the initial 50 days of the crop's growth with no further irrigation thereafter to enable drought to coincide with flowering and grain filling. Such amount of moisture stress is projected to achieve 15-20 % (1-2 t/ha) of grain yield obtainable under well-watered conditions (Bänziger *et al.,* 2000). This stress level delays silking and causes ear abortion in drought-susceptible genotypes.

3.5 Seed preparation and planting

A seed preparation file (SeedPrep) was generated using spreadsheet-based software, Fieldbook 8.4.7 (Vivek *et al.,* 2007), for use in preparing seed labels, field maps and recording files for both crosses and trials. For the crosses, each donor and recipient as paired crosses to facilitate reciprocal hand-

pollination. Trials were planted following an alpha (0,1) lattice design where each replication was divided into incomplete bocks, each consisting of six plots.

3.6 Traits measured and/or derived

Kernel quality and agronomic characteristics utilised in this study are illustrated in Table 3.3. Agronomic traits measured on a plot basis included grain field, anthesis and silking dates at all locations. Endosperm modification scores, protein, lysine and tryptophan content were measured only for the Muzarabani optimum trial. Derived traits included anthesis-silking interval (ASI) and lysine content.

Table 3.3 Measured and derived traits

Trait	Procedure
Grain yield (GY)	Calculated from shelled grain weight per plot adjusted to 12.5% grain moisture basis and converted to tons per hectare.
Anthesis date (AD)	Taken as number of days after planting when 50% of the plants start shedding pollen.
Silking date (SD)	Taken as number of days after planting when 50% of the plants start producing silks.
Anthesis-silking interval (ASI)	Derived from anthesis date and silking date as follows: ASI= SD – AD interval (ASI)
Endosperm modification (MOD)	A combined modification percentage obtained by summing up the number of kernels in modification classes 1, 2, and 3 in a 100-kernel plot sample is recorded.
Percent protein (PROT)	Percentage of protein in the grain is recorded.
Percent tryptophan (TRP)	Percentage of tryptophan in the grain is recorded.
Percent lysine (LYS)	Percentage of lysine in the grain is recorded.

3.7 Light-table assessment

Kernel endosperm modification scores were assessed at CIMMYT Harare by means of a random 100-kernel sample obtained from F_2 ears selfed from each optimum trial plot (Pixley and Bjarnason, 2002). Light-tabling was done for each plot to pick out kernels with the *o2o2* genotype by using the degree of opaqueness as a secondary trait. Gradation in the opaqueness was scored on a 1 to 5 scale under a light table, where 1 = completely modified (i.e., translucent, normal phenotype); 2 = 75% modified; 3 = 50% modified; 4 = 25% modified; and 5 = completely opaque (Pixley and Bjarnason, 2002). For each 100-kernel plot sample, the number of kernels falling in each class was recorded under the light table

and recorded. A combined modification percentage was obtained by summing up the number of kernels in modification classes 1, 2, and 3. A good donor, on the basis of endosperm modification, is one with a high combined percentage (about 90%) of 1, 2, and 3 modification scores over a range of recipient lines (Vivek, *et al.*, 2008).

3.8 Protein analysis

Under the light-table, 20 class 2 kernels were randomly drawn from F_2 grain obtained by selfing two random ears from each optimum trial plot. The F_2 grain samples were analysed by CIMMYT Soil and Plant Analysis Laboratory in Mexico for kernel endosperm protein content and quality (tryptophan) following procedure described by Villegas (1975) and Villegas *et al.* (1984). The process, as outlined by Kevin and Bjarnason (2002), involves finely grinding whole-grain samples, defatting the resulting flour, and then calorimetrically determining the concentrations of nitrogen and tryptophan for duplicate samples. Lysine concentration was not measured because the procedure is more costly than that for tryptophan, and because lysine and tryptophan concentrations in the protein of *o*2 endosperm are significantly correlated (r = 0.99) (Cordova and Krivanek, 2006, cited by Vivek *et al.,* 2008). The calorimetric reaction for lysine determination in maize kernels is also time-consuming and its reproducibility is affected by many factors (Tsai *et al.,* 1972). Lysine content was indirectly inferred from the colorimetric analysis of tryptophan content (Villegas *et al.*, 1984)

3.9 Data analysis

All the values for kernel quality characteristics (i.e., endosperm modification, protein, lysine and tryptophan) were transformed using arcsine transformation as illustrated by Zar (1974) before subjecting them to analysis of variance (ANOVA). PROC GLM (SAS Institute, 2007) was used to perform the analysis of variance (Table 3.4) per environment and across environments to assess the genotype x environment performance of the crosses under both yield and non-yield limiting conditions. The statistical model underlying each observation for the individual site analysis is defined as:

$$Y_{ijk} = \mu + m_i + f_j + mf_{ij} + e_{ijk}$$

where Y_{ijk} is the phenotypic measurement of the progeny of the i^{th} male crossed with j^{th} female in the k^{th} replication

μ is the general mean of all genotypes

m_i is the effect of the i^{th} male

f_j is the effect of the j^{th} female

mf_{ij} is the interaction of of the i^{th} male with the j^{th} female

e_{ijk} is the error associated with each observation.

$Y_{ijk} = \mu + m_i + f_j + mf_{ij} + s_k + ms_{ik} + fs_{jk} + e_{ijk}$

where Y_{ijk} is the observed value of the i^{th} male crossed with j^{th} female at the k^{th} location

μ is the general mean of all genotypes

m_i is the effect of the i^{th} male

f_j is the effect of the j^{th} female

mf_{ij} is the interaction of of the i^{th} male with the j^{th} female

s_k is the effect of the k^{th} site

ms_{ik} is the effect of the i^{th} male in site k

fs_{jk} is the effect of the j^{th} female in site k

e_{ijk} is the error associated with each observation.

Table 3.4. Skeleton ANOVA of genotypes grown in different environments

Source of variation	Degrees of freedom	Mean squares	
		Observed	Expected
Environments (E)	e – 1		
Replicates (r) /E	(r – 1)e		
Genotypes (G)	n – 1	$MS_{Genotypes}$	$V_\varepsilon + rV_{GE} + reV_{Genotype}$
Genotypes X E	(n – 1)(e – 1)	MS_{GE}	$V_\varepsilon + rV_{GE}$
Pooled error	(n – 1)(r – 1)e	MS_{Error}	V_ε

The general combining ability (GCA) variance and effects of parents and the specific combining ability (SCA) variance and effects of hybrids (Table 3.5) were estimated via line x tester analysis (Kempthorne, 1957), cited by Singh and Chaudhary (1985), where females were designated as lines and males or donors as testers. Thus the term "female" is occasionally used to refer to lines, while "male" is used interchangeably to refer to tester and donor in the foregoing discussion. A fixed effects model was assumed for the genotypes (donors, lines and hybrids) and environments in the current study. The following underlying statistical model was assumed in the combining ability analysis:

$Y_{ijkl} = \mu + m_i + f_j + (mf)_{ij} + p_{ijk} + r_l + e_{ijkl}$

where Y_{ijkl} is the observed value of the progeny of the i^{th} male crossed with j^{th} female in the k^{th} replication.

μ is the overall population mean.

m_i is the effect of the i^{th} mother (effect of the GCA of the i_{th} mother).

f_j is the effect of the j^{th} father mated to the i^{th} mother (effect of the GCA of the i^{th} father).

$(mf)_{ij}$ is the interaction between the i^{th} mother and the j^{th} father (effect of the SCA of the i^{th} mother and the j^{th} father).

r_l is the effect of the l^{th} replication.

e_{ijk} is the experimental error.

Significance of environment, hybrid, parent, interaction (site x entry, site x line, site x donor, line x donor, and site x line x donor) mean squares were estimated with F tests at 5 % and 1 % probability levels, respectively, for all measured and/or derived traits. Line and donor GCA and hybrid SCA effects were tested for significance using the t test at $P < 0.05$ and $P < 0.01$ probability levels, respectively. Least significant difference ($LSD_{0.05}$) was used to determine the superiority, in terms of mean performance, GCA and SCA effects, of parental lines and hybrids over their respective counterparts for the different traits under the three environments (i.e., optimum, drought, and MSV conditions).

Table 3.5. Skeleton ANOVA for NCDII

Source	Degrees of freedom	Mean squares	
		Observed	**Expected**
Between females (f)	(f – 1)	MS_F	$\sigma^2_w + p\sigma^2_{fm} + mp\sigma^2_f$
Between males (m)	(m – 1)	MS_M	$\sigma^2_w + p\sigma^2_{fm} + fp\sigma^2_m$
Females X males (fm)	(f – 1)(m – 1)	MS_{MF}	$\sigma^2_w + p\sigma^2_{fm}$
Within full sib families (p)	fm(p – 1)	MS_W	σ^2_w

Table adapted from Kearsey and Pooni (1996)

σ^2_w = variation within full sibs;

σ^2_f = variation between females

σ^2_m = variation between males

σ^2_{fm} = variation due to interaction between females and males

CHAPTER 4

RESULTS

4.1 Analysis of variance

Analysis of variance (ANOVA), general (GCA) and specific combining ability (SCA) analysis, heterosis and heritability estimates for grain endosperm modification and protein content and quality were based on laboratory analysis results obtained from grain samples drawn from one site (i.e., optimum) to minimise shipment and chemical analysis costs in Mexico

4.1.1 Kernel quality characteristics and grain yield under optimum conditions

Mean squares obtained from analysis of variance for grain endosperm modification and protein content and quality are presented in Table 4.1. No ssignificant differences due to crosses, lines, testers and their interaction were detected for all kernel quality characteristics and grain yield under optimum conditions. For MOD, the estimate of variance due to females was positive while variances due to males, and female x male interaction were both negative. The variance due to female x male interaction was negative for the remaining traits except PROT where it was zero, while that due to males was zero for TRP, LYS and PROT, but 0.01 for GY. Negative variance components are considered not to be significantly different from zero. Male variance was negative for LYS and GY whereas for TRP and PROT it was zero.

Table 4.1 Mean squares and variances for kernel quality characteristics and grain yield under optimum conditions

Source of variation	DF	Characteristic				
		MOD	TRP	LYS	PROT	GY
Cross	34	70.67	0.85	4.34	2.91	3.54
Line (F)	6	173.68	0.44	0.50	2.00	1.09
Tester (M)	4	48.4	0.55	4.78	2.8	5.54
Line*Tester (FM)	24	52.38	0.64	4.24	2.55	3.82
Error (MSe)	66	99.06	0.89	4.88	2.52	4.40
σ^2_{FM} ^		-1.33	-0.01	-0.02	0.00	-0.02
σ^2_M		-0.02	0.00	0.00	0.00	0.01
σ^2_F		0.69	0.00	-0.02	0.00	-0.02

σ^2_M = variation between males.

σ^2_F = variation between females.

σ^2_{FM} = variation due to interaction between females and males.

4.1.2 Endosperm modification and some agronomic traits under varying environments

The mean squares and variances due to crosses, lines, donors and their interactions for endosperm modification relative to some agronomic traits studied under varying environments are presented in Table 4.2. No significant differences were detected for all quality and agronomic traits studied under optimum environment. However, significant differences ($P<0.05$) among crosses, lines, and donors were observed for all traits under drought stress conditions, while interactions were non-significant. Under artificially induced MSV disease, significant ($P<0.05$) differences among crosses were observed for GY and AD only. Highly significant ($P<0.01$) differences were also detected among lines for AD and ASI, and due to interaction for GY, whereas differences among lines for GY, among donors for all traits, and similarly interactions were significant for AD were not observed under MSV disease. Highly significant ($P<0.01$) differences were observed due to both crosses and lines for AD and ASI, and due to donors for AD, while non-significant differences due to interaction for all traits, due to crosses, lines and donors for GY, and due to donors for ASI were detected across all environments.

The variance among donors was positive for all traits under all environments, except for MOD under optimum, GY under drought stress and across all environments, and GY and ASI under MSV disease conditions. Variances among lines were positive for both AD and ASI under all but optimum environments. It was also positive for MOD and GY under optimum and drought stress conditions, respectively, but was negative or zero for GY under all environments except drought stress. However variance for interaction was positive for AD and ASI under all environments except MSV disease, but negative for MOD under optimum conditions. For grain yield interaction variance was positive under MSV and across environments, but was negative under optimum and drought conditions.

Table 4.2 Mean squares and variances for endosperm modification and some agronomic traits under varying environments

Source of variation	Optimum (1)[€]					Drought (2)				MSV disease (1)				Across all environments (4)			
	DF	MOD	GY	AD	ASI	DF	GY	AD	ASI	DF	GY	AD	ASI	DF	GY	AD	ASI
Cross	34	70.67	3.54	7.52	11.91	34	0.78**	31.27**	11.83**	34	4.41*	17.14*	8.63	34	2.85	33.42**	12.08**
Line (F)	6	173.68	1.09	7.81	8.53	6	1.77**	43.51**	30.79**	6	3.38	42.2**	20.83**	6	3.56	79.18**	29.98**
Tester (M)	4	48.4	5.54	0.27	18.82	4	1.60*	128.61**	17.90**	4	3.52	21.06	6.01	4	1.69	91.35**	14.63
Line*tester (FM)	24	52.38	3.82	8.65	11.60	24	0.41	11.79	6.2	24	4.82**	10.23	6.01	24	2.98	13.69	8.31
Error (MSe)	66	99.06	4.40	6.50	10.80	140	0.63	11.61	5.87	70	2.68	10.53	6.14	280	2.04	10.06	7.17
σ^2_{FM} [Σ]		-1.33	-0.02	0.06	0.02		-0.01	0.01	0.01		0.06	-0.01	0.00		0.03	0.10	0.03
σ^2_M		-0.02	0.01	-0.03	0.03		0.00	0.48	0.05		-0.01	0.04	0.00		-0.01	0.32	0.03
σ^2_F		0.69	-0.02	0.00	-0.02		0.01	0.18	0.14		-0.01	0.18	0.08		0	0.37	0.12

[Σ] σ^2_M = variation between males.

σ^2_F = variation between females.

σ^2_{FM}= variation due to interaction between females and males.

[€] Numbers in parenthesis refer to total number of sites

4.1.3 Contribution to total variance

The proportional contributions of lines (females), testers (males/donors) and their interaction (crosses), to total variance for kernel quality and some agronomic characteristics under optimum environment are presented in Figure 4.1. It is evident from the graphical illustration that the contribution of line x tester interaction to total variance was higher than that of lines and donors for all traits. The 41 % proportional contribution of lines to the total variance for MOD was about six times the 7.6 % contributed by donors. However, a completely opposite scenario was observed for LYS whereby donors contributed 12.2 % to total variance relative to 1.9 % from lines. The contributions of lines and donors to total variance for TRP and PROT were similar. Donors alone contributed approximately equally for MOD (7.6 %) and TRP (7.2 %), and GY (18.4 %) and ASI (18.6 %). The proportional contribution of donors to the total variance for GY and ASI was almost three times that made by lines. With a contribution of 18.3 %, lines contributed far more than donors whose contribution was only 0.4 % to total variance for AD. The highest contribution to total variance was made by line x donor interaction which was 81.2 % whilst the lowest contribution of 0.4 % came from donors for AD. The proportional contribution of line x donor to total variance was relatively lower for kernel quality than agronomic characteristics. Donors were relatively more consistent in their contribution to total variance across traits than lines. The proportional contribution of donors exceeded that of lines by more than five times for GY and one and half times for ASI, respectively.

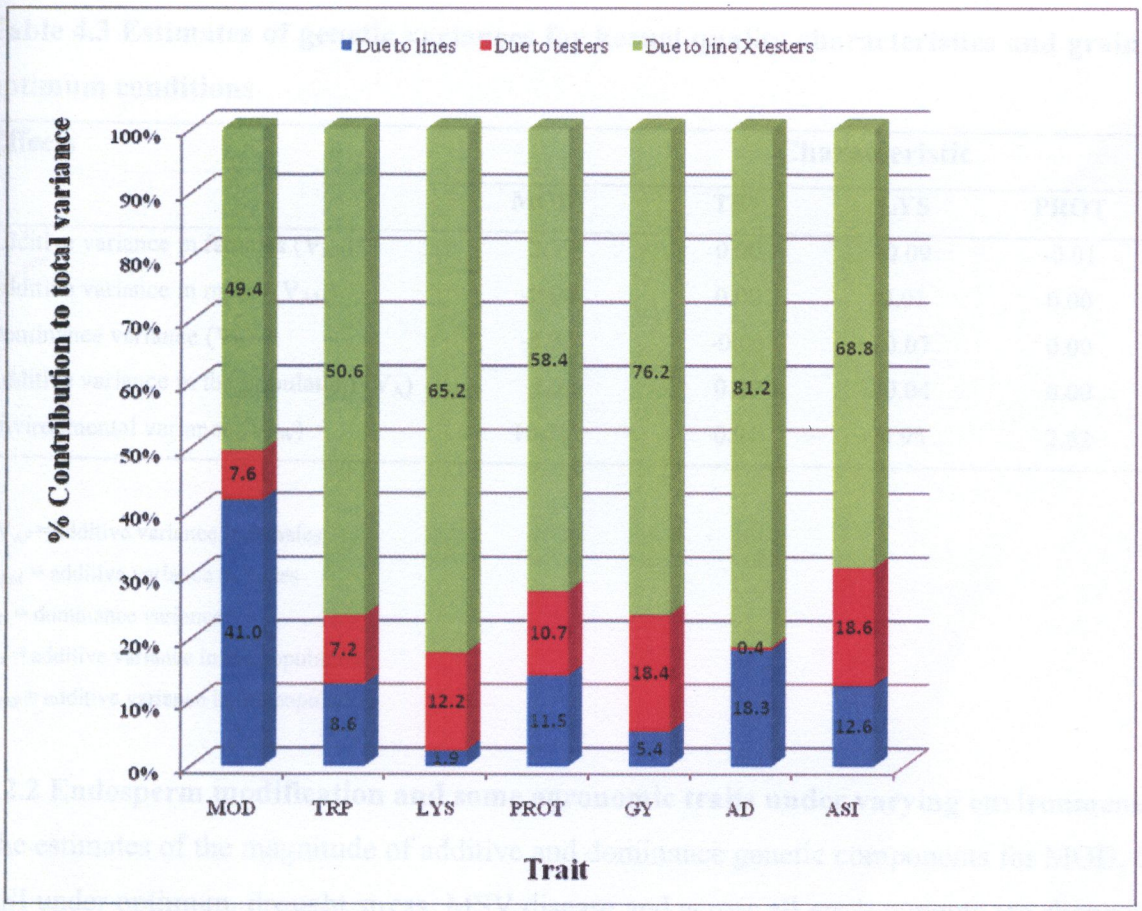


Figure 4.1 Proportional contribution of lines, testers and their interactions to total variance under optimum environment

4.2 Gene action

4.2.1 Kernel characteristics and grain yield under optimum conditions

The estimates of additive and dominance genetic components for kernel quality characteristics and GY under optimum conditions are presented in Table 4.3. Positive additive variance was detected within females (lines) and within the population for MOD, whereas dominance was negative for this trait. Both additive and dominance variances were, however, negative or zero for the rest of the kernel quality traits, except for LYS which was positive for additive variance in males. For GY, additive variance within males was positive while dominance variance and additive variance in females and the population were either negative or positive. The magnitude of environmental variance, on the other hand, was positive and relatively much higher than additive and dominance variance for all quality traits and grain yield, and ranged from 0.91 (TRP) to 10.38 (MOD).

Table 4.3 Estimates of genetic variances for kernel quality characteristics and grain yield under optimum conditions

Effects	Characteristic				
	MOD	TRP	LYS	PROT	GY
Additive variance in females (V_{AF})[ψ]	2.77	0.00	-0.09	-0.01	-0.06
Additive variance in males (V_{AM})	-0.06	0.00	0.01	0.00	0.03
Dominance variance (V_D)	-5.33	-0.03	-0.07	0.00	-0.07
Additive variance in the population (V_A)	1.35	0.00	-0.04	0.00	-0.02
Environmental variance (V_{EW})	10.38	0.91	4.95	2.52	4.46

[ψ] V_{AF} = additive variance in females

V_{AM} = additive variance in males

V_D = dominance variance

V_A = additive variance in the population

V_{EW} = additive variance in the population

4.2.2 Endosperm modification and some agronomic traits under varying environments

The estimates of the magnitude of additive and dominance genetic components for MOD, GY, AD and ASI under optimum, drought stress, MSV disease and across all environments are shown in Table 4.4. Positive additive variance within females was observed for all traits under drought, MSV disease and across all environments, except GY under MSV disease, but was negative for all traits excluding MOD under optimum conditions. Positive additive variance in males detected for all characters under drought, MSV disease and across all environments, with the exception of GY under MSV disease and across all environments. Under optimum conditions, it was however positive for GY and ASI and negative for MOD and AD. Additive variance in the population was positive for all characters under drought, MSV disease and across all environments apart from GY under MSV disease, positive for MOD and ASI and negative for GY and AD under optimum conditions. Positive dominance variance was detected for all traits under all environments except MOD and GY under optimum conditions, GY under drought and AD and ASI under MSV disease. Under all environments and for all traits, the magnitude of environmental variance was positive and relatively very high.

Table 4.4 Estimates of genetic variances for endosperm modification and some agronomic traits under varying environments

Effects	Optimum (1)[€]				Drought stress (2)			MSV disease (1)			Across all environments (4)		
	MOD	GY	AD	ASI	GY	AD	ASI	GY	AD	ASI	GY	AD	ASI
V_{AF}[ψ]	2.77	-0.06	-0.02	-0.07	0.03	0.73	0.56	-0.03	0.73	0.34	0.01	1.50	0.50
V_{AM}	-0.06	0.03	-0.14	0.12	0.02	1.91	0.19	-0.02	0.18	0.00	-0.02	1.27	0.10
V_D	-5.33	-0.07	0.25	0.09	-0.03	0.02	0.04	0.24	-0.03	-0.01	0.11	0.41	0.13
V_A	1.35	-0.02	-0.08	0.02	0.03	1.32	0.38	-0.03	0.45	0.17	0.00	1.38	0.30
V_{EW}	102.38	4.46	6.36	10.72	0.64	10.93	5.65	2.51	10.33	6.07	1.96	9.06	6.92

[ψ] V_{AF} = additive variance in females

V_{AM} = additive variance in males

V_D = dominance variance

V_A = additive variance in the population

V_{EW} = environmental variance

[€] Numbers in parenthesis refer to total number of sites.

4.3 Heritability

4.3.1 Kernel characteristics and grain yield under optimum conditions

Heritability estimates in the broad sense and narrow sense for kernel quality characteristics and grain yield under optimum environment are presented in Table 4.5. Both narrow sense and broad sense heritability were very low (≤ 0.05) for all quality traits and grain yield. A value of 0.03 was observed for narrow sense heritability due females for MOD, whereas PROT gave a broad sense heritability value of 0.05. The ratio or relative magnitude of narrow sense heritability to broad sense heritability is a measure of fixable genetic variation to total genetic variation. Narrow sense to broad sense heritability ratios of 0.23 and 0.21 were obtained for TRP and LYS respectively, while grain yield gave a ratio of 0.06. The ratio was however negative for MOD and PROT. Narrow sense heritability due to females was positive, though low for MOD while that due to males negative or zero for all traits apart from GY.

Table 4.5 Heritability estimates for kernel quality characteristics and grain yield under optimum conditions

Heritability	Characteristic				
	MOD	TRP	LYS	PROT	GY
Narrow sense heritability (h^2 ns)	0.01	0.00	----[Ω]	0.00	0.00
Narrow sense heritability due to females (h^2_f)	0.03	----	----	0.00	----
Narrow sense heritability due to males (h^2_m)	0.00	0.00	0.00	0.00	0.01
Broad sense heritability h^2 (bs)	----	----	----	0.05	----
NS/BS Ratio	-----	0.23	0.21	----	0.06

4.3.2 Endosperm modification and some agronomic traits under varying environments

Broad sense and narrow sense heritability estimates for MOD, GY, AD and ASI under optimum, drought stress, MSV disease and across all environments are given in Table 4.6. The magnitude of average narrow sense heritability ranged from -0.01 for AD under optimum conditions to 0.13 for the same trait across all environments. It was very low (<0.05) for all traits under optimum and MSV disease conditions, medium for AD under drought stress (0.11) and across all environments (0.13), but was low for ASI (0.06) under drought stress. Narrow sense heritability due to females was low (<0.10) for all traits under all environments, except across all environments where it was moderate (0.14) for AD. However, narrow sense heritability due to males was very low for all traits under all environments with the exception of AD which was medium under drought (0.16) and across all environments (0.12). Broad sense heritability was high for GY (0.55) and AD (0.33) under drought stress, medium for GY (0.21), AD (0.21) and ASI (0.13) under MSV, while it was moderate for GY (13 %) and very high for ASI (1.20) across all environments. The ratio of narrow sense heritability to broad sense heritability was positive for all traits under all environments, with the exception of AD under optimum conditions and across all environments and GY under MSV and across all environments where it was negative.

Table 4.6 Heritability estimates for endosperm modification and some agronomic traits under varying environments

Heritability	Optimum (1)				Drought stress (2)			MSV disease (1)			Across all environments (4)		
	MOD	GY	AD	ASI	GY	AD	ASI	GY	AD	ASI	GY	AD	ASI
h^2 (ns)[Ψ]	0.01	0.00	----	0.00	0.04	0.11	0.06	----	0.04	0.03	0.00	0.13	0.04
h^2_f(ns)	0.03	----	0.00	----	0.05	0.06	0.09	----	0.07	0.05	0.01	0.14	0.07
h^2_m(ns)	0.00	0.01	----	0.01	0.03	0.16	0.03	----	0.02	0.00	----	0.12	0.01
h^2 (bs)	----	----	0.05	0.03	0.08	0.55	0.33	0.21	0.21	0.13	0.13	----	1.20
NS/BS Ratio	----	0.06	----	0.06	0.49	0.20	0.19	----	0.21	0.20	----	----	0.03

[Ψ] h^2 (ns) = average narrow sense heritability.

h^2_f(ns) = narrow sense heritability due to females.

h^2_m(ns) = narrow sense heritability due to males.

h^2 (bs) = broad sense heritability

[Ω] ---- = indicates negative value

[€] Numbers in parenthesis refer to total number of sites.

4.4 Heterosis

Mid-parent heterosis for the crosses was determined for all kernel quality traits and GY whereby positive heterosis was desirable for these traits. Estimates for mid-parent heterosis for the five best endosperm-modified hybrids, where the degree of heterosis was dependent on cross and character studied, are presented in Table 4.7. There was consistency in the magnitude of the mean and mid-parent heterosis for MOD except for the cross L2/L12, which despite having the highest mean for MOD, gave the second highest heterosis for this trait. Heterosis for MOD ranged from 4.24 (L2/L11) to 8.11 (L5/L12) among the five best modified hybrids whereas it was as low as -7.46 (L1/L10). In addition to giving the second highest heterosis for MOD, L2/L12 gave positive heterosis for LYS (0.31) and PROT (0.50), while negative values were realised for TRP and GY.

Among the five most modified crosses, L5/L12 gave desirable heterosis for all traits except LYS GY, while L7/L8 had desired heterosis for MOD and TRP, but undesirable heterosis for LY, PROT and GY. Apart from giving the second highest heterosis for PROT among the most modified crosses, L2/L11 had desirable heterosis for MOD and GY, but negative heterosis for TRP and LYS. Consistently desirable heterosis was obtained from L7/L10 for all traits except TRP, and it gave the highest heterosis for PROT among the top five most modified crosses. Despite being in the bottom five hybrids considered, L3/L12 gave the best heterosis for LYS (0.80) and GY (2.02), and the second best heterosis for TRP (0.50). The cross L6/L11, regardless of being in the bottom five, gave the third highest heterosis for TRP (0.43), the second highest heterosis for LYS (0.81), and the fourth highest heterosis for PROT.

Table 4.7 Mid-parent heterosis estimates of selected hybrids for kernel quality characteristics and grain yield under optimum conditions

Cross	Characteristic					
	Mean MOD	MOD	TRP	LYS	PROT	GY
			Five best endosperm modified hybrids			
L2/L12[A]	94.81	7.53	-0.05	0.31	0.50	-0.52
L5/L12	93.07	8.11	0.14	-1.62	0.85	-1.88
L7/L10	92.80	6.19	-0.05	0.56	1.08	1.26
L7/L8	92.25	6.01	0.60	-0.10	-0.33	-0.49
L2/L11	91.28	4.24	-0.48	-0.08	1.07	1.05
			Five least endosperm modified hybrids			
L6/L11	77.12	-4.39	0.43	0.81	0.78	0.19
L3/L12	76.50	-5.31	0.50	0.98	0.27	2.02
L5/L11	75.78	-5.38	0.01	-0.90	-0.06	-1.13
L1/L8	69.89	-6.11	-0.46	-0.46	0.48	-0.44
L1/L10	68.43	-7.46	-0.25	-0.40	0.62	1.37

4.5 Estimates of general combining ability (GCA) effects

Variation in general combining ability effects (GCA) was estimated among lines and donors for kernel quality characteristics, grain yield and flowering traits to identify the best quality protein maize (QPM) inbred lines (donors) for use in the conversion of non-QPM inbred lines to QPM. Positive GCA effects were desirable for all kernel quality traits and GY. For flowering traits, negative values were desirable for ASI since this is associated with good pollen-silk synchronisation and tolerance to drought stress, while for AD negative GCA effects are preferred given that breeding for earliness is one of the objectives of the country's national program. Summary of results for analysis of GCA effects for these traits under stress and non-stress environments are presented in Tables 4.8 to 4.11.

4.5.1 Kernel quality characteristics and grain yield under optimum conditions

The GCA effects for kernel quality characteristics and GY for the donors and lines under optimum conditions are presented in Tables 4.8 and 4.9. The donor L8 had GCA effects for TRP, LYS and PROT in the desired direction, but undesirable GCA effects for MOD and GY, whereas L9 gave positive GCA effects for all traits except PROT and GY. Undesirable GCA effects for all traits were obtained for L10 and L11, except GY and MOD, respectively. Desirable GCA effects for all characters, excluding MOD, were observed for L12. The donors L9 and L12 had the highest desirable GCA effects for MOD, whereas L10 was the only donor with desirable GCA effects for GY.

Table 4.8 Donor GCA effects for kernel quality characteristics and grain yield under optimum conditions

Donor	Characteristic				
	MOD	TRP	LYS	PROT	GY
L8	-1.95	0.26	0.44	0.37	-0.07
L9	1.52	0.03	0.48	-0.03	-0.15
L10	-1.12	-0.17	-0.38	-0.27	0.88
L11	0.11	-0.09	-0.55	-0.43	-0.39
L12	1.53	-0.02	0.03	0.37	-0.33
$^{\neq}S.E._{gj}\pm$	1.88	0.18	0.41	0.28	0.41
LSD (0.05)	3.75	0.36	0.83	0.56	0.82

$^{\neq}$ gj represents the general combining ability effects of the j^{th} donor

The line L1 had undesirable GCA effects for all traits as opposed to L2 which gave desired GCA effects for all traits except LYS. In addition, L2 had the highest GCA effects for TRP (0.21) and the

TRP were realised from L3, and it had the best GCA effects for PROT (0.46) and GY (0.31). In spite of having the best GCA effects for GY, L4 had negative GCA effects for all Kernel quality traits. The line L5 though having negative GCA effects for MOD, it consistently gave positive GCA effects for the remaining characters. In addition to having the second best GCA effects for PROT (0.31), L6 had positive GCA effects for LYS, but negative GCA effects for the rest of the traits. Consistently desirable GCA effects for all traits except PROT were obtained for L7 and additionally had the best GCA effects for MOD.

Table 4.9 Line GCA effects for kernel quality characteristics and grain yield under optimum conditions

Line	Characteristic				
	MOD	TRP	LYS	PROT	GY
L1	-5.00	-0.20	-0.15	-0.75	-0.30
L2	4.40	0.21	-0.02	0.13	0.09
L3	-1.60	-0.19	0.12	0.46	0.31
L4	-1.23	-0.07	-0.36	-0.12	0.10
L5	-0.87	0.14	0.18	0.06	0.20
L6	-1.11	-0.04	0.10	0.31	-0.46
L7	4.97	0.14	0.12	-0.14	0.06
¥ $S.E._{gi}\pm$	2.30	0.22	0.51	0.34	0.50
LSD (0.05)	4.59	0.44	1.01	0.68	1.00

¥ gi represents the general combining ability effects of the i^{th} line

4.5.2 Endosperm modification and some agronomic traits under varying environments

The estimates of donor and line GCA effects for MOD, GY, AD and ASI obtained under optimum, drought stress, MSV disease and across all environments are depicted in Tables 4.10 and 4.11. It is evident that the donor L8 had desirable (i.e., negative) GCA effects for AD and ASI under all environments except optimum conditions for AD and MSV disease for ASI, whereas desirable (i.e., positive) GCA effects were realised for GY under all environments excluding optimum conditions. Undesirable (i.e., positive) GCA effects for AD and ASI were observed for L9 under all environments except optimum conditions, whereas negative GCA effects for GY were realised under all environments apart from drought stress. The donor L10 gave undesirable GCA effects for AD and ASI under all environments except optimum conditions for AD and MSV disease for ASI, while GCA effects for GY were negative under all environments except MSV disease. The GCA effects for AD were desirable under all environments except MSV whereas that for ASI were desirable under drought

and MSV disease, but not desirable under optimum and across all environments. In addition to having desirable GCA effects for AD and ASI under all locations, L12 had desirable GCA effects for GY under drought and MSV disease.

Table 4.10 Donor GCA effects for endosperm modification and some agronomic traits under varying environments

Donor	Optimum (1) €				Drought (2)			MSV (1)			Across all environments (4)		
	MOD	GYD	AD	ASI	GYD	AD	ASI	GYD	AD	ASI	GY	AD	ASI
L8	-1.95	-0.07	0.20	-1.06	0.07	-1.01	-0.63	0.19	-0.16	0.46	0.02	-0.49	-0.46
L9	1.52	-0.15	-0.04	-0.30	0.06	1.13	0.49	-0.01	0.84	0.70	0	0.76	0.34
L10	-1.12	0.88	-0.04	0.28	-0.35	2.41	0.87	0.43	0.22	-0.45	0.2	1.25	0.39
L11	0.11	-0.39	-0.09	1.47	0.13	-0.59	-0.22	-0.67	0.74	-0.40	-0.19	-0.12	0.15
L12	1.53	-0.33	-0.04	-0.39	0.08	-1.94	-0.51	0.06	-1.64	-0.30	-0.02	-1.39	-0.42
¥ S.E. gj ±	1.88	0.41	0.50	0.64	0.11	0.37	0.65	0.32	0.63	0.48	0.10	0.33	0.35
LSD (0.05)	3.75	0.82	0.99	1.28	0.22	0.74	1.28	0.64	1.26	0.96	0.20	0.64	0.69

¥ gj represents the general combining ability effects of the j^{th} donor.

€ Numbers in parenthesis refer to total number of sites.

For line L1, undesirable GCA effects were observed for GY, AD and ASI under all environments except optimum conditions for ASI. Desirable GCA effects for L2 were observed for GY under optimum and MSV disease conditions but not for drought and across all locations, whereas GCA effects were undesirable for AD and ASI for all environments except ASI under optimum conditions. Consistently desirable GCA effects were obtained for AD, ASI and GY under all locations for L3. Desirable GCA effects for AD and GY, and undesirable GCA effects for ASI were observed for L4 under all environments. For L5, desirable GCA effects for AD and ASI and negative GCA effects for GY were realised under all environments except, while undesirable GCA effects for ASI under drought and positive GCA effects for GY under optimum conditions were also realised for this line. Negative GCA effects for AD, ASI and GY under all locations except drought for GY and optimum conditions for ASI were observed for line L6. Undesirable GCA effects for AD and GY and negative GCA effects for ASI were realised under all environments except optimum conditions for ASI and GY.

Table 4.11 Line GCA effects for endosperm modification and some agronomic traits under varying environments

Line	Optimum (1) €				Drought (2)			MSV (1)			Across all environments (4)		
	MOD	GYD	AD	ASI	GYD	AD	ASI	GYD	AD	ASI	GY	AD	ASI
L1	-5.00	-0.30	0.86	-0.20	-0.15	1.33	1.23	-0.16	2.44	1.39	-0.09	1.20	0.90
L2	4.40	0.09	0.59	-0.13	-0.11	1.63	1.07	0.05	1.57	1.39	-0.88	1.63	0.85
L3	-1.60	0.31	-0.74	-0.67	0.42	-0.43	-0.95	0.48	-0.70	-0.28	0.45	-0.57	-0.71
L4	-1.23	0.10	-0.41	0.13	0.19	-0.60	0.45	0.61	-0.83	0.66	0.19	-0.60	0.42
L5	-0.87	0.20	-0.94	-0.73	-0.16	-0.96	0.21	-0.03	-0.36	-1.41	-0.06	-0.80	-0.42
L6	-1.11	-0.46	-0.01	1.53	0.07	-1.56	-1.42	-0.12	-2.63	-1.34	-0.12	-1.44	-0.66
L7	4.97	0.06	0.66	0.07	-0.27	0.57	-0.59	-0.84	0.50	-0.41	-0.27	0.57	-0.37
¥S.E. gi ±	2.30	0.50	0.61	0.79	0.12	0.74	0.49	0.39	0.78	0.59	0.21	0.56	0.49
LSD (0.05)	4.59	1.00	1.22	1.57	0.23	1.47	0.97	0.78	1.55	1.18	0.42	1.10	0.96

¥ gi represents the general combining ability effects of the i^{th} line

€ Numbers in parenthesis refer to total number of sites

4.6 Estimates of specific combining ability (SCA) effects

4.6.1 Kernel quality characteristics and grain yield under optimum conditions

The specific combining ability effects for kernel quality traits and GY under optimum conditions are given in Table 4.12. It is evident that the majority of the crosses had highly significant SCA effects for MOD with ten of them having positive SCA effects for this trait. Lysine and tryptophan had one cross each with significant SCA effects while GY had two crosses with significant SCA effects and all crosses had non-significant SCA effects for TRP. The top five crosses with respect to significant positive SCA effects for MOD were L5/L12 (7.78), L3/L11 (6.52), L1/L11 (5.23), L2/L12 (4.56), and L7/L8 (4.50). The crosses L7/L12, L5/L11 and L3/L12 had amongst the highest SCA effects for MOD though in the undesirable direction. In addition to having the highest SCA effects for MOD, L5/L12 had positive though non-significant SCA effects for TRP and PROT. The cross L6/L9, in addition to having highly significant ($P<0.01$) positive SCA effects for MOD, had the highest and third highest positive, though non-significant, SCA effects for TRP and LYS respectively. L7/L10 gave positive, though non-significant SCA effects for LYS, PROT and GY in addition to having the sixth highest SCA effects for MOD. Despite having the highest and significant positive SCA effects for GY, L5/L9 had negative SCA effects for TRP, LYS and PROT. However L3/L11, in addition to giving the second highest and significant SCA effects for GY, gave positive SCA effects for TRP and LYS, irrespective of them being non-significant. L5/L8 had highly significant ($P<0.01$) positive SCA effects for LYS, and in addition its SCA effects for TRP and GY were in the desired direction though non-significant. Besides giving the third highest and significant positive SCA effects for MOD, L1/L11 gave highly significant negative SCA effects for PROT and positive though not significant SCA effects for TRP.

Table 4.12 Hybrid SCA effects for kernel quality characteristics and grain yield under optimum conditions

Cross	Characteristic				
	MOD	TRP	LYS	PROT	GY
L1/L8 [A]	-2.63**	-0.49	-0.61	0.67	-0.26
L1/L9	1.35	0.08	1.30	1.69	-0.61
L1/L10	-4.40**	-0.07	-0.14	1.13	1.07
L1/L11	5.23**	0.13	-0.43	-2.90	-0.25
L1/L12	1.33	0.38	0.45	-0.04	0.10
L2/L8	-4.04**	0.26	0.24	0.01	-1.13
L2/L9	-4.97**	0.13	-0.79	-0.84	1.05
L2/L10	2.39*	0.29	0.02	-0.64	-0.40
L2/L11	1.98	-0.54	0.20	1.22	1.20
L2/L12	4.56**	-0.14	0.30	0.25	-0.40
L3/L8	0.47	-0.22	-0.69	0.12	0.38
L3/L9	-0.46	-0.33	-0.55	0.05	-0.19
L3/L10	0.87	0.42	-0.04	-0.87	-1.20
L3/L11	6.52**	-0.48	0.35	0.84	-0.97
L3/L12	-5.27**	0.61	0.91	-0.14	2.03
L4/L8	2.65**	0.30	0.34	0.46	0.72
L4/L9	2.79**	-0.19	-0.09	-0.02	-1.09
L4/L10	-0.33	-0.04	-0.34	-0.10	-0.21
L4/L11	-3.18**	0.34	1.16	-0.35	0.86
L4/L12	-2.01*	-0.41	-1.10	0.01	-0.18
L5/L8	-1.67	0.71	3.07**	-0.36	0.94
L5/L9	1.32	-0.40	-0.50	-0.22	2.54
L5/L10	-2.51*	-0.38	-0.16	-0.19	-0.30
L5/L11	-5.00**	-0.01	-0.72	0.13	-1.03
L5/L12	7.78**	0.07	-1.72	0.63	-1.81
L6/L8	1.15	-0.96	-1.97	-0.40	0.49
L6/L9	2.82**	0.76	1.45	0.11	-1.07
L6/L10	0.16	-0.17	-0.02	-0.58	0.25
L6/L11	-3.89**	0.50	1.03	0.84	0.61
L6/L12	-0.31	-0.13	-0.52	0.03	-0.64
L7/L8	4.50**	0.41	-0.38	-0.45	-0.49
L7/L9	-3.63**	-0.08	-0.43	-0.42	-1.13
L7/L10	4.27**	-0.04	0.69	1.29	0.78
L7/L11	0.42	0.08	-1.59	0.26	0.01
L7/L12	-5.64**	-0.37	1.69	-0.68	0.88
[ξ] S.E. sij ±	4.59	0.45	1.01	0.69	1.00
LSD (0.05)	9.17	0.89	2.02	1.37	2.00

[A] The first line is the female and the second line is the male or donor.

[ξ] Represents the specific combining ability effects of the cross between the i^{th} line and the j^{th} donor.

4.6.2 Endosperm modification and some agronomic traits under varying environments

The specific combining ability effects of the different crosses for MOD relative to GY, AD and ASI und optimum, drought stress, MSV disease and across all locations are shown in Table 4.13. Indications a clearly that some of the crosses had significant SCA effects under certain environments whereas no significant SCA effects were detected for all the crosses for GY under drought stress and across a locations and ASI under drought stress. Under optimum conditions significant ($P<0.05$) positive SC effects for GY were observed for L3/L12 and L5/L9 whereas for ASI significant negative SCA effec were obtained from L3/L11 and L6/L9 while that for L6/L11 were significant positive. Under the sam environment and for AD, significant negative SCA effects were realised for L2/L11, L5/L9 and L6/L1 while that for L1/L8, L2/L9 and L5/L10 were significant positive. Significant positive SCA effects for A were consistently detected for L2/L9, L3/L11, L4/L8 and L7/L10 under drought stress, while significar negative SCA effects for L4/L18 and significant positive SCA effects for L4/L12 were identified for G under MSV disease. Significant negative SCA effects for L1/L8, L4/L12, and L6/L9, and significar positive SCA effects for L2/L11, L4/L8 and L6/L12 were distinguished for AD under MSV, whil significant positive SCA effects for L1/L9 and significant negative SCA effects for L1/L11 were foun under the same environment. On the other hand, positive significant SCA effects for L4/L8 and significar negative SCA effects for L6/L11 were observed for AD and ASI, respectively, across all locations.

In addition to having the third best SCA effects for MOD, L1/L11 gave desirable and significant SCA effects for ASI under MSV disease. It also gave desirable though non-significant SCA effects for AS under optimum, drought stress and across all locations, as well as desirable SCA effects for AD unde optimum conditions, despite being non-significant. Apart from being among the top five crosses in terms of SCA effects for MOD, L2/L12 gave desirable SCA effects though non-significant for ASI under al environments and AD under optimum and MSV disease conditions. L4/L8, in addition to giving significant positive SCA effects for MOD, it gave desirable though non-significant SCA effects for ASI under optimum MSV and across all locations. Desirable SCA effects for ASI under optimum, drought and across all locations, in spite of being non-significant, were detected for L5/L12 whose SCA effects were the best for MOD among all crosses. L6/L9 proved to be a good cross in the sense that, in addition to having desirable and significant SCA effects for MOD and ASI under optimum conditions, and AD under MSV, its GCA effects were consistently desirable though non-significant for ASI under drought, MSV and across all environments. In addition to being the sixth best endosperm-modified cross, L7/L8 consistently gave desirable SCA effects for ASI under drought stress, MSV and across all environments and AD under optimum, drought and across all locations.

Table 4.13 Hybrid SCA effects for endosperm modification and some agronomic traits under varying environment

Hybrid	Optimum (1) €				Drought (2)			MSV (1)			Across all environments (4)		
	MOD	GY	AD	ASI	GY	AD	ASI	GY	AD	ASI	GY	AD	ASI
L1/L8 Å	-2.63*	-0.26	2.33	1.39	-0.06	-0.05	-0.26	1.03	-2.10*	-1.39	0.16	0.32	-0.12
L1/L9	1.35	-0.61	0.57	0.96	0.23	0.14	0.29	0.59	0.56	4.04**	0.06	0.64	1.40
L1/L10	-4.40**	1.07	-1.76	-1.28	0.29	-0.64	0.08	-1.37	2.51*	-1.15	-0.02	0.15	-0.56
L1/L11	5.23**	-0.25	-1.71	-1.47	-0.14	0.02	-0.16	-1.35	0.99	-2.20**	-0.52	0.12	-0.99
L1/L12	1.33	0.10	0.57	0.39	-0.36	0.25	-0.04	1.10	-1.96	0.70	0.34	-1.46	0.26
L2/L8	-4.04**	-1.13	0.27	0.66	0.16	-1.68	-1.43	1.30	-1.57	1.94	0.28	-1.45	-0.07
L2L9	-4.97**	1.05	3.50	-0.10	-0.23	2.51*	0.96	-0.71	0.43	-0.63	0.08	1.96	0.29
L2/L10	2.39*	-0.40	-0.16	0.99	-0.12	-1.27	1.57	0.20	-1.62	-0.49	-0.04	-1.37	0.91
L2/L11	1.98	1.20	-2.11	-1.53	0.16	0.23	0.67	0.22	3.52**	-1.20	0.12	0.18	-0.35
L2/L12	4.56**	-0.40	-1.50	-0.01	0.02	0.46	-1.45	-1.01	-0.76	0.37	-0.41	0.73	-0.69
L3/L8	0.47	0.38	0.27	0.19	0.02	-0.79	0.93	0.49	1.03	-1.06	0.27	-0.07	0.25
L3/L9	-0.46	-0.19	-0.83	0.10	0.06	-0.76	-1.02	-0.88	0.03	-1.30	-0.24	-0.58	-0.81
L3/L10	0.87	-1.20	-0.50	0.52	0.21	0.45	0.43	0.59	-0.69	1.18	-0.10	-0.07	0.64
L3/L11	6.52**	-0.97	-0.11	-2.33*	-0.39	2.79**	-0.81	-0.89	-0.88	1.47	-0.66	1.15	-0.62
L3/L12	-5.27**	2.03	1.17	1.52	0.10	-1.69	0.48	0.70	0.50	-0.30	0.73	-0.43	0.55
L4/L8	2.65**	0.72	0.27	-0.94	-0.34	2.21*	0.03	-3.09**	3.83**	-0.32	-1.05	2.13	-0.30
L4/L9	2.79**	-1.09	-1.50	0.96	0.28	-0.93	-1.76	0.08	-0.84	-0.56	0.02	-1.05	-0.78
L4/L10	-0.33	-0.21	-0.83	0.72	-0.33	-0.55	-0.14	-0.27	0.11	1.25	-0.20	-0.45	0.42
L4/L11	-3.18**	0.86	1.55	-1.13	0.29	-0.55	0.79	1.22	-1.08	0.87	0.80	-0.15	0.33
L4/L12	-2.01*	-0.18	0.50	0.39	0.09	-0.19	1.08	2.05*	-2.03*	-1.23	0.39	-0.48	0.33
L5/L8	-1.67	0.94	-0.20	0.26	-0.30	1.08	1.26	0.06	0.03	0.74	-0.23	0.50	0.88
L5/L9	1.32	2.54*	-2.63*	1.50	-0.02	-0.56	0.81	0.58	1.36	-0.16	0.84	-0.60	0.74
L5/L10	-2.51*	-0.30	3.04**	-0.08	0.09	-1.18	-0.74	-0.01	-1.35	-0.35	-0.01	-0.17	-0.48
L5/L11	-5.00**	-1.03	0.42	-1.27	0.34	-0.51	-0.31	0.62	-1.54	-0.40	0.14	-0.54	-0.57
L5/L12	7.78**	-1.81	-0.63	-0.41	-0.10	1.18	-1.02	-1.25	1.50	0.17	-0.75	0.81	-0.57
L6/L8	1.15	0.49	-2.80**	-1.68	0.15	-0.15	0.23	0.25	-1.37	0.68	0.37	-1.12	-0.14
L6/L9	2.82**	-1.07	0.44	-2.77**	-0.12	0.20	-0.56	1.61	-2.04*	-1.23	-0.08	-0.30	-1.28
L6/L10	0.16	0.25	0.44	-1.34	0.04	0.92	-0.77	-0.18	1.25	0.58	0.05	0.88	-0.58
L6/L11	-3.89**	0.61	0.82	7.13**	-0.31	-1.91	0.32	0.12	-0.61	0.20	0.09	-0.90	2.00*
L6/L12	-0.31	-0.64	1.10	-1.34	0.25	0.94	0.78	-1.81	2.77**	-0.23	-0.43	1.44	0.00
L7/L8	4.50**	-0.49	-0.13	0.12	0.37	-0.62	-0.77	-0.04	0.16	-0.59	0.10	-0.30	-0.50
L7/L9	-3.63**	-1.13	0.44	-0.64	-0.20	-0.60	1.28	-1.27	0.50	-0.16	-0.70	-0.06	0.44
L7/L10	4.27**	0.78	-0.23	0.46	-0.17	2.29*	-0.44	1.04	-0.22	-1.02	0.32	1.03	-0.36
L7/L11	0.42	0.01	1.15	0.60	0.05	-0.05	-0.51	0.05	-0.41	1.27	0.04	0.16	0.21
L7/L12	-5.64**	0.88	-1.23	-0.54	-0.06	-1.02	0.44	0.21	-0.03	0.50	0.24	-0.83	0.21
ξ S.E. sij ±	4.59	1.00	1.22	1.57	0.22	1.16	0.95	0.78	1.55	1.18	0.35	0.74	0.67
LSD (0.05)	9.17	2.00	2.43	3.13	0.44	2.29	1.88	1.56	3.09	2.36	0.69	1.46	1.31

€ Numbers in parenthesis refer to total number of sites.

Å The first line is the female and the second line is the male or donor.

ξ Represents the specific combining ability effects of the cross between the i^{th} line and the j^{th} donor.

4.7 Hybrid mean performance for kernel quality and some agronomic characteristics

Mean grain quality performance varied among hybrids and among individual quality attributes for the grain samples drawn and analysed from the optimum environment. On the other hand, mean agronomic performance varied among hybrids and among traits within individual environments and across locations.

4.7.1 Kernel quality characteristics and grain yield under optimum conditions

Mean performance of the crosses for kernel quality and GY under optimum conditions are given in Table 4.14. The overall mean for kernel quality traits was 83.87 % for MOD, 0.06 % for TRP, 0.294 % for LYS, and 12.63 % for PROT whereas that for GY was 8.03 tha^{-1} under these conditions. The highest mean (94.81 %) for MOD was obtained from L2/L12 while L1/L10 gave the lowest mean (68.43 %) for this trait. The cross L5/L8 gave the highest mean for both TRP and LYS while the lowest mean for TRP and LYS was obtained from L3/L11 and L7/L11, respectively. The highest mean for PROT was realised from L5/L12 whereas L1/L11 gave the poorest mean for this character. The cross L5/L9 gave the best mean for GY while the mean for L5/L12 was the lowest for this trait.

In addition to giving the highest mean for MOD, L2/L12 also gave means for TRP (0.061 %), LYS (0.298 %) and PROT (13.46 %) that were above the respective overall means for these traits, though its mean for GY was however lower than the overall mean for this trait. The cross L5/L8 outperformed all the other crosses in terms of TRP (0.07 %) and LYS (0.354 %) and furthermore its means for PROT and GY were above the overall means for the respective traits despite its mean for MOD being below the overall mean. Besides being the highest yielder, L5/L9 gave means that surpassed the overall means for MOD (81.35 %) and LYS (0.295 %), while its mean for TRP (0.059 %) was marginally lower than the overall mean. L5/L12 proved to be an outstanding cross in the sense that, in addition to outperforming the other crosses by occupying first and second position for PROT and MOD, respectively, its mean for TRP (0.062 %) exceeded the overall mean for this trait.

Table 4.14 Hybrid means for kernel characteristics and grain yield under optimum conditions

Cross	Characteristic				
	MOD	TRP	LYS	PROT	GY
L1/L8 [A]	69.89	0.057	0.289	12.96	7.40
L1/L9	81.08	0.060	0.320	13.69	6.96
L1/L10	68.43	0.057	0.283	12.75	9.68
L1/L11	84.26	0.059	0.275	8.28	7.08
L1/L12	80.95	0.062	0.299	12.07	7.49
L2/L8	81.77	0.066	0.303	13.22	6.92
L2L9	85.01	0.064	0.289	11.73	9.02
L2/L10	90.47	0.063	0.287	11.72	8.60
L2/L11	91.28	0.057	0.287	13.70	8.92
L2/L12	94.81	0.061	0.298	13.46	7.39
L3/L8	79.70	0.059	0.291	13.70	8.65
L3/L9	83.20	0.056	0.294	13.20	7.99
L3/L10	81.47	0.061	0.289	11.83	8.02
L3/L11	89.75	0.054	0.292	13.58	6.97
L3/L12	76.50	0.064	0.310	13.45	10.03
L4/L8	83.22	0.065	0.300	13.45	8.78
L4/L9	87.64	0.059	0.294	12.40	6.89
L4/L10	80.25	0.058	0.276	12.06	8.80
L4/L11	77.98	0.062	0.297	11.61	8.60
L4/L12	81.62	0.056	0.270	12.86	7.62
L5/L8	77.70	0.070	0.354	12.64	9.10
L5/L9	86.35	0.059	0.295	12.40	10.62
L5/L10	77.69	0.057	0.287	12.17	8.81
L5/L11	75.78	0.061	0.276	12.29	6.80
L5/L12	93.07	0.062	0.270	13.82	6.08
L6/L8	81.35	0.055	0.270	12.87	7.99
L6/L9	87.77	0.066	0.326	13.08	6.35
L6/L10	81.09	0.057	0.321	11.95	8.71
L6/L11	77.12	0.064	0.303	13.45	7.79
L6/L12	84.00	0.059	0.287	13.45	6.60
L7/L8	92.25	0.067	0.295	12.29	7.53
L7/L9	87.30	0.061	0.295	11.94	6.81
L7/L10	92.80	0.060	0.300	13.58	9.75
L7/L11	90.26	0.061	0.261	12.26	7.70
L7/L12	84.98	0.059	0.327	12.07	8.64
Mean	83.87	0.060	0.294	12.63	8.03
LSD (0.05	7.80	0.009	0.039	2.20	3.42

[A] The first line is the female and the second line is the male or donor.

4.7.2 Endosperm modification and some agronomic traits under varying environments

The mean performance of the crosses for MOD relative to the agronomic characteristic studied under yield-limiting and non-stress environments are given in Table 4.15. The overall mean for GY ranged from 1.35 t/ha under drought stress to 8.03 t/ha under optimum conditions, whereas the overall mean for AD was as high as 106 days under drought stress to as low as 70 days under optimum conditions. However, anthesis-silking interval was fairly stable under varying environments and ranged from 2.2 to 2.3 days. Under optimum conditions, L5/L9, L2/L9 and L6/L11 gave the highest means for GY, AD and ASI respectively, while the lowest means were obtained from L5/L12 for GY, L5/L9 for AD and L4/L8 for ASI. The highest means under drought were given by L4/L11 for GY, L2/L9 and L7/L10 for AD, and L2/L10 for ASI, while the lowest means were obtained from L1/L10 for GY, L6/L11 for AD and L7/L8 for ASI under the same environment. However under MSV disease, the highest mean for GY was given by L4/L12, that for AD by L2/L11 and that for ASI by L1/L9, while the lowest means for GY, AD, and ASI were obtained from L4/L8, L4/L12 and L5/L10, respectively under the same environment. Across all locations, the highest and lowest means for GY were given by L3/L12 and L7/L9, respectively, while L2/L9 gave the highest mean for AD and that for ASI was given by L6/L8. However L1/L9 and L6/L9 gave the highest and lowest means, respectively for ASI across all locations.

Despite giving GY means that were consistently lower than the respective overall means under all environments, L5/L12 gave the second best mean for MOD and its means for AD and ASI were desirably lower than the respective overall means under all environments. L2/L11 gave GY means above the overall means under optimum and drought stress, as well means for AD and ASI under optimum, and ASI under MSV that were desirably below the respective overall means. This was in addition to L2/L11 giving the fifth best mean for MOD under optimum conditions. Apart from having the best mean for MOD, L2/L12 gave means for AD that were lower than overall means under optimum and MSV disease, and means for ASI that were below the overall means under all environments except MSV disease. In addition to giving the fourth highest mean for MOD, L7/L8 gave means for ASI that were consistently below the overall means under all locations, whereas its mean for GY under drought stress was above the overall mean while its AD under the same environment was below the overall mean. L7/L10 gave means for GY that were consistently above overall means under all locations, while its means for ASI were below the overall means under all environments except optimum conditions. This was in addition to L7/L10 having the third highest mean for MOD.

Table 4.15 Hybrid means for endosperm modification and some agronomic traits under varying environments

Cross	Optimum (1) €				Drought (2)			MSV (1)			Across all environments (4)		
	MOD	GY	AD	ASI	GY	AD	ASI	GY	AD	ASI	GY	AD	ASI
L1/L8 Å	69.89	7.40	73.00	2.33	1.21	105.83	2.67	6.48	73.67	2.67	4.08	89.58	2.58
L1/L9	81.08	6.96	71.00	2.67	1.50	108.17	4.33	5.84	77.33	8.33	3.95	91.17	4.92
L1/L10	68.43	9.68	68.67	1.00	1.15	108.67	4.50	4.32	78.67	2.00	4.07	91.17	3.00
L1/L11	84.26	7.08	68.67	2.00	1.20	106.33	3.17	3.24	77.67	1.00	3.18	89.75	2.33
L1/L12	80.95	7.49	71.00	2.00	0.93	105.20	3.00	6.42	72.33	4.00	4.21	86.91	3.00
L2/L8	81.77	6.92	70.67	1.67	1.47	104.50	1.33	6.95	73.33	6.00	4.20	88.25	2.58
L2/L9	85.01	9.02	73.67	1.67	1.07	110.83	4.83	4.75	76.33	3.67	3.98	92.92	3.75
L2/L10	90.47	8.60	70.00	3.33	0.77	108.33	5.83	6.10	73.67	2.67	4.06	90.08	4.42
L2/L11	91.28	8.92	68.00	2.00	1.53	106.83	3.83	5.02	79.33	2.00	3.83	90.25	2.92
L2/L12	94.81	7.39	68.67	1.67	1.34	105.71	1.43	4.51	72.67	3.67	3.47	89.54	2.00
L3/L8	79.70	8.65	69.33	0.67	1.87	103.33	1.67	6.57	73.67	1.33	4.74	87.42	1.33
L3/L9	83.20	7.99	68.00	1.33	1.89	105.50	0.83	5.01	73.67	1.33	4.19	88.17	1.08
L3/L10	81.47	8.02	68.33	2.33	1.64	108.00	2.67	6.92	72.33	2.67	4.55	89.17	2.58
L3/L11	89.75	6.97	68.67	0.67	1.52	107.33	0.33	4.34	72.67	3.00	3.59	89.00	1.08
L3/L12	76.50	10.03	70.00	2.67	1.96	101.50	1.33	6.65	71.67	1.33	5.15	86.17	1.67
L4/L8	83.22	8.78	69.67	0.33	1.28	106.17	2.17	3.14	76.33	3.00	3.15	89.58	1.92
L4/L9	87.64	6.89	67.67	3.00	1.89	105.17	1.50	6.10	72.67	3.00	4.19	87.67	2.25
L4/L10	80.25	8.80	68.33	3.33	0.87	106.83	3.50	6.20	73.00	3.67	4.18	88.75	3.50
L4/L11	77.98	8.60	70.67	2.67	1.97	103.83	3.33	6.59	72.33	3.33	4.78	87.67	3.17
L4/L12	81.62	7.62	69.67	2.33	1.72	102.83	3.33	8.14	69.00	1.33	4.55	86.08	2.58
L5/L8	77.70	9.10	68.67	0.67	0.97	104.67	3.17	5.64	73.00	2.00	3.72	87.75	2.25
L5/L9	86.35	10.62	66.00	2.67	1.22	105.17	3.83	5.96	75.33	1.33	4.76	87.92	2.92
L5/L10	77.69	8.81	71.67	1.67	0.93	105.83	2.67	5.81	72.00	0.00	4.12	88.83	1.75
L5/L11	75.78	6.80	69.00	1.67	1.66	103.50	2.00	5.35	72.33	0.00	3.87	87.08	1.42
L5/L12	93.07	6.08	68.00	0.67	1.17	103.83	1.00	4.20	73.00	0.67	3.16	87.17	0.83
L6/L8	81.35	7.99	67.00	1.00	1.65	102.83	0.50	5.74	69.33	2.00	4.26	85.50	1.00
L6/L9	87.77	6.35	70.00	0.67	1.37	105.33	0.83	6.90	69.67	0.33	3.78	87.58	0.67
L6/L10	81.09	8.71	70.00	2.67	1.12	107.33	1.00	5.55	72.33	1.00	4.12	89.25	1.42
L6/L11	77.12	7.79	70.33	12.33	1.25	101.50	1.00	4.75	71.00	0.67	3.76	86.08	3.75
L6/L12	84.00	6.60	70.67	2.00	1.76	103.00	1.17	3.54	72.00	0.33	3.41	87.17	1.17
L7/L8	92.25	7.53	70.33	1.33	1.53	104.50	0.33	4.74	74.00	1.67	3.83	88.33	0.92
L7/L9	87.30	6.81	70.67	1.33	0.95	106.67	3.50	3.30	75.33	2.33	3.00	89.83	2.67
L7/L10	92.80	9.75	70.00	3.00	0.58	110.83	2.17	6.05	74.00	0.33	4.24	91.42	1.92
L7/L11	90.26	7.70	71.33	4.33	1.27	105.50	1.00	3.97	74.33	2.67	3.55	89.17	2.25
L7/L12	84.98	8.64	69.00	1.33	1.11	103.17	1.67	4.85	72.33	2.00	3.93	86.92	1.67
Mean	83.87	8.03	69.61	2.20	1.35	105.56	2.33	5.42	73.50	2.21	3.99	88.55	2.26
LSD (0.05)	7.80	3.42	4.15	5.35	1.28	5.50	3.91	2.67	5.29	4.04	2.30	5.10	4.30

€ Numbers in parenthesis refer to total number of sites.

Å The first line is the female and the second line is the male or donor.

CHAPTER 5
DISCUSSION

5.1 Analysis of variance

No ssignificant differences due to crosses, lines, testers and their interaction were detected for MOD, TRP, LYS, PROT and GY under optimum conditions in the current study (Table 4.1). These results are in conformity with findings by Pixley and Bjarnason (1993), and Pixley and Bjarnason (2002) in which no significant differences due to crosses were identified for GY, MOD, TRP and PROT in the grain. According to Pixley and Bjarnason (2002), the use of only eight QPM genotypes in their study, and the fact that genetic variation for TRP is generally very small may have largely contributed to lack of significant differences in their work. Only five QPM parents were used in the current study as opposed to the eight used by Pixley and Bjarnason (2002), which may support the likelihood of failing to detect significant differences in this study. According to Gomez and Gomez (1984), nonsignificant F test in the analysis of variance indicates the failure of the experiment to detect any differences among treatments. The same authors further indicate that this outcome does not, in any way, prove that all treatments are the same, because the failure to detect treatment differences based on the nonsignificant F test could be the result of either a very small or nil treatment difference or a very large experimental error.

The analysis of mean squares for optimum conditions in Table 4.1 and 4.2 indicates that: (i) the values for mean square error were large relative to those for mean squares for the other sources of variation for the respective traits, which may have contributed to the nonsignificant F test, and (ii) means for quality and agronomic traits (Tables 4.14 and 4.15) seem to have minimum differences among themselves. Since the heterotic grouping of the inbred lines used in this study was not verified, the donors used may belong to the same heterotic group by virtue of having shared the same donor(s) during conversion, which may have caused very small or nil treatment differences. Sampling error may have also contributed to lack of significant differences for the kernel quality traits, given that analysis for MOD, TRP, LYS, and PRO was conducted only on two ears sampled per plot.

Negative variances due to either males, females or their interaction were observed for the kernel quality and agronomic traits under the varying environments as depicted in Tables 4.1 and 4.2. Akbar *et al.,* (2009) working with bread wheat using Line x Tester analysis also obtained negative variances for grain yield and flowering traits. According to Singh and Chaudhary (1985), negative estimates of variance, though not theoretically expected, may occur unless an experiment is so conducted that sampling errors are minimised, and the assumptions of the mating design used are fulfilled in the

of variance are obtained, it is advisable to regard them as not existing, implying that variances that are positive will be responsible for the inheritance of the character under consideration. Therefore it may be safely assumed that negative variances observed in this study may have been due to sampling error (Singh and Chaudhary, 1985; Akbar *et al.,* 2009).

Nonetheless, variance due to females was more important than that due to males in the phenotypic expression of MOD. Since variance due to female x male interaction was negative and that due to males and females zero for TRP, this suggests that both maternal and paternal variance were important in the expression of TRP. Variance due to males was positive and therefore more important in the expression of LYS given that the other variances were negative. However variance due to males played a more dominant role in influencing GY than that due to females and male x female interaction. Under optimum conditions, variance due to donors was important for GY and ASI while variance due to line x tester interaction was important for AD and ASI (Table 4.2). Under severe moisture stress, variance due to males and that due to line x male interaction were important for AD and ASI, while variance due to females was important for all traits. Variance due to males was important for AD under MSV disease while variance due to line x tester interaction was important for GY under the same environment. Under different environments, variance due to males and that due to females were important for AD and ASI while variance due to line x tester interaction was important for all traits.

Significant differences observed under drought in the performance of crosses for AD and ASI, and lines and donors for GY, AD and ASI (Table 4.2) indicates that there were differential responses among different crosses, lines and donors to severe moisture stress. The significant differences detected under MSV disease in the performance of crosses for GY and lines for flowering traits indicates the existence of variability in the response and tolerance to MSV disease among the different crosses and lines used in this study. There was line x tester interaction observed under MSV disease for GY which is an indication that each specific cross was unique from the other and that the donors showed distinct combining ability effects due to the effect of MSV. These findings are in accordance with Narro *et al.,* (2003) who reported significant line x tester interaction under artificially induced MSV. Under different environments, crosses and lines differed significantly for AD while donors differed significantly for ASI, and these results are in conformity with findings by Betran *et al.,* (2003) who concluded that days to flowering varied with change in environment.

It is evident from Figure 4.1 that the proportional contribution of maternal and paternal (line x tester) interaction to total variance was higher than that of lines and donors for all traits. These findings are

donors were greater than their respective line x tester interactions. This reveals its dominant influence on the phenotypic expression of these traits under non-yield limiting conditions. The contribution of lines to the total variance for MOD was about six times that of donors, which confirms that endosperm hardness is recovered in the progeny from the non-QPM parent through the action of endosperm modifier genes in the donor parent. However, a completely opposite scenario was observed for LYS whereby donors contributed more to total variance than lines, which indicates that the synthesis of enhanced levels of lysine is due to the activity of the *opaque-2* gene and amino acid modifier genes that occur in the donor. Lines and donors simultaneously contributed almost equally to total variance for PROT and this is in conformity with Vivek *et al.,* (2008) whereby there are significant difference in terms of protein content between conventional maize and QPM. The proportional contribution of donors to the total variance for GY and ASI was almost three times that made by lines, and donors were relatively more consistent in their contribution to total variance across traits than lines. This is consistent with the assertion by Vivek *et al.,* (2008) that a donor must not only be good in its ability to modify kernel phenotype, but also be "elite" in all other aspects.

5.2 Gene action

Positive additive variance was detected within females (lines) and within the population for MOD (Table 4.3), which suggests that additive gene action was more predominant than non-additive genetic effects. Hence, the best performing F_1 may be produced by crossing with the highest GCA effects (Teklewold and Becker, 2005). These results confirm findings by Bhatnagar *et al.,* (2005) in which endosperm hardness was more governed by additive genetic effects than non-additive gene action. Additive type of genetic variation in modified endosperm texture was also reported by Glover and Mertz (1987). Non-negative additive variance was also observed for TRP and LYS which suggests the occurrence of a preponderance of additive gene action in the expression of these quality traits. These findings corroborate results obtained by Pixley and Bjarnason (1993) which revealed that additive gene action was predominant over non-additive genetic effects in the expression of TRP in the grain. Therefore additive genetic variation is responsible for the inheritance of kernel quality traits and according to Dabholkar (1999) such variation can be exploited through conventional breeding procedures, such as pedigree method, to realise substantial genetic gain. Karademir *et al.,* (2007) advised that when additive gene effects are substantial and environmental effects are small, selection in early generations may be more appropriate, and therefore this approach may be ideal in the process of converting normal endosperm maize to QPM. In addition, Karademir *et al.,* (2007) also imply that additive genetic effects are more vulnerable to environmental effects, especially when they are small as is the case with kernel quality traits, and therefore environmental effects should be kept small to enhance genetic progress when selecting for a trait that is governed by additive gene effects.

According to Teklewold and Becker (2005), the predominance of additive genetic variance for a trait also means that, besides hybrid and synthetic breeding, opportunity exists for genetic improvement by accumulating favourable alleles from the inter-regional variability through selection, which is a strategy worthy of exploring in QPM conversion.

Positive additive variance under optimum conditions and drought stress were detected for GY while dominance variance was more important under MSV disease and across locations for the same trait (Table 4.4). This implies that additive genetic effects predominantly played a role in influencing GY under optimum conditions and drought stress, while the phenotypic expression of this trait under MSV disease and across environments was characterised by a preponderance of dominance gene action. The predominance of dominance genetic effects on GY across environments is agreeable with findings by Pixley and Bjarnason (1993), and Bhatnagar *et al.,* (2005). Positive dominance variance observed under optimum conditions and positive additive variance under MSV disease for AD, indicates that dominance gene action was predominant in the expression of this trait under optimum conditions while additive genetic effects had a principal influence on AD under MSV disease. The implications thereof in breeding for early maturity are that more genetic gain is achievable through selection for this trait in later generations as opposed to having this done in early generation.

The preponderance of dominance variance for AD under optimum conditions denotes that non-additive gene effects were largely responsible for the expression of this trait, hence selection in early generations will bring no or slow genetic improvement (Teklewold and Becker, 2005). It was observed in this study that both additive and dominance gene action played a major role in the expression of AD under severe moisture stress and across environments, which implies that both early and later generation selection are effective in breeding for early maturity under these environments. In environments with a prevalence of additive gene action upon the expression of AD, additive variance due to females was more paramount, which implies that in selecting donors for use in a QPM conversion program, the maturity of the line is more important than that of the donor. Both additive and dominance variance were important for ASI under all environments except MSV disease, which implies that both additive and non-additive gene action were equally involved in influencing this trait under these environments. Similar findings were made by Esmail (2007) in bread wheat where both additive and dominance genetic components played a role in the inheritance of days to heading. Therefore, to maximise selection advance, procedures which are known to be effective in shifting gene frequency when both additive and non-additive genetic variances are involved would be preferred (Esmail 2007). It therefore means that more genetic gain is realisable by conducting selection for good ASI in both early and later generations. The predominance of additive

detected under all environments besides optimum conditions for ASI indicated the existence of maternal additive gene action in influencing this trait. The implication this scenario has on breeding is that the genetic progress realised in selecting for this trait is dependent on the choice of female parents.

5.3 Heritability

Broad sense heritability provides information about the correspondence between genotypic and phenotypic variance and if it is large, the character concerned is regarded as highly heritable, while if small, environmental agency is considered as mostly responsible for the phenotypic manifestation of the character (Dabholkar, 1999). Heritability estimates in the current study were categorised in accordance with Robinson (1966) as low (5 to 10 %), medium (10 to 30 %), and high (30 to 60 %). Heritability estimates in the broad sense and narrow sense, as well as the ratios of narrow sense to broad sense heritability as depicted in Table 4.5, were either very low (≤ 0.05) or negative for all quality traits and grain yield under optimum conditions. The low heritability could be due to the influence of large environmental variance relative to genotypic variance, whereas the negative heritability revealed the occurrence of sampling error (Dabholkar, 1999). These findings are in agreement with results obtained by Vogel *et al.,* (1981) in which narrow sense heritability estimates determined by variance component analysis were zero or very small, and this was attributable to large environmental variance components. According to Kearsey and Pooni (1996), heritability for quantitative traits in plants is dependent on the absolute size of the genetic variation and type of population under study and the ambient conditions (i.e., environmental variation) in which the trait was measured. This observation by these workers is consistent with what was alluded to by Pixley and Bjarnason (2002) in which they attributed lack of significant differences for quality traits to use of a small number of genotypes and the inherently low genetic variability associated with such traits. Mashiringwani (1993) in his study of floral traits in bread wheat attributed the occurrence of a low (< 30 %) ratio of narrow sense heritability to broad sense heritability to epistatic gene action. Findings by these authors may be helpful in explaining the low heritabilities detected for kernel quality traits in this study.

The magnitude of heritability values and the relative contribution to fixable genetic effects to total genetic variation were dependent on the environment (Kearsey and Pooni, 1996), which emphasises the importance of estimating heritabilities at a number of environments. Under drought stress, both narrow sense and broad sense heritability were as low as less than 0.10 for GY. The low value of heritability estimate of 0.07 obtained for GY under drought stress in the current study is consistent with the assertion by Bänziger *et al.* (2004) that in experiments under drought, grain yield has very low heritabilities. This is also in support of recommendations by Bänziger *et al.*, (2000) that grain yield and

secondary traits are supposed to be simultaneously employed when screening for drought tolerance to enhance breeding progress for high GY under such adverse environment.

Narrow sense heritability for AD was moderate and chiefly due to donor effects, implying that donors were the principal determinants of maturity under drought. Broad sense heritability for AD was however high and almost similar in magnitude to that obtained by Hallauer and Miranda (1981) which justifies the use of early maturity as a strategy in breeding for drought escape. Broad sense heritability for ASI was high as opposed to narrow sense heritability, which is consistent with recommendations of ASI by Bänziger *et al.* (2000) as one of the secondary traits that may be effectively employed in a drought breeding program. This is largely because the heritability of suitable secondary traits is less or not affected by stress (Bänziger and Lafitte, 1997).

High broad sense heritability for flowering characteristics (AD and ASI) detected under drought in the current study reveals that variation for these traits was successfully transmitted to the progeny (Saleem *et al.,* 2008). The ratio of narrow sense heritability to broad sense heritability was moderate under drought stress, which illustrates the importance of the relative contribution of fixable genetic effects to total genetic variation under these conditions for GY. Under MSV disease, narrow sense heritability was very low while broad sense heritability was moderate for all traits, which reflects the low contribution of fixable genetic effects for these traits under MSV disease. However under different environments, narrow sense heritability was medium for AD, but very low for ASI, whereas broad sense heritability was moderate for GY but very high at 1.2 for ASI. Heritability values greater than 1 were also obtained for plant height (i.e., 1.31) by Vogel *et al.,* (1981) while working with Indiangrass. The low narrow sense heritability estimates obtained in the current study suggest non-fixable component variation governing these traits and therefore, F_1 populations should be exploited to utilise the components of variation (Ceyan *et al.,* 2008). Thus, the kernel quality and agronomic traits involved in the current study can be improved by making selections among recombinants through segregating populations (Ceyan *et al.,* 2008).

5.4 Heterosis

Mid-parent heterosis of each cross was calculated as the difference between the F_1 hybrid mean and the average of its parents (Falconer and Mackay, 1997), and hybrids were ranked according to the mean performance of their MOD. A positive value for mid-parent heterosis was desirable for all kernel quality traits and GY, and from Table 4.7, it is evident that the degree of heterosis was dependent on cross and character studied. According to Mashiringwani (1993), the presence of high and desirable heterosis for a particular trait indicates the possibility of utilizing non-genetic additive

obtain superior homozygous segregates in subsequent generations. An analysis of the estimates for mid-parent heterosis for the five best endosperm-modified hybrids, clearly shows that there was consistency in the magnitude of the mean and mid-parent heterosis for MOD except for the cross L2/L12 which despite having the highest mean for MOD, gave the second highest heterosis for this trait. This seems to suggest that there is a possibility of a positive correlation between the mean performance and mid-parent heterosis for MOD among the parental lines used in this study. Given that L2/L12 gave positive heterosis for LYS and PROT in addition to giving the second highest heterosis for MOD, it implies that these three traits may be successfully selected for in a QPM conversion program. On the other hand, more genetic progress is likely to be made by selecting for MOD concurrently with TRP and PROT using L5/L12 as a source population given that desirable heterosis for these three traits was detected in this cross.

If the objective of the conversion program is to ensure that variability for high GY is maintained among the segregates, L2/L11 would be a more appropriate cross given that apart from having desirable heterosis for MOD and PROT, it was associated with desirable heterosis for GY. Consistently desirable heterosis was obtained from L7/L10 for all traits except TRP, and it gave the highest heterosis for PROT among the top five most modified crosses, which implies that this cross will be appropriate in breeding for MOD, LYS and PROT. Despite being in the bottom five hybrids considered, L3/L12 gave the best heterosis for LYS and GY, and the second best heterosis for TRP, while L6/L11, regardless of being in the bottom five also, gave the third highest heterosis for TRP, the second highest heterosis for LYS, and the fourth highest heterosis for PROT. For these reasons, these two crosses may be worth considering in breeding for those kernel quality traits in which they outperformed the best modified crosses in this study.

5.5 General combining ability analysis

The GCA effects for kernel quality characteristics and GY for the donors and lines under optimum conditions are presented in Tables 4.8 and 4.9. The donor L8 had desirable GCA for TRP, LYS and PROT, L9 for MOD and LYS, L10 for GY, L11 for MOD, and L12 for MOD and PROT, and were therefore good general combiners for the respective traits (Table 4.8). Desirable and significant GCA effects were also detected for QPM inbred lines by Pixley and Bjarnason (1993) for GY, PROT and TRP, Pixley and Bjarnason (2002) for GY and MOD, and Xingming *et al.,* (2001) for GY. The donor L11 was the only good general combiner for GY, but was a poor combiner for all quality traits, while the rest of the donors except L11were good combiners for at least three of the quality traits. This is consistent with the fact that there is, generally a negative correlation between yield and quality in plants. Therefore L8, L9 and L12 would be the most appropriate donors given that L8 was a good

general combiner for TRP, LYS and PROT, L9 for MOD, TRP and LYS, and L12 for MOD, LYS and PROT, that is, each of the three donors was a poor general combiner for only one quality trait.

The lines L2 and L7 had desirable GCA for MOD, L2, L5 and L7 for TRP, L3, L5, L6 and L7 for LYS, L2,L3, L5 and L6 for PROT, and L2, L3, L4, L5 and L7 for GY, and were thus good general combiners for the respective traits. Given that L2 was a good general combiner for MOD, TRP, PROT and GY, L3 for LYS, PROT and GY, L5 for TRP, LYS, PROT and GY, and L7 for MOD, TRP, LYS and GY, L2, they will be the most ideal lines since they combined well for GY and at least two kernel quality traits. MOD had the highest number of general good combiners for donors whilst for lines the highest number was for GY. An analysis of the *per se* calorimetric data for the parental lines used in this study clearly indicates that the non-QPM inbred line, L1 had TRP and LYS levels that were as good as those for QPM lines, and it also had the highest protein content among (Table 3.1). Surprisingly, it turned out to be a poor general combiner for all the quality traits including GY. This emphasises the important role played by amino acid modifier genes which are only associated with QPM, and whose function is to ensure that high levels of TRP and LYS are maintained in the grain endosperm during conversion (Moro *et al.,* 1996).

The donors L8 and L12 were good general combiners for MOD, AD and ASI under optimum conditions, L8, L11 and L12 for GY, AD and ASI under drought stress. Under MSV, L8 was a good combiner for GY and AD, L10 for GY and ASI and L12 for GY, AD and ASI, whereas across all environments, L8 was a good combiner for GY, AD and ASI, and L12 for AD and ASI. The donor L12 was the best donor in the sense that it was a good combiner for MOD and at least two traits under all environments, while L8 was the second best donor given that it was a good combiner for a minimum of two traits under all environments except optimum conditions. In addition to being a good general combiner for MOD, L12 was a desirable general combiner for earliness and good pollen-silk synchronization. The donors L9 and L12 were good general combiners for MOD, AD and ASI, but poor combiners for GY, implying that there was a positive correlation between good MOD and earliness, and MOD and ASI, but a negative correlation between MOD and GY. This is not surprising since earliness is closely associated with low GY. The lines L2, L3 and L5 were good combiners for at least 3 traits under optimum conditions, while L3, L4 and L6 were good combiners for a minimum of two characters under both drought stress and across all environments. The line L3 was a good combiner for at least two traits under all environments, L4 and L6 under all environments besides optimum conditions, and L5 under all environments except drought stress. This reflected the good stability of these lines for certain traits under varying environments. Therefore L3 was the best

in breeding for high GY, early maturity and good ASI. In addition to being a good combiner for MOD, L2 was a good combiner for at least one agronomic trait under some of the environments.

5.6 Specific combining ability analysis

Positive SCA effects were desirable for the quality traits as well as GY. The majority of the crosses had highly significant SCA effects for MOD with ten of them having positive SCA effects for this trait, while PROT and LYS had one cross each with significant SCA effects under optimum conditions (Table 4.12). Significant SCA effects for GY and PROT were reported by Pixley and Bjarnason (1993), while significant SCA for GY and MOD were observed by Pixley and Bjarnason (2002). The top five crosses with respect to significant positive SCA effects for MOD were L5/L12, L3/L11, L1/L11, L2/L12 and L7/L8, and it is not surprising that these crosses also had the highest mid-heterosis for this trait. All the donors involved in these crosses except L8, had good GCA for MOD. Of the recipients, L1, L3 and L5 were poor general combiners for MOD while L2 and L7 were good combiners for this trait. It is therefore not surprising that despite the donor L8 being a poor general combiner for MOD, it managed to produce a cross which had high SCA effects and heterosis for this trait due to the predominant influence of L7. This illustrates the importance of heterosis and maternal effects in influencing the expression of a trait. The cross L2/L12 comprised of parents that were good general combiners for MOD, which may imply that desirable SCA effects are likely to be realised by crossing parents which are good general combiners for the trait under consideration. In addition to having desirable SCA effects for MOD, L5/L12 and L6/L9 had positive though non-significant SCA effects for TRP and PROT, and TRP and LYS respectively, which may be due to the fact that the parents involved were good general combiners for the respective traits.

Desirable, though non-significant SCA effects for LYS, PROT and GY, and the sixth highest SCA effects for MOD associated with L7/L10 may be attributed to L7 which was a good general combiner for MOD, TRP, LYS and GY while L10 was also a good combiner for GY. The cross L5/L9 had significant positive SCA effects for GY but negative SCA effects for TRP, LYS and PROT, despite L5 having good GCA effects for the three traits and L9 being a good combiner for TRP and LYS. This implies that these parental lines were not capable of transmitting desirable genes for better kernel quality performance to their progeny. These findings confirm the conclusion arrived at by Tyagi and Lai (2005) that a parent with good GCA effects for a particular trait(s) may not necessarily produce better hybrids. Dabholkar (1999) attributed the behaviour whereby good X good general combiners produce poor cross combination to intra- and/or inter-allelic interaction of genes governing the phenotypic expression of the character. Despite both parents comprising the cross being poor general combiners for MOD, L4/L8 had significant positive SCA effects for this trait, which is in conformity

with Tyagi and Lal (2005) that a parent with poor GCA might produce better hybrids, which could be as a result of intra- and/or inter-allelic interaction of genes concerned with the character, according to Dabholkar (1999). The implications of the findings in the current study are that the selection of lines for use in breeding for a particular trait should be based on both the GCA and SCA effects of the respective lines involved in the improvement of the trait.

Significant SCA effects were observed under certain environments whereas non-significant SCA effects were detected for all crosses for GY under drought stress (Table 4.13). Lack of significant SCA effects for GY under drought stress were attributed to low genotypic variance for this trait under stress environments by Bänziger and Cooper (2001). Under optimum conditions, significant positive SCA effects for GY observed for L3/L12 and L5/L9, significant negative SCA effects for ASI and AD detected for L3/L11 and L6/L9, and L2/L11, L5/L9 and L6/L11, respectively, and may be attributed to the desirable general combining ability associated with the respective parents for the individual crosses. Significant positive SCA effects for AD consistently detected for L2/L9, L3/L11, L4/L8 and L7/L10 under drought stress were due to the good GCA effects of the respective parents, except for L2/L9 and L7/L10 whose parents were both poor combiners for AD. This outcome is justifiable according to Tyagi and Lal (2005) who argue that bad combiners may give desirable SCA for a trait. The cross L4/L12 gave significant positive SCA effects for GY, and L1/L8, L4/L12, and L6/L9 had significant negative SCA effects for AD under MSV disease, whereas significant negative SCA effects for AD and ASI were distinguished for L6/L11 across all locations. This was due to the fact that either of the parents or both were good general combiners for the respective characteristics under MSV disease.

In addition to having the third best SCA effects for MOD, L1/L11 gave desirable and significant SCA effects for ASI under all environments, and desirable SCA effects for AD under optimum conditions. This was attributable the involvement of good general combiners in the respective cross combinations except under optimum conditions where both parents were poor combiners for ASI. Apart from being among the top five crosses in terms of SCA effects for MOD, L2/L12 gave desirable SCA effects for ASI under all environments and AD under optimum and MSV disease conditions as a result of the occurrence of cross combinations with desirable GCA effects for either both or one of the parents. One of the parents for the well-modified cross, L4/L8, was a good general combiner across all environments for ASI, while poor GCA effects was realised for both parents under MSV disease for ASI, and therefore it is not surprising that this cross had desirable though non-significant SCA effects for ASI under MSV.

Desirable SCA effects for ASI under optimum, drought stress and across all locations, though non-significant, detected for L5/L12 whose SCA effects were the best for MOD among all crosses, may be

attributed to good GCA effects for either one of the parents or both for this trait. L6/L9 proved to be a good cross in the sense that, in addition to having desirable and significant SCA effects for MOD and ASI under optimum conditions, and AD under MSV, its SCA effects were consistently desirable though non-significant for ASI under drought, MSV and across all environments. This was as result of at least one of it parents being a good general combiner for ASI under these environments. In addition to being the sixth best endosperm-modified cross, L7/L8 consistently gave desirable SCA effects for ASI under drought stress, MSV and across all environments and AD under optimum, drought and across all locations. This may be attributed to the fact that one of its parents or both had desirable GCA effects for ASI under these environments, except under optimum conditions where both parents were poor general combiners.

5.7 Hybrid mean performance

The cross L2/L12 gave the highest mean for MOD, but did not necessarily have the highest SCA effects for this trait, but was nonetheless the fourth best endosperm-modified cross (Tables 4.12 and 4.14). Both parents comprising this cross had desirable GCA effects for this trait (Tables 4.8 and 4.9), which may be responsible for both the high mean and desirable SCA effects associated with this trait. The cross L1/L10 gave the lowest mean for MOD, which may be attributed to the fact that it had one of the least and undesirable SCA effects for this trait, and both parents were characterised by undesirable GCA effects for MOD. The levels of overall means, as a percentage of total protein in whole grain flour, obtained in this study fall within the range found across QPM genetic backgrounds by Moro *et al.,* (1996), cited by Krivanek *et al.,* (2006), and Vivek *et al.,* (2008) for LYS, and by CIMMYT (2005), cited by Krivanek *et al.,* (2006), and Vivek *et al.,* (2008) for TRP.

The cross L5/L8 gave the highest mean for both TRP and LYS due to the fact that it had good SCA effects for both traits, and both parents were good general combiners for the two traits. The lowest mean for TRP and LYS was obtained from L3/L11 and L7/L11, respectively, which may be due to the fact that both crosses had poor SCA effects for these traits and that both donors involved in the cross combinations had undesirable GCA effects for the respective traits. The highest mean for PROT was realised from L5/L12, which was attributable to the desirable SCA associated with this cross for PROT, and the desirable GCA effects of both parents involved in the cross. On the other hand, L1/L11 gave the poorest mean for PROT possibly due to the undesirable SCA effects characteristic of this cross, compounded by the poor GCA of both parents for this character. The cross L5/L9 gave the best mean for GY despite having undesirable SCA effects for this trait, which may not be surprising given that the line L5, involved in this cross had good GCA for this trait. The mean for L5/L12 was the lowest for GY probably as a result of its SCA effects being undesirable, though the line or female

involved had good GCA for this trait. However given that the donor involved in this cross combination had undesirable GCA for GY, this may be responsible for the low GY.

In addition to giving the highest mean for MOD, L2/L12 also gave means for TRP of 0.061 %, LYS of 0.298 % and PROT of 13.46 % that were above the respective overall means for these traits, though its mean for GY was however lower than the overall mean for this trait. The cross L5/L8 outperformed all the other crosses in terms of TRP at 0.07 % and LYS at 0.354 % and furthermore its means for PROT and GY were above the overall means for the respective traits despite its mean for MOD being below the overall mean. Besides being the highest yielder, L5/L9 gave means that surpassed the overall means for MOD of 81.35 % and LYS of 0.295 %, while its mean for TRP of 0.059 % was marginally lower than the overall mean. L5/L12 proved to be an outstanding cross in the sense that, in addition to outperforming the other crosses by occupying first and second position for PROT and MOD, respectively, its mean for TRP of 0.062 % exceeded the overall mean for this trait.

Under optimum conditions, L5/L9 gave the highest mean for GY, which was consistent with the fact that this cross had the fourth highest SCA for this trait under these conditions, while the lowest mean observed for GY came from L5/L12 whose SCA was poor. The low and desirable means obtained from L5/L9 for AD and L4/L8 for ASI were as a result of the desirable SCA associated with these crosses under optimum conditions. The highest mean under drought was given by L4/L11 for GY, which may be due to the high SCA associated with the cross. However L1/L10 gave the lowest mean GY under drought despite having desirable SCA under this environment, which may be attributed to the poor GCA of both constituent parents. The implications of this finding are that constituent parents, both with poor GCA for GY may produce desirable SCA for this trait in the resultant cross combination, but may not necessarily give high mean GY under drought stress. The lowest and desirable means obtained from L6/L11 for AD and L7/L8 for ASI under the same environment may be attributed to the desirable SCA associated with the respective traits under drought stress. However under MSV disease, the highest mean for GY was given by L4/L12, which may have been due to the significant and desirable SCA associated with this cross. The lowest mean GY under this environment was obtained from L4/L8, which is not surprising given that significant and the poorest SCA was given by this cross under MSV disease. However what is surprising is that both constituent parents were good general combiners for this trait, which may imply that even though both parents comprising a cross are good general combiners for a particular trait, there is a likelihood of obtaining both undesirable SCA and a low mean GY under MSV disease.

The lowest means for AD and ASI obtained from L4/L12 and L5/L10, respectively under MSV disease are associated with desirable SCA detected for this cross. Across all locations, the highest and lowest means for GY were given by L3/L12 and L7/L9, respectively, while L6/L8 gave the lowest mean for AD and L6/L9 for ASI. The high mean GY for L3/L12, and the low or desirable AD and ASI means obtained for L6/L8 and L6/L9, respectively, are consistent with the desirable SCA effects associated with the respective crosses, the low mean GY realised for L7/L9 are in conformity with the poor SCA observed for this cross across all environments.

Despite giving the second best mean for MOD, L5/L12 gave GY means that were consistently lower than the respective overall means under all environments, which is consistent with the persistently poor SCA effects noticeable under all environments. By consistently giving high means and desirable SCA effects for MOD, coupled with acceptable means for agronomic traits under most of the environments, the crosses L2/L11, L2/L12, L7/L8 and L7/L10 have proved to be good combinations which can be successfully used in the QPM conversion program.

CHAPTER 6

CONCLUSIONS

Appropriate donor inbred lines were successfully identified on the basis of general and specific combining ability effects and mid-heterosis. On the basis of GCA effects for kernel quality and agronomic traits, CML511 was the best donor because in addition to being a desirable general combiner for MOD, LYS and PROT, it gave good GCA effects for more agronomic traits under more environments than the other donors. CZL082 was the second best donor mainly because it was a good general combiner for MOD, TRP and LYS. On the other hand, HX482P was the best general combiner among the lines because it gave desirable GCA effects for more kernel quality and agronomic traits under more environments than its counterparts, followed by L7 which outperformed the other lines in terms of the number of traits with desirable GCA effects. In terms of SCA effects, CZL082 was the best specific combiner among the donors because it was a constituent parent in more cross combinations with desirable SCA for kernel quality and agronomic characteristics under more environments than the other donors. CML511 was the second best donor, given that it was involved in cross combinations that had the best SCA effects for MOD and LYS. L7 was the most outstanding line because it gave the highest number of traits with desirable SCA effects for agronomic traits in addition to MOD. In addition, CML511 was a constituent parent in separate cross combinations with the highest means for MOD and PROT and the second highest mean for MOD.

On the basis of mid-parent heterosis, CML511 was the best donor because it was one of the parents in the crosses with the highest and second highest positive mid-parent heterosis for MOD. EL77P and L7 had the best mid-parent heterosis whereby they were constituent parents in the crosses that constituted the best five hybrids in terms of mid-parent heterosis. The mid-parent heterosis of two of the five poorest crosses, in terms of the mean for MOD, was higher for TRP, LYS and PROT than that for the best modified hybrids. CML511 was a constituent parent in one of these crosses, which further demonstrates its superiority as a highly competent donor on the basis of mid-parent heterosis for kernel quality.

Additive genetic effects were more preponderant in the control of all kernel quality traits studied than dominance gene action, implying that selection in early generations may be more appropriate for these characteristics. Dominance gene action was predominant in influencing GY under MSV disease and across all environments, while additive genetic effects were more important in controlling this trait under optimum conditions and drought stress. Days to flowering was controlled mainly by dominance gene action under optimum conditions, whereas additive genetic effects were predominant in controlling the same trait under MSV disease. Both additive and dominance gene action were

preponderant in the control of ASI under all environments apart from MSV, which implies that selection in both early and later generations is effective when breeding for the trait. Maternal additive gene action was predominant in the genetic control of ASI under all environments besides MSV disease, which suggests that genetic progress in breeding for this trait is dependent on the choice of female parents.

The low narrow sense and broad sense heritability detected for all kernel quality traits including GY imply that the genetic progress realized when breeding for these traits is very slow. The narrow sense heritability found medium for AD and low for ASI under drought stress implies that moderate genetic progress is achievable for fixable genetic variation when selecting for AD under severe soil moisture stress. The high broad sense heritability detected for both AD and ASI indicates that accelerated genetic progress is obtainable for non-fixable genetic variation during selection for these traits under drought stress. Under different environments, the medium narrow sense heritability detected for AD indicates that fixable genetic variation is moderately heritable for days to flowering under these conditions. The broad sense heritability found medium for GY and very high for ASI under drought stress implies that the inheritance of non-fixable genetic variation is average for GY, but very high for ASI under this environment. Therefore the inheritance of kernel quality traits was generally very low while that for agronomic traits ranged from very low to very high depending on the trait and the environment in which the trait was measured.

CZL082 was the best donor in terms of desirable GCA and SCA effects for both kernel quality and agronomic characteristics, while CML511 was the most outstanding donor in relation to desirable GCA and SCA effects, and mean performance. Therefore the two donors have a high likelihood of being successfully used in the QPM conversion program together for the purpose of complimenting each other and maintaining genetic diversity among the segregants. It is however necessary to further validate the superiority of these donor lines by conducting more combing ability analyses with more non-QPM inbred lines before they can be permanently adopted by the national program.

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