# Relationship of molecular genetic distance of seven quality protein maize (*Zea mays* L.) inbred lines with specific combining ability and grain yield of hybrids

by

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A thesis submitted in partial fulfillment of the requirements for the degree of **Master of Science (M. Sc.) in Crop Science (Plant Breeding)**

Department of Crop Science
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University of Zimbabwe

**2007**

The undersigned certify that they have read and recommend to the Department of Crop Science, the thesis entitled:

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## ABSTRACT

Quality protein maize (QPM) with the *opaque-2* gene has double the amount of the essential amino acids (tryptophan and lysine) as compared to normal endosperm maize but the gene is associated with undesirable agronomic traits. The aim of this study was to generate genetic information for use in developing breeding strategies for QPM in Southern Africa. Specific objectives were: i) to study the combining ability and type of gene action controlling yield in crosses among QPM inbred lines from Mexico and Southern Africa ii) to determine the relationship between combining ability for grain yield of QPM hybrids and molecular genetic distances between the parent inbred lines. Seven QPM inbred lines were crossed in a diallel mating design. The genetic distances between the maize inbred lines was quantified by using 62 simple sequence repeat (SSR) markers. The lines and the derived F1 hybrids were also evaluated for tryptophan and protein content. The F1 hybrids were evaluated in replicated trials at five locations. The quality of QPM inbred lines was variable with hybrids made up of high quality lines exhibiting high quality values. There was significant variation among the hybrids for yield and QPM traits. While both the general combining ability (GCA) and the specific combining ability (SCA) were significant, the GCA effects were found to be more important than SCA for yield in this study. QPM inbred line SC1 from Southern Africa had the highest GCA value and consistently appeared as one of the parents of the best yielding $F_1$ hybrids. Preponderance of GCA effects indicated that additive gene effects were found to be more important in conferring high yield. Cluster analysis of the lines based on the SSR markers revealed five groups that were in conformity with pedigree information. The correlation between genetic similarity (GS) and SCA was not significant, low and negative (-0.035) suggesting low predictive value. As a result, use of SSR as a predictive tool for heterotic grouping in breeding maize hybrids should be confirmed by phenotypic data from the field evaluations.

## DEDICATION

This dissertation is dedicated to my wife Lovejoy, and my daughters: Makomborero, who has developed interest in the interpretation of the data, and Tinomutenda, who did not want to miss the opportunity of switching on my laptop and opening my dissertation file as soon as I got into my study room.

## ACKNOWLEDGEMENTS

This project will not have succeeded without the valuable help I received from my supervisors, Mr. C. Musvosvi, Dr. B. Vivek (CIMMYT- Zimbabwe), Dr. C. Mutengwa (Fort Hare University) and Dr. J. Derera (University of Kwazulu-Natal). I am also indebted to Dr. Warburton (CIMMYT-Mexico) for her role in reviewing the molecular aspect of this study; her critical comments and encouragement contributed immensely towards its successful completion. I acknowledge the guidance I received from Dr. K. Pixley (CIMMYT- Mexico) from the designing stage to the final write up phase.

I would like to acknowledge the support of the management at Seed Co Ltd, and the Nippon Foundation who made it possible for this project to be conducted through a grant I received from them. Use of computer software and hardware from CIMMYT needs special acknowledgement as that gesture immensely contributed to the fruitful completion of this work. I wish to also acknowledge the help that I received from my colleagues and support staff at Seed Co (RARS, and KRC), and the support staff at ART and CIMMYT- Zimbabwe Research Stations.

The level of tolerance and endurance expressed by my wife Lovejoy, daughters (Makomborero and Tinomutenda) and my mother deserve special recognition during the two years that I have been studying, particularly during various periods when the study demanded my time most. I also would like to express my appreciation of the encouragement I got from my brothers, Simon, Shadreck and Shusha, and my sister Jane.

Lastly, I would like to express my gratitude to the Almighty God whose hand was with me throughout, without which, this would have been impossible to achieve.

# CONTENTS

**Page**

# TABLES

**Page**

# FIGURES

**Page**

# APPENDICES

**Page**

## LIST OF ABBREVIATIONS

| | |
|---|---|
| ABC | - Applied Biotechnology Centre |
| AD | - Anthesis Date |
| ANOVA | - Analysis of Variance |
| ART | - Agricultural Research Trust |
| ASI | - Anthesis to Silking Interval |
| CIMMYT | - International Maize and Wheat Improvement Centre |
| CML | - CIMMYT Maize Line |
| DMP | - Days to Mid-pollen |
| DMS | - Days to Mid-silk |
| DNA | - Deoxyribonucleic Acid |
| EHT | - Ear Height |
| ER | - Ear Rot |
| ET | - *Exserohilium turcicum* |
| FAO | - Food and Agriculture Organisation |
| GCA | - General combining ability |
| GD | - Genetic Distance |
| GLS | - Grey Leaf Spot |
| KRC | - Kadoma Research Centre |
| MRD | - Modified Roger's Distance |
| PCR | - Polymerase Chain Reaction |
| PIC | - Polymorphism Information Content |
| PHT | - Plant Height |
| PMPH | - Pelmitic Mid Parent Heterosis |
| PLS | - *Phaeosphaeria* Leaf Spot |

| | |
|---|---|
| QPM | - Quality Protein Maize |
| RARS | - Rattray Arnold Research Station |
| RFLP | - Restriction Fragment Length Polymorphism |
| RL | - Root Lodging |
| SCA | - Specific Combining Ability |
| SL | - Stalk Lodging |
| SSA | - Sub-Saharan Africa |
| SSR | - Simple Sequence Repeats |
| UPGMA | - Unweighted Pair Group Mean Average |

# Chapter 1

## INTRODUCTION

Cases of malnutrition are prevalent in developing countries, especially in sub-Saharan Africa (SSA). It is mainly a result of inadequate food volume or poor nutritive value of the consumed food. Productivity of the staple food crops such as maize is low relative to yield potential in SSA. The little available grain tends to have nutritionally inadequate levels of essential amino acids such as lysine and tryptophan. Although agronomically superior varieties are available within the Southern African region, they have poor nutritive attributes. The identification of maize mutants that enhance the nutritive quality of maize brought significant hope as it has double the amount of essential amino acids (lysine and tryptophan) than in normal endosperm maize. There are challenges in breeding and improving maize for the QPM trait. The use of *opaque-2* gene that confers high lysine and tryptophan levels in maize has proven to be difficult. The gene has been associated with other undesirable agronomic traits such as low yield, high lodging, ear rots, poor storability and susceptibility to many foliar diseases in tropical and subtropical environments. The need to develop varieties with superior agronomic and nutritive qualities using improved breeding sources becomes of paramount importance. In addition to current conventional methods, use of molecular tools in fingerprinting and genetic diversity has become important in accelerating the variety development process in modern plant breeding programs, worldwide, which should result in rapid generation of QPM hybrids in SSA. It is important, therefore, to investigate if there is a possibility to incorporate this tool in the QPM breeding

program in Zimbabwe by assessing the association between the phenotypic data from specific combining studies and genetic distance data from molecular studies in QPM.

Cases of malnutrition are prevalent in these areas and are caused by both inadequate quantity of food and poor nutritive value of the maize that is grown and consumed. Maize protein is deficient in two essential amino acids, lysine and tryptophan (Bhatia and Robson, 1987). Quality protein maize, which has a high leucine – isoleucine ratio, is especially important for young children, pregnant or lactating women and the sick (Pixley and Bjarnason, 2002). The net protein utilization of maize is low, with a biological nutritional value that is equivalent to 40 % of that of milk; therefore, inclusion of supplementary sources of lysine and tryptophan (such as legumes and animal products) becomes imperative (Krivanek *et al*., 2007). These amino acids are also important for the proper nutrition of monogastric farm animals such as pigs and poultry. The alternative protein sources such as milk, chicken meat and eggs are generally more expensive than QPM and are beyond the reach of the poor in SSA. In general QPM has a higher yield potential (>6 t $ha^{-1}$) than the alternative plant sources of lysine and tryptophan such as legumes (<3 t $ha^{-1}$).

In order to improve the nutritive value of maize, the *opaque-2* gene was selected among several high lysine mutants of maize by researchers and is now being used in plant breeding research programs (Bjarnason and Vasal, 1992). Historically, the *opaque-2* gene has been associated with

pleiotropic effects such as low grain yield, ear rot and slow dry-down that have discouraged maize growers from adopting these nutritive cultivars and have complicated breeding efforts (Darrigues, Lamkey and Scott, 2006). Breeding efforts were made to improve agronomic traits of *opaque-2* maize, which resulted in the development of maize that confers 60 % to 100 % more lysine and tryptophan content relative to normal maize with flavor and agronomic traits that resemble normal maize.

The resultant increase in essential amino acids, the increase in digestibility and the increase in nitrogen uptake relative to the normal maize, increases the biological value (the amount of nitrogen that is retained in the body) from 40 % to 57 % of levels found in milk in normal endosperm maize to 80 % to 90 % levels in QPM.

Interest in QPM has gradually increased since the recognition of the work by International Maize and Wheat improvement Centre (CIMMYT) scientists who were recognized by being awarded the 2000 World Food Prize. The honour was on the successful work in developing hard endosperm *opaque-2* maize varieties by recurrent selection for hardness, high lysine and high tryptophan. This resulted in the development of competitive cultivars in terms of yield and other traits, relative to normal endosperm genotype used as checks (Pixley and Bjarnason, 2002). Their efforts have resulted in the availability of several QPM materials (inbred lines, hybrids and open pollinated varieties) that are adapted to Latin American environments and can be used as a source of the *opaque-2* gene in other parts of the world where QPM breeding programs are

being undertaken. Successful or ongoing efforts to produce QPM varieties can be found in Africa, including in Kwazulu-Natal in South Africa (Gevers and Lake, 1992), Ghana and in Uganda (Krivanek *et al*., 2007). There are also other upcoming programs such as Seed Co in Zimbabwe, national breeding programs in Zimbabwe, Ethiopia and Mozambique in southern and eastern Africa. Despite that effort, only a few varieties have been released in the region. Varieties must be developed that are adapted to all local environments. Particular attention should be paid to improving agronomic weaknesses in QPM. Therefore, there is a need to focus on development of germplasm for Zimbabwe and other regions of Southern Africa.

Considerable work on maize improvement in the sub-Sahara Africa region has led to the availability of superior traditional normal endosperm maize cultivars. It is important to find the most efficient method to use sources of the *opaque-2* gene (which are exotic to this part of the world), in local QPM breeding programs as the local lines and traditional normal endosperm cultivars are converted to QPM via backcrossing. High potential lines adapted to the Southern African region are available, and if they can be converted to QPM, alleviating the shortfalls of *opaque-2* maize should not be difficult. The research work done elsewhere on the type of gene action for yield, protein, lysine and tryptophan content in protein and other quantitative traits usually pertain to the genetic materials and the test environments used (Falconer, 1988). Therefore, the genetic information generated by this study will help those involved in QPM breeding to develop products with superior agronomic and

nutritional properties, using the germplasm and environments pertinent to southern African region that will immensely improve the socio-economic status of the people in this region.

Information or knowledge about germplasm diversity and genetic relationships between breeding materials plays an important role in crop improvement. Heterosis has been associated with mating of parents of wide diversity which is desirable while inbreeding depression has been associated with mating of parents that are closely related. In cross-pollinating crops, such as maize, the later increases homozygosity, reducing vigour and productiveness resulting in traits being fixed (Hallauer and Miranda, 1988). In addition, lack of genetic diversity in breeding materials leads to genetic vulnerability to new diseases and retardation of breeding progress, as improvement of any trait requires genetic variation on which selection can act. Various methods are used to assess genetic diversity, including analysis of data from agronomic performance, biochemistry, and molecular data (Mohammadi and Prasanna, 2003).

It is against this background that a study on the relationship between the combining ability for grain yield and endosperm modification, and the relationship between combining ability for yield and genetic distances among seven Seed Co and CIMMYT QPM lines was done. Besides giving an in depth understanding on the type of gene action, this effort was used to develop new products to help alleviate the nutritive shortfalls

within the region. The study also attempted to establish the relationship between the genetic distance and genetic parameters for grain yield among these QPM inbred lines.

## 1.1 Justification

Almost every meal eaten by the people living in the third world countries such as in SSA has maize either as a sole component (such as porridge taken at breakfast) or forming the bulk of the meal. Production of maize in such parts of the world can be regarded as livelihood as it forms an important part of the nutrition of the inhabitants of most of these regions. The analysis in this update focuses on maize, which is the major staple in the most seriously affected countries. The Southern African Development Community (SADC) Regional Early Warning Unit (REWU) estimates that 60 % of the average diet is constituted by maize and its contribution in Zambia, Malawi and Swaziland while in Zimbabwe and Lesotho, it is between 40 % and 60 %. These statistics make maize synonymous with food in this region. Its importance can further be illustrated by the fact that in Africa, maize constitutes about 20 % of the total daily calories and provides 17 % to 60 % of the total protein of human consumption, with even higher protein estimates for the most vulnerable members of community such as those who are sick and weaning children (Krivanek, Groote, Gunaratna, Diallo and Friesen, 2007). In Southern and Eastern Africa, per capita consumption of maize is 100 kg and provides 50 % and 30 % of calories, respectively (Pandey, 1999). Maize is also used as animal feed, including both ruminant (that is, cows, goats) and monogastric animals (that is, pigs, poultry).

More than 20 million hectares are under maize cultivation in SSA where monocropping is generally practised by smallholder farmers, (Pandey, 1999) where it plays a very important role in human and animal nutrition. In 1993, 23 million metric tonnes (Mt) were produced in SSA and this quantity was complimented by imports of 2.5 Mt (Pandey, 1999). According to Food and Agriculture Organisation (FAO, 2005) and World Food Program (WFP, 2005), Zimbabwe imported 1.2 Mt of maize in 2005 to compliment local production. As the population continues to rise, the production levels are expected to increase to 54 Mt, with imports equally rising to 3.1 Mt by 2020 (Pandey, 1999). There is therefore need to improve both productivity and quality of maize to close the gap between production and consumption, while reducing cases of malnutrition.

## 1.2 Research Goal

The goal of this research is to contribute towards alleviation of problems of malnutrition in SSA by developing QPM hybrids.

### 1.2.1 Main objective

The main objective of this study is to facilitate QPM breeding by generating genetic information that can be of use in the QPM breeding programs.

### 1.2.2 Specific objectives

The specific objectives of the study were as follows:

- To study the combining ability for grain yield and endosperm modification in experimental hybrids among QPM inbred lines from Mexico and Southern Africa;
- To determine the relationship between molecular genetic distances and combining ability for grain yield in QPM inbred lines.

### 1.2.3 Research Hypotheses

The following hypotheses were tested in this study:

- Additive and non-additive gene effects are important in the inheritance of yield and endosperm modification in the southern African and CIMMYT experimental and elite QPM lines under study;
- There is a significant and positive association between SCA effects of the hybrids for grain yield and the molecular genetic distances among the QPM inbred lines under study.

# Chapter 2

# LITERATURE REVIEW

## 2.1 Improvement of Maize for QPM Traits

Breeding work on high lysine content has concentrated on improvement of the original *opaque-2* maize for agronomic traits, while maintaining a high level of protein and the essential amino acids. Several populations have been improved by backcross and recurrent selection techniques at CIMMYT-Mexico, in Ghana, etc. (National Research Council, 1988; Bjarnason and Vasal, 1992; Villegas, Vasal and Bjarnason, 1992; Krivanek *et al.*, 2007). The resultant *opaque-2* germplasm, has superior protein quality similar to the earlier *opaque-2* maize (Pixley and Bjarnason, 1993). The accumulation of modifier genes for the *opaque-2* mutation resulted in the development of vitreous (translucent) kernels and agronomic performance that matches that of the normal endosperm maize (Bjarnason and Vasal, 1992).

## 2.2 Gene Action

### 2.2.1 Modifier genes

The *opaque-2* mutation gene increases the percentage of lysine in the grain as it reduces the synthesis of zein and during the synthesis of a number of endosperm proteins (Larkins, Moro, Lopes, Habben, Clore and Dannenhoffer, 1995). The *opaque-2* gene in QPM changes the

composition of protein in maize endosperm by increasing the content of lysine and tryptophan (Mertz, Bates and Nelson, 1964). The *opaque-2* maize has less zein (prolamines) content (5 % to 27 % in contrast to 54 % to 59 % in normal endosperm maize), which significantly improves the nutritional quality of the *opaque-2* maize (Babu, Mani and Gupta, 2004). It achieves this by coding for a protein required for the transcription of the 22 kDa zein structural genes and 22 kDa zein synthesis is almost completely repressed in homozygous recessive *opaque-2* maize endosperm (Kata, Taylor Bockholt and Smith, 1994).

The opaque appearance and the soft endosperm that characterize the maize with the *opaque-2* gene, delayed adoption of such maize as it results in split pericarp kernels, which in turn, results in increased susceptibility to pests, and poor processing characteristics. The QPM is a result of exploitation of genes that confer the phenotypic features of normal maize kernels while maintaining the higher nutritive value associated with the *opaque-2* gene carrier. Bauman, (1975) and Larkins *et al.* (1995) suggest that development of QPM should be done following simultaneous selection of multiple loci: *o2, o2* modifiers, and genes governing the synthesis of lysine-rich proteins. That multiple loci promote formation of multiple protein bodies which forms a vitreous phenotype. Lopes and Larkins (1996) confirmed earlier findings by Bauman (1975) that the frequency of vitreous types differ from one population to the other, with modifiers having more effect on flint- than dent-grain and they disputed earlier observations that the vitreouseness is influenced by the environment. Although multiple genes have been associated with vitreouseness, Bauman (1975) and Lopes and Larkins (1995) have observed that 1/16 of the progenies from selfing the F2 had a phenotype similar to that of

the parents, which indicates that two genes were involved. There was a negative correlation between protein quality and vitrouseness (Bauman, 1975; Lopes and Larkins, 1995; Hunter, Beatty, Singletary, Hamaker, Dilkes, Larkins and Jung, 2002). Accumulation of 27-kda gama-zein storage protein has been found to be always associated with endosperm modification (Lopes and Larkins, 1995).

### 2.2.2 Combining Ability Effects

Additive gene action was reported to play a relatively greater role than dominance for percent protein in grain, and percent tryptophan or lysine in protein for different *opaque*-2 germplasm (Singh, Singh, Singh and Bahl, 1977; Motto, Lorenzoni, Gentinetta, Maggiorre and Salamini, 1978; Wessel-Beaveret, Lambert and Dudley, 1985). The work done by Sreeramulu and Bauman (1970) showed that general combining ability (GCA) effects were significant while specific combining ability (SCA) effects were not significant for percent lysine in grain and in protein. Bjarnason, Pollmer and Klein, (1976), reported significant GCA for percent protein and lysine in grain, but significant SCA only for percent lysine in protein.

In studies conducted by Dudley, Lambert and Alexander (1971), Dudley, Alexander and Lambert, (1975), broad sense heritability for percent protein in grain ranged from 54 % to 73 % among half sib families. A narrow sense heritabilty of 68 % for percent protein among half-sibs was reported by Motto (1979), who also obtained a low heritability value of protein tryptophan content. Among the S1 families, heritability estimates

of 68 % and 84 % were reported by Wessel-Beaveret *et al*. (1985). These levels of heritability were generally moderate to high supporting the predominance of additive gene affects.

The reported heritability estimates for percent lysine in protein were 0 % to 70 % (Dudley *et al*., 1971), 2 % to 29 % (Dudley *et al*., 1975) and 70 % and 76 % (Wessel-Beaveret *et al*., 1985). Various ranges of heritability estimates for percent lysine in grain have been reported, including 17 % to 72 % (Dudley *et al*.,1971); 7 % to 47 %, (Dudley *et al*., 1975) and 76 % (Wessel-Beaveret *et al*., 1985). High heritability estimates suggest that additive gene effects are more important than non-additive gene effects, which agrees with studies in which GCA effects were found to be significant while the SCA effects were not in normal endosperm maize (Betran *et al*., 2003; Long, Bänziger and Smith, 2004; Makumbi, 2005)

The heritability that was reported for percent tryptophan in protein was 27 % (Motto, 1979). In the study by Pixley and Bjarnason (1993), most of the genetic variability for tryptophan concentration in protein among QPM hybrids was additive and interactions of genotype by location effects (G x E) were small. Of the four diallel studies conducted by Pixley and Bjarnason (1993), two of them had significant GCA effects for tryptophan concentration in grain and none exhibited significant differences for SCA effects. The interaction for genotype by location was

significant for all the diallel studies. For grain yield, Pixley and Bjarnason (1993) found significant GCA effects in three trials and significant SCA effects in one trial only.

Generally, studies on the inheritance of the gene controlling endosperm type (normal translucence vs. opaque appearance) have indicated that inheritance is complex (Bjarnason and Vasal, 1992; Larkins, *et al*., 1995; and Pixley and Bjarnason, 2002). However, this brief review of literature suggests that additive gene action is more important than non-additive in conferring high tryptophan and lysine concentration. This implies that selection of lines characterized as good for these traits will consistently display the desired endosperm and pass this trait on to their offspring, the phenotype of which can be predicted if the parent performance is known.

This survey of the literature suggests that additive gene action is more important than non-additive in conferring high tryptophan and lysine concentration and genes conferring vitrouseness or modification of the endosperm. It also suggests that GCA effects for yield were more important than SCA effects. The implication of this is that selection of lines characterized as good for these traits will give rise to products that exhibit that trait or the offspring can be predicted if the parent performance is known. With the better understanding of the GCA and SCA effects of these inbreds and hybrids, respectively, for kernel modification and agronomic traits such as yield, maize breeders in the region would possibly be more efficient in generating QPM hybrids and even open-pollinated varieties exhibiting acceptable grain and agronomic

characteristics (Long *et al*., 2004). Perhaps, the small to large G x E effects reported by Motto (1979) suggests that performance of QPM hybrids might not be stable, and that hybrid ranking might change in each environment.

## 2.3 Genotype and Environment Interactions

The *opaque-2* maize was originally unacceptable, until vigorous work was done to eliminate the chalky appearance that characterizes it and overcoming other undesirable genes that are linked with it. It has been observed that the modifier genes are influenced by the environment condition, in certain genetic background, (Lopes and Larkins, 1995). Betran *et al*. (2003) reported significant interaction between genotype and the environment (G x E) in the analysis of variance for grain yield of both hybrids and inbred lines, in normal endosperm maize which may also apply to the QPM maize. In QPM germplasm, grain yield and protein concentration in grain was found to be least stable as G x E interactions and sums of square for deviation from linear regression were found to be greatest (Pixley and Bjarnason, 2002).

## 2.4 Diallel mating design

The mating designs attempt to partition and estimate the magnitude of the variance due to genotypic, environmental or interaction between the genotypic and environmental effects. Such designs *per se* generate progenies that are then evaluated in various types of evaluation designs. It is the output from this evaluation which is partitioned into various components. It is therefore a pre-requisite that genotypes are evaluated in different environments to determine their general performance and the total variability for the linear model becomes $Q^2_p = Q^2_g + Q^2_e + Q^2_{eg}$

where $Q^2_p$ = phenotypic variance, $Q^2_g$ = genotypic variance, $Q^2_e$ = environmental variance and $Q^2_{eg}$ variance due to genotype by environment interaction. This assumes there is no correlation of genotype and environments which can be minimized by properly designed experiments (Hallauer and Mirranda, 1988). In a diallel mating design, crosses are produced in all possible combinations or pairs for a number of parents and can be used for inbred parents or broad genetic base varieties. The crosses are evaluated in replicated trials and inference on the type of gene action is made. It provides information on the nature and amount of genetic parameters and general and specific combining ability of parents and their crosses respectively (Singh and Chaudhary, 2004; Makumbi, 2005). It is important to consider whether the parents are reference genotypes or whether they are random genotypes coming from a reference population. Griffing (1956) and Cockerham (1963) coined these parent types as fixed model or model I and random model or model II, respectively. In model I, the parents are the population while in model II the parents are a sample from a population. In the current study only a few parents were used and represented a sample of the lines used in the region hence a fixed effects model was adopted. The results therefore pertain to this set of germplasm.

There are four types of diallel mating systems which Griffing (1956) listed as methods I, II, III and IV, depending on the type of genotypes involved. Method I has the parents, the F1 hybrids and their reciprocals, while Method II involves F1s and parents but no reciprocals. Method III has F1s and reciprocals but without parents; while Method IV has F1s only without parents and without reciprocals. Methods that exclude parents in maize are commonly used because parents are inbred lines which complicate field designs due to poor vigour of the parents (F = 1) as compared to the crosses (F = 0) where F is the degree of correlation between uniting gametes which measures the increase in homozygosity or

the likeliness that two genes on a locus are alike, also known as inbreeding coefficient (Hallauer and Miranda, 1988). The analyses that include parents are used for the open pollinated, synthetic and composite varieties, because the parents have some reasonable vigour comparable to their progeny, especially in self-pollinated crops displaying inbred vigour. Methods that exclude reciprocals on the other hand disregard maternal effects.

The number of crosses rapidly increases with the increase in the number of parents, more so with diallel mating designs. However, the diallel mating design assumes all possible crosses among a set of parents. Diallel mating designs have been extensively used in the genetic studies to determine the importance of gene action in maize (Melchinger, Lee, Lamkey, and Woodman, 1990; Dudley, Saghai Maroof and Rufene, 1991; Pixley and Bjarnason, 1993; Reif, Melchinger, Xia, Warburton, Hoinsington, Vasal, Srinivasan, Bohn and Fritch, 2003; Betran, Ribaut, Beck and Gonzalez de Leon, 2003; Makumbi, 2005; Bhatnagar, Betran and Rooney, 2004. In the current study, a seven by seven (7 x 7) parent diallel Method IV was used as the heterotic pattern was not well established to enable proper classification of males and females.

## 2.5 Other Mating Designs

There are other mating designs that could have been used but were not chosen because of several reasons weighing against them, but in favour of the diallel. The designs that could have been used include those that are population based such as biparental progenies which involves mating at random of pairs of individuals in the population. Alternatively, parent offspring regression where measurements are made from the reference

population which can be the source population, such as broad genetic base or the F2 population from the cross of inbred lines could have been used.

Another mating design where different sets are used as males or females is the North Carolina Design II (NCDII). It is important when number of lines involved increases to levels that make diallel cumbersome. As such, it is more preferred than diallel as twice as many parents can be used than in a diallel mating design, besides the fact that two independent additive variance ($Q^2_A$) can be determined while able to estimate dominance variance ($Q^2_D$). It is suitable where the germplasm can be classified as males or females by, for instance heterotic groups or testers. This has been used by Menkir and Ayodele (2005).

Some designs with special strengths include the nested designs or design I which is suitable for estimation of genetic components of variance for a reference population, and the design III that estimates average magnitude of dominance of genes affecting the traits (Hallauer and Miranda, 1988).

The diallel mating design was used in this study, as it allows for all possible crosses among a set of parents which is particularly important when no clear cut demarcation procedure can be followed to classify the germplasm. Besides that, the number of lines involved was so few such that

they could be accommodated comfortably by the diallel mating design. It is applicable to situations where the parents are derived from either the population or are themselves a population (Hallauer and Miranda, 1988).

## 2.6 Breeding methods for improving QPM traits

Traditional breeding methods such as recurrent selection have been used to improve maize in terms of level of protein. Dudley *et al*. (1975) reported an increase from 4.4 % to 26.6 % of protein in a maize population after 70 cycles of selection. Protein quality in terms of lysine content was successfully improved through two cycles of selection by Zuber and Helm (1972) according to Darrigues *et al*. (2006).

Mutation breeding has been at the centre stage of the effort by CIMMYT scientists. A combination of *sugary-2* and *opaque-2*, both being mutants, was preferred as it overcame issues of kernel hardness which also overcame ear rot problems associated with *opaque-2* maize, but problems of other agronomic traits remained unresolved (Vasal, 2001). Of late, emphasis shifted to use of mutants in combination with genetic modifier genes which has culminated in development of high quality protein maize which yield just as well as the normal endosperm maize (Vasal, 2001).

Use of transgenic technology in the improvement of maize for nutritional aspects has been reported. For instance, improvement for methionine has been achieved through accumulation of the product of the *Dzs10* gene by replacing the 3' untranslated region (UTR) with a sequence of the cauliflower mosaic virus that would enhance the level of expression in maize endosperm cells (Darrigues *et al*., 2006). Synthesis of a porcine α-lactalbumin gene with good digestibility, bioavailability, and amino acid balance in maize kernels gave rise to a 20 % increase in lysine levels. However, molecular approaches in improving the amino acid balance were hindered by the unstable expression of the modified protein in the target host (Darrigues *et al*., 2006), as well as occasional non-acceptance of genetically modified cultivars by the general public and government regulation bodies.

## 2.7 Genetic Distance

Molecular tools have been used in the improvement of normal endosperm maize in several other ways which can also be applied to QPM. For instance, simple sequence repeats (SSRs) have been successfully utilized to determine genetic similarities and relationships in maize (Senior, Murthy, Goodman and Stuber, 1998; Warburton, Xia, Xianchun, Crossa and Hoinsington, 2002; Prasanna, Mohammadi, Sudan, Nair, Garg, Rathore, Setty, Kumar, Zaidi and Singh, 2002; Reif *et al*., 2003). The high level of polymorphism associated with SSRs provides the highest potential for large-scale fingerprinting of maize genotypes. The ability of SSRs to be analysed by automated systems, high level of accuracy that characterize them and their repeatability, make SSRs more preferable than other molecular methods for studies of genetic relationships.

Knowing the level of genetic diversity within a set of germplasm (as measured by molecular markers, for example) is useful because a low level of diversity within a breeder's pool may indicate that gain from selection will not be optimal. Low levels of diversity within the set of cultivars grown within a region may point to a risk of genetic uniformity and large scale susceptibility to disease (genetic vulnerability), should a new biotic or abiotic stress such as disease or drastic climatic change respectively, break out. Finally, when seeking to expand the genetic base of a set of germplasm (breeding pool or cultivars in a region, for example), one should seek new variation from a new source that itself is very diverse. Molecular markers, such as SSRs, can verify this. Senior *et al.* (1998), observed an average of five alleles per locus in a study of temperate maize inbred lines. In the same study, the Polymorphism Information Content (PIC) values, (a measure of allele diversity at a locus which is comparable to gene diversity), ranged from 0.17 to 0.92 with an average of 0.59. In a study of tropical and subtropical maize inbred lines, Xia, Reif, Melchinger, Fritch, Hoinsington, Beck, Pixley, and Warburton (2005) reported a PIC range of 0.16 to 0.88, with an average of 0.64.

A high correlation between genetic distance and combining ability or heterosis can be used to predict the level of combining ability expected to be achieved in the hybrid of the lines that have been measured with the markers, thus saving the time and expense of making and testing hybrids that are not predicted to be high yielding. In temperate maize, Dudley *et al.* (1991) reported a significant but low correlation of Modified

Rogers' distance (MRD), a measure of genetic distance that range from zero (no diversity) to one (no similarity) with SCA for yield (0.35 with 66 loci and 0.25 with 29 loci). The MRD is described as being equivalent to the square root fraction of the heterozygous loci of the hybrids with homozygous loci. Also in temperate maize, Betran *et al.*, (2003) found significant positive correlation between SCA and mid parent ($r$ = 0.47) and high-parent ($r$ = 0.31) heterosis. Reif *et al*. (2003) found a significant ($P<0.01$) correlation between $MRD^2$ and Pelmitic Mid Parent Heterosis (PMPH) of 0.63. Similar mixed results for using GD as a predictor of hybrid performance has also been reported in other crops. Gutierrez, Basu, Saha, Jenkins, Shoemaker, Cheatham, and McCarthy (2002), reports that in sunflower (*Helianthus annus* L.) there was significant correlation between the hybrid performance and GD. The same authors report no correlation between measures of diversity and hybrid performance of wheat and very low correlation ($r$ = 0.07) between yield of F2 hybrids, heterosis and GD. A correlation value that is high can be used to predict the level of relationship or SCA if one of these values is known.

It has been observed that inbred lines that are indistinguishable using isozyme or zein profiles can be isolated using SSR analysis as they are PCR based, codominant, locus-specific, highly reproducible, hyper variable, informative and are relatively easy to use.

Among several uses of these molecular tools, is determination of genetic diversity through determination of genetic distance. Genetic distance is any quantitative measure of genetic difference, either at the sequence level, or allelic frequency level, that is calculated between individuals,

populations or species (Mohammadi and Prasanna, 2003). The measures of genetic distance or genetic similarity (GS) that use binary data are i) Nei and Li's (1979) coefficient ($GD_{NL}$), ii) Jaccard's coefficient ($GD_J$), iii) simple matching coefficient ($GD_{SN}$), and Modified Rogers' distance (MRD).

Although the relationship between GD and SCA has been reported to be significant, the relationship has generally been weak ($r < 0.5$). A similar trend has been observed in other crops implying that GD data cannot readily replace evaluation of hybrids for SCA. The reason is that often heterosis is found among lines within the same heterotic group because there are many factors that contribute to heterosis which include dominance theory, over dominance theory and biochemical factors and then molecular factors.

# Chapter 3

## MATERIALS AND METHODS

## 3.1 Plant Materials

Eleven maize inbred lines were chosen for this study and are listed in Table 3.1. However, some lines were later dropped from the study as it was not possible to make all crosses with them, leaving only seven (inbred lines 1 to 7) for the combining ability study. The lines were derived from the program in which elite lines from Southern Africa (Regional) were being converted to QPM using the backcross breeding method, using QPM donors from CIMMYT-Mexico and CIMMYT-Zimbabwe. The seven lines come from the Seed-Co's improvement program to convert normal endosperm elite lines to QPM, and represent major heterotic groups that are used within southern Africa (Table 3.1).

In the winter of 2006, these eleven lines were crossed in all possible combinations at Muzarabani to form single cross hybrids in a diallel mating design. Muzarabani is a winter site in Zimbabwe which lies at an altitude of 600m above sea level and is characterised by high temperatures, even during winter, which makes it a suitable site for off-season breeding work in order to attain two seasons within a year. The lines were planted in plots measuring 0.75m wide and 4m long. Where a line was used as a female, two rows were provided to ensure adequate seed for the subsequent trials. The male rows were planted at three different dates, a week before the females were planted, on the same date with females, and a week after the females, to ensure pollen and silk synchronization.

**Table 3.1:** The Quality Protein Maize (QPM) inbred line code, heterotic group and origin of the lines used in the study

| | Line | Heterotic Group[†] | Source |
|---|---|---|---|
| 1 | SC10 | P | Seed Co |
| 2 | SC1 | S | Seed Co |
| 3 | SC5 | S | Seed Co |
| 4 | SC2 | P | Seed Co |
| 5 | SC4 | N3 | Seed Co |
| 6 | WWO1408 | O | South Africa |
| 7 | CML511 | A | CIMMYT-Zimbabwe |
| 8[*] | SC7 | W | Seed Co |
| 9[*] | SC9 | O | Seed Co |
| 10[*] | CML159 | B | CIMMYT-Mexico |
| 11[*] | CML175 | B | CIMMYT-Mexico |

*, not included in the combining ability analysis

[†], P, Natal Potchefstroom Pearl; S, Southern Cross; N3, Salisbury White; O, miscellaneous; A, Tuxpeno, Kitale or BSSS; W, M37W; B, ETO, Ecuador 573 or Lancaster

Lines were crossed to each other in all possible combinations without reciprocals, in a half diallel mating design as maternal effects were not anticipated. Where reciprocals did exist, the seed was bulked together. This followed Griffing (1956) Method IV.

### 3.2 Laboratory analysis for protein and tryptophan content

Seed of the lines were scored for endosperm modification at the Seed Co Rattray Arnold Research Station seed laboratory by randomly assessing 100 kernels from 20-32 cobs of inbred lines and a one kilogram sample of the shelled F2 grain drawn randomly from each F2 population. Each kernel was evaluated on a back-lit (candling) table and after visually assessing the sample, assigned scores ranging from 1 to 5, where 1 = completely modified (that is, transluscent, normal phenotype); 2 = 75 % modified; 3 = 50 % modified; 4 = 25 % modified; and 5 = completely opaque as described by Bjarnason and Vasal (1992) and Pixley and Bjarnason (2002). The visual rating of the kernels is presented in Figure 3.1 adapted from Krivanek *et al.* (2007). The lines and the F2 grain were analysed for the protein and tryptophan content, whose values were used to derive at the quality index through expression of tryptophan content as a proportion of the protein content.

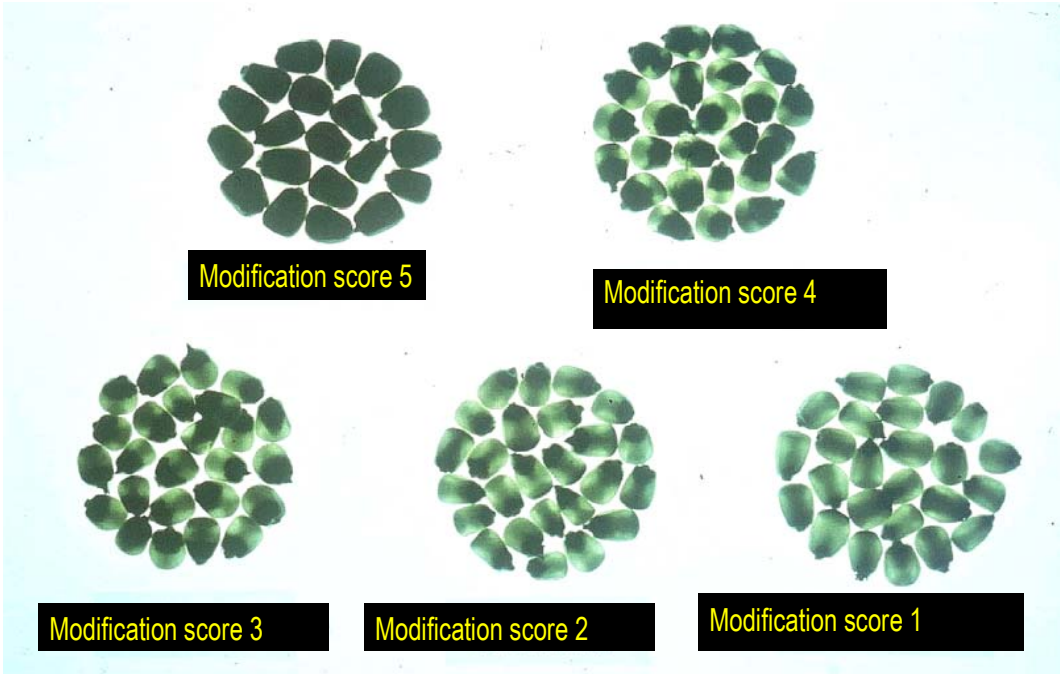


**Figure 3.1 : Endosperm modification scores (Adapted from Krivanek, 2007)**

For the determination of protein, nitrogen (N) content was determined using the Micro-KjelDahl method (National Institute of Nutrition, 1983), calculated as follows:

$$\text{Nitrogen \%} = \frac{(\text{Volume of HCl used for titration in the sample (ml)} - (\text{Volume of HCl used for titration in blank})}{\text{g of sample}} \times \frac{\text{normality HCl} \times 14.0067}{1000} \times 100$$

The protein determined as follows:

Protein % = % of nitrogen x conversion factor for maize (6.25)

Tryptophan content was determined by the method described by Villegas, Ortega, and Bauer (1984) adapted by CIMMYT-Mexico Soils and Plant Analysis Laboratory protocol from 20 kernel samples. As the values of lysine and tryptophan are usually highly correlated, only the tryptophan content was determined as lysine, which is more costly to analyse, is assumed to be four times the value of tryptophan. Due to this well established relationship between these amino acids in the protein of the *opaque*-2 maize endosperm (Pixley and Bjarnason, 2002), tryptophan may be used as a single parameter for the evaluation of the nutritional quality of the protein.

Tryptophan content was measured using the simple and reproducible colorimetric method published by Villegas (1975) and Villegas *et al.* (1984). It has been used by the CIMMYT laboratory for several years for determination of tryptophan (and indirectly, lysine) content. It is based on the Hopkins-Cole reaction in which one molecule glyoxylic acid and two molecules of tryptophan form a colored compound with a maximum absorption at 560 nm. The intensity of color was measured with a spectrophotometer such that increased intensity of color implied more tryptophan. The tryptophan content of the sample was calculated from the standard curve as follows:

Tryptophan % = Factor x Reading (Optical density)

The quality index was determined by expressing the tryptophan content as a percentage of the protein content.

### 3.3 Determination of F1 Hybrid Yield and Agronomic Performance

To determine the performance of the F1 hybrids in terms of yield and content of lysine and/or tryptophan, the 21 F1 hybrids that had been generated in the winter of 2006 were evaluated in replicated trials planted at five sites in summer, 2007. These were planted at two different dates at the Rattray Arnold Research Station (RARS), RARS-Early and RARS-late, Kadoma Research Centre (KRC), at CIMMYT station which is near Harare (CIMMYT-Harare) and the Agricultural Research Trust (ART) farm (Table 3.2). Endosperm modification score was done at two sites only, RARS-late and ART, while protein quality traits were measured at RARS-early only as cost was prohibitive.

Optimal management of the trials was done so as to attain the maximum potential yield in all the sites.

### 3.3.1 Experimental Design

At all sites, trials were laid out as a 10x6 alpha lattice (0.1) design (Patterson, Williams, and Hunter, 1978), an incomplete block design. The trial was conducted with three replications. In all environments, plants were planted in two rows of 4m spaced at 0.75m. The seeding rate was high and the plants were thinned, resulting in a target population of 53,300 plants per hectare. Check entries were normal commercial hybrids with normal endosperm, and various experimental QPM hybrids.

**Table 3.2**: **Locations and environments used to evaluate F1 hybrids.**

| Location | Latitude | Longitude | Altitude (masl)[†] |
|---|---|---|---|
| RARS[‡] Early | 17°40' S | 31°13' E | 1341 |
| RARS Late | 17°40' S | 31°13' E | 1341 |
| CIMMYT-Harare | 17°48' S | 31°02' E | 1470 |

| | | | |
|---|---|---|---|
| ART Farm | 17°41' S | 31°0'4E | 1468 |
| KRC | 18°20' S | 30°60'E | 1149 |

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†masl, metres above sea level

‡RARS, Rattray Arnold Research Station; CIMMYT, International Maize and Wheat Improvement Centre; ART, Agricultural Research Trust; KRC, Kadoma Research Centre

## 3.4 Crop Husbandry

### 3.4.1 Land Preparation

Between June and August 2005, the sites were deep ploughed at a depth of 25cm and disc harrowed just prior to planting, immediately followed by clod breaking by rolling. Planting stations were established by hand held hoe following the pre-marked wire chain.

### 3.4.2 Fertilizer Application

A compound fertilizer was applied and incorporated by the disc harrow as basal application prior to planting. All the sites received optimum fertilizer of 400 kg $ha^{-1}$ basal application of maize fertilizer (8 % N, 16 % $P_2O_5$, and 8 % $K_2O$). Top dressing was achieved by an application of

ammonium nitrate (34.5 % N) at 400 kg $ha^{-1}$ which was split into two applications. The first application was done four weeks after crop emergence (WACE) and the second was done at eight WACE).

### 3.4.3 Weed and Pest Control

Pre-emergence herbicides were applied at all the sites according to Long *et al*. (2004). Atrazine, 50 % flowable, was applied at a rate of 4.5 l $ha^{-1}$ together with Dual 960EC at 1.8 l $ha^{-1}$. Hand weeding was used to control weeds that emerged later. Furadan was applied at planting to protect the crop from early pest damage at 4 kg $ha^{-1}$. As stalk borer (*Busseola fusca*, Fuller.) is known to be a problem pest, prophylactic application of contact insecticide Dipterex 2.5 % granules into the whorl was done at four WACE.

## 3.5 Measurements

Yield data were obtained following hand harvesting and weighing the actual grain yield after shelling and moisture determination, which was used for standardization of the moisture content to 12.5%. Grain moisture was measured with a moisture meter (Burrows, Model 750, Seedburo Equipment Company, Chicago, Illinois, USA). Other agronomic traits were recorded to enable correlation analysis with traits of primary importance (Pixley and Bjarnason, 2002). Disease scores were subjectively assigned on a 1 to 9 scale where 1 = no symptoms observed, while 9 = completely blighted foliage by *Cercospora zeae-maydis*, Tehon and Daniels, (GLS), *Exserohilium turcicum* (ET), *Puccinia sorghi* (PS),

*Phaeosphaeria spp*, Henn (PLS), and grain disease (ER) caused by *Fusarium* and *Diplodia spp*. Days from planting to when 50 % of plants had 2 cm to 3 cm silk length or had started to shed pollen were recorded as days to mid silking (SD) and days to mid pollen (AD) respectively, with the difference between the two being the days to anthesis-silking interval (ASI) i.e., ASI = SD-AD.

Plant height (PHT) and cob (ear) height (EHT) was measured from the plant base to the last node and from the plant base to the node bearing the upper most ear, respectively. Stalk and root lodging were the proportion of plants at harvest with stalks broken below the ear level (SL) or with an inclination of $30^{o}$ or more at the base of the plant (RL), respectively (Poehlman and Sleper, 1995) and their summation constituted the total lodging (TL). Finally, ear position is the relative position of the ear on the plant and husk cover is the proportion of the ears that had exposed ear tips.

### 3.7 Formation of F2 Grain for Quality Analysis

In order to form F2 grain that represent the actual product of the F1 seed derived from the diallel crosses, full sib pollinations were carried out in trials under controlled pollinations at RARS to prevent the xenia effects which is the immediate effects of foreign pollen parent on the grain (Poehlman and Sleper, 1995; Weingartner, Kaeser, Long and Stamp, 2002) that characterize the *opaque-2* recessive gene and affects the quality of the subsequent grain. This was achieved by hand pollinating the first four plants (two planting stations with two plants per station from the

two row plot) of the F1 hybrids in trials. Plant to plant full-sib pollination method was used where pollen from a plant within the plot was used to pollinate another plant within the same plot. This was only done at RARS-early in the first replication. The pollinated ears were not included in the yield evaluation and the area was proportionally reduced.

At harvest, yield data were collected and a sample of the hand-pollinated seed that represents the actual grain was sent for quality analysis where tryptophan and protein were determined together with the parent QPM inbred lines. Unlike the F1, the F2 quality is of importance as it is directly used by the farmers.

### 3.8 Phenotypic Data and Diallel Analyses

The data for the F1 hybrids were first subjected to analysis of variance (ANOVA) with REML on FIELDBOOK (Banziger and Vivek, 2007), for each site to determine if yield and all other quantitative traits were significantly different from one another, (Hallauer and Miranda, 1988). The locations that were significantly different ($P<0.05$) were further subjected to combined ANOVA using the adjusted means in order to determine the environmental effects. Cultivar effects were considered fixed while locations were random.

A diallel analysis following a standard combining ability model where variation among crosses or entries were split between that due to general combining ability (GCA) effects and that due to specific combining ability (SCA) effects, were subsequently done using adjusted means of significant sites after removing check entries based on a method described by Kempthorne (1957). The analysis of variance was done for individual sites or environments and then across sites (combined). This analysis followed Method IV, Model II of Griffing, (1956) to obtain estimates of GCA and SCA effects for each line for both yield and endosperm modification.

### 3.9 Statistical Model

The value of a cross (i x j) in Griffing's, (1956) analysis were expressed by:

$$X_{ij} = \mu + g_i + g_j + s_{ij} + e_{ijk;}$$

Where $\mu$ = general mean; $g_i + g_j$ = general combining ability effects of the ith and jth parent, respectively; $s_{ij}$ = specific combining ability effect of the cross i x j; and $e_{ijk}$ = experimental error for the $x_{ijk}$ observations (k =1,2,…,r; i = j = 1,2,…,n)

Furthermore, correlation coefficients between SCA and mean yield, SCA and genetic distances, and genetic distance and yield, were determined within environments and on combined data by PROC CORR of SAS (SAS Institute, 1997).

## 3.10 Quantifying the Genetic Distances Among the Quality Protein Maize (QPM) Inbred Lines Using Simple Sequence Repeat (SSR) Markers

To determine genetic diversity, the eleven white-grained QPM inbred lines listed in Table 3.1 were sent to the Applied Biotechnology Centre (ABC) at CIMMYT-Mexico where marker analysis using SSR was done following Warburton *et al*. (2002).

### 3.10.1 DNA Extraction

The inbred lines were grown in the greenhouse where the youngest leaves of plants were collected 10 days after planting. Deoxyribonucleic Acid (DNA) was extracted from these fresh leaves with a sap extractor (MEDU Erich Pollahne; Am Weingarten Germany) according to the Applied Biotechnology Centre's Manual of Laboratory Protocols (CIMMYT, 2005) and modified from Clarke, Moran, and Appels (1989). Fluorescent oligonucleotides were bought from commercial producers. The DNA was qualified and quantified on an agarose gel using a size standard (λ DNA cut with *Hind* III).

### 3.10.2 Polymerase Chain Reactions

The SSR markers that were used were referenced from Warburton *et al*. (2002) and are listed online in MaizeGDB at http://www.agron.missouri.edu. The forward primers were labeled at the 5' end with either 6-carboxyfluorescein (6-FAM), tetrachloro-6-

carboxyfluorescein (TET), or hexachloro-6-carbofluoroscein (HEX). The PCR reactions were done in 10-µL volumes with 2-µL of template DNA (the output of sap extractor was diluted five times with distilled and deionised water), 0.4 to 4 pmols each of 1 to 4 primers, 1X PCR buffer, 0.25 m*M* dNTPs, 1.5 to 2.5 m*M* $MgCl_2$ and 0.75 U *Taq* polymerase.

A Peltier Thermal cycler (MJ Research) was used for the PCR reactions with the following profile: 94°C for 2 minutes, followed by 30 cycles of: 94°C for 30 seconds of denaturing, X°C for 1 min, and 72°C for 1 min; followed by extension at 72°C for 5 min. X°C refers the annealing temperature, which was specific for each primer combination and ranged from 52°C to 60°C (values of X are reported in Warburton *et al*. 2002.). Primers were multiplexed to increase efficiency according to Warburton *et al*. (2002).

### 3.10.3 Electrophoresis and Determination of Amplified Segments

Samples containing two PCR reactions (0.5 µL of each), 0.3 µL GeneScan 350 or 500 internal lane standard (Applied Biosystems, Foster City, CA) labeled with N, N, N, N-tetramethyl-6-carboxyrhodamine (TAMRA), and 30 % (v/v) formamide were denatured by heating at 95°C for 5 min, kept on ice, then loaded on 4.5 % (w/v) denaturing (6 M urea) acrylamide:bisacrylamide (29:1) gels (36 cm well-to-read). By multiloading two multiplexed PCR reactions, on average, five simple sequence repeats (SSR) markers were run in each lane of the gel simultaneously. DNA

samples were electrophoresed in 1X TBE buffer (pH 8.3) at constant voltage (3.00 kV) for 2.5h on an ABI Prism 377 DNA Sequencer (Perkin Elmer, Foster City, CA) as described by Warburton *et al*. (2002).

### 3.10.4 Analysis of Genetic Diversity Data

Fragment sizes were automatically calculated with GeneScan 3.1 (Perkin Elmer/Applied Biosystems) using the Local Southern sizing method. The GeneScan data were appended to a table with Genotyper 2.1 (Perkin Elmer/Applied Biosystems), which converts peak sizes to alleles using pre-set Category functions. For each individual, peaks were assigned as present (1) or absent (0), and the binary data used for further analyses of relationships. Similarity matrices were constructed using the Simple Matching similarity coefficient (Kaufman and Rousseeuw, 1990). Dendograms were created from the similarity matrix using the Unweighted Pair Group Mean Average (UPGMA) method (Rohlf, 1997) to visualize the patterns of diversity in the set of lines. The similarity matrix and the dendrogram were created in NTSYSpc 2.01 (Rohlf, 1997). To determine the level of association, a correlation analysis was run by SAS (1997), to determine the correlation coefficients between the genetic distance and SCA, and genetic distance and yield.

## 3.11 Relationship of genetic distance with SCA and yield of hybrids

The Jaccard's genetic similarity derived from the SSR was correlated with the adjusted means of yield from the ANOVA of individual sites and combined analysis, after removing the check and non-diallel entries, the SCA values obtained from the analysis. The correlation coefficients derived was used to establish the relationship between these parameters.

# Chapter 4

# RESULTS

## 4.1 Genotypic Performance *Per Se*

### 4.1.1 Quality of the QPM inbred lines and the F1 hybrids

The original 11 QPM inbred lines were analysed for endosperm quality, and their quality data in terms of tryptophan and protein levels, and the quality index, are in table 4.1. Half of the QPM inbred lines that were analysed, (SC5,WW014 and CML511, and the inbred lines that were excluded from the genetic studies SC9, CML159 and CML175) had values of the quality index (percentage of tryptophan divided by protein content) of 0.70, 0.80, 0.86, 0.74, 0.69 and 0.85, above that described as being QPM (Table 4.1).

The F1 hybrids from the crosses SC5 x WWO1408 and WWO1408 x CML511 had tryptophan levels of 0.066 and 0.073 and quality indices of 0.74 and 0.81 respectively (Appendix 4.1) which were within acceptable range.

**Table 4.1**: Tryptophan, protein and quality index of lines in the diallel study

| Line # | Line Code | Tryptophan % | N % | Protein % | Quality Index |
|---|---|---|---|---|---|
| 1 | SC10 | 0.035 | 1.50 | 9.35 | 0.38 |
| 2 | SC1 | 0.044 | 1.32 | 8.27 | 0.53 |
| 3 | SC5 | 0.061 | 1.39 | 8.68 | 0.70[a] |
| 4 | SC2 | 0.064 | 1.10 | 6.90 | 0.93[a] |
| 5 | SC4 | 0.045 | 1.60 | 9.99 | 0.45 |
| 6 | WWO1408 | 0.063 | 1.28 | 7.97 | 0.79[a] |
| 7 | CML511 | 0.081 | 1.58 | 9.66 | 0.86 [a] |
| 8 | SC7 | 0.045 | 1.57 | 9.83 | 0.46 |
| 9 | SC9 | 0.079 | 1.42 | 8.87 | 0.89[a] |
| 10 | CML159 | 0.063 | 1.21 | 7.55 | 0.83[a] |
| 11 | CML175 | 0.084 | 1.59 | 9.91 | 0.85[a] |

[a], Quality index acceptable as QPM lines.

### 4.1.2 Performance of F1 hybrids in replicated yield trials

In the individual site analysis, there was highly significant variation ($P<0.001$) among the F1 hybrids for yield (Table 4.3) and AD, at all five sites. The other agronomic traits were variable: for example, the hybrids varied significantly ($P<0.001$) at all other sites except at RARS-late ($P<0.05$) for plant height and at CIMMYT-Harare for AD, while for RL, significant differences ($P<0.01$) were observed only at ART farm, and SL at RARS-late and ART farm while at other sites, the differences were not significant ($P>0.05$). Cob diseases score (ER) was significant ($P<0.01$) at RARS-early, CIMMYT-Harare, RARS-late and at ART farm, HT at KRC, RARS-late and at ART-Farm, PLS at RARS-early and GLS at both RARS-Late and at ART-Farm. The highest mean grain yield among the hybrids of 9.25 tons $ha^{-1}$ was recorded at KRC while the lowest was 8.61 tons t $ha^{-1}$ recorded at the CIMMYT-Harare site (Table 4.8). At all the sites, the best entries were commercial checks except at ART-Farm where a QPM check hybrid, (CML159 X SC5) had the highest mean yield. The commercial check hybrid SC633 was the highest at RARS early, KRC, and ART farm. Of the diallel study F1 hybrids in Table 3.1, (hybrids from the crosses among the seven inbred lines), the cross SC1 X SC10 had the highest mean yield at CIMMYT-Harare and at ART-Farm (10.89 t $ha^{-1}$ and 11.67 t $ha^{-1}$ respectively). The cross SC1 X SC2, SC1 X CML511 and SC1 X SC4 had the highest mean yield at RARS early, KRC and RARS-late with respective means of 11.16 t $ha^{-1}$, 11.80 t $ha^{-1}$ and 10.60 t $ha^{-1}$.

The mean kernel endosperm modification scores and the subjective scores assigned to the whole plot that was only recorded at RARS-late and at ART-Farm, were highly significant ($P<0.001$). The QPM inbred line SC5 appeared in the hybrids SC5 X SC1, SC5 X SC4 and SC5 X CML511 that had the lowest mean modification scores of 1.4, 1.4 and 1.3, respectively Table 4.1.

The combined ANOVA showed highly significant ($P<0.001$) differences among the 21 entries and the check varieties, for AD and EH, with $P<0.01$ for EPO, while PHT and ER were significant at $P<0.05$, GYG, lodging (both RL and SL) and ASI were not significantly different. Entry and environment interactions were highly significant ($P<0.001$) for the traits GYG, AD and PHT and not significant for RL, ASI, EH, SL, ER and EPO (Table 4.4). The highest yielding F1 hybrid was the commercial check SC633 (11.69 ton $ha^{-1}$). The next four hybrids had an experimental QPM inbred line SC5 as one of the parents (Table 4.2).

**Table 4.2**: Means of yield and all the other traits across the five sites for the 21 diallel F1 hybrids

| Entry | Parent 1 | Parent 2 | Pedigree | GYG† t/ha | AD d | ASI d | PHT cm | EPO # | RL % | SL % | EPP # | ER % | GLS 1-5 | PS 1-5 | ET 1-5 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 9 | 2 | 5 | SC1 X SC4 | 10.3 | 74 | -0.5 | 253 | 0.5 | 4.5 | 1.9 | 1.101 | 2.6 | 2.2 | 2.2 | 1.1 |
| 8 | 2 | 4 | SC1 X SC2 | 10.1 | 73 | 1.7 | 251 | 0.5 | 1.7 | 4.3 | 1.260 | 2.6 | 1.9 | 2.3 | 1.9 |
| 1 | 1 | 2 | SC10 X SC1 | 10.1 | 73 | 0.1 | 244 | 0.5 | 3.5 | 3.4 | 1.021 | 1.6 | 1.9 | 1.9 | 1.5 |
| 10 | 2 | 6 | SC1 X WWO1408 | 9.9 | 71 | 0.8 | 241 | 0.5 | 2.0 | 2.2 | 1.000 | 2.8 | 1.3 | 1.6 | 1.3 |
| 7 | 2 | 3 | SC1 X SCSC4 | 9.8 | 75 | 1.0 | 257 | 0.5 | -1.1 | 0.7 | 1.021 | 2.7 | 1.0 | 1.1 | 1.9 |
| 2 | 1 | 3 | SC10 X SCSC4 | 9.8 | 70 | 1.2 | 243 | 0.5 | 0.5 | 8.1 | 0.975 | 2.3 | 1.2 | 1.2 | 1.8 |
| 14 | 3 | 6 | SCSC4 X WWO1408 | 9.3 | 70 | 1.6 | 248 | 0.5 | -0.1 | 0.2 | 1.021 | 2.4 | 1.1 | 1.0 | 1.3 |
| 19 | 5 | 6 | SC4 X WWO1408 | 9.2 | 69 | 1.1 | 241 | 0.5 | 0.3 | -0.4 | 1.235 | 2.5 | 1.7 | 1.3 | 1.2 |
| 12 | 3 | 4 | SCSC4 X SC2 | 9.2 | 69 | 1.9 | 257 | 0.5 | 3.2 | 0.3 | 1.165 | 2.2 | 1.8 | 1.4 | 2.4 |
| 11 | 2 | 7 | SC1 X CML511 | 9.1 | 73 | -0.2 | 255 | 0.5 | -0.2 | 7.9 | 1.276 | 2.3 | 1.1 | 3.3 | 1.4 |
| 4 | 1 | 5 | SC10 X SC4 | 9.0 | 70 | 1.1 | 255 | 0.5 | 2.0 | 3.3 | 0.942 | 1.9 | 1.7 | 1.1 | 1.7 |
| 16 | 4 | 5 | SC2 X SC4 | 9.0 | 69 | 0.8 | 251 | 0.5 | -0.9 | 3.0 | 1.088 | 1.8 | 3.0 | 1.5 | 2.1 |
| 15 | 3 | 7 | SCSC4 X CML511 | 9.0 | 73 | 1.5 | 251 | 0.6 | -0.4 | 1.4 | 1.343 | 2.6 | 1.1 | 1.8 | 1.4 |
| 20 | 5 | 7 | SC4 X CML511 | 8.8 | 72 | 1.0 | 253 | 0.5 | -0.6 | 6.4 | 1.045 | 2.0 | 2.0 | 2.5 | 1.1 |
| 5 | 1 | 6 | SC10 X WWO1408 | 8.5 | 69 | 1.1 | 237 | 0.5 | 7.9 | 2.2 | 0.925 | 2.7 | 1.0 | 1.1 | 1.5 |
| 21 | 6 | 7 | WWO1408 X CML511 | 8.4 | 70 | 1.3 | 233 | 0.5 | 3.1 | 0.2 | 1.027 | 2.7 | 1.1 | 2.0 | 1.4 |
| 18 | 4 | 7 | SC2 X CML511 | 8.3 | 68 | 4.2 | 237 | 0.5 | -1.4 | 10.2 | 1.142 | 2.1 | 1.0 | 2.8 | 1.7 |
| 6 | 1 | 7 | SC10 X CML511 | 8.3 | 71 | -0.4 | 251 | 0.5 | 4.6 | 6.8 | 1.238 | 2.3 | 1.0 | 2.7 | 1.2 |
| 17 | 4 | 6 | SC2 X WWO1408 | 8.3 | 68 | 0.7 | 225 | 0.6 | -0.1 | 2.4 | 1.198 | 2.1 | 2.0 | 1.2 | 1.4 |
| 3 | 1 | 4 | SC10 X SC2 | 8.0 | 69 | 0.8 | 239 | 0.5 | 0.9 | -0.2 | 0.942 | 2.0 | 1.0 | 1.3 | 2.1 |
| 13 | 3 | 5 | SCSC4 X SC4 | 7.7 | 72 | 1.3 | 238 | 0.5 | 3.9 | 2.4 | 1.047 | 3.1 | 1.5 | 1.3 | 1.8 |
| Mean | | | | 9.1 | 71 | 1.0 | 246 | 0.5 | 3.0 | 3.1 | 1.081 | 2.4 | 1.5 | 1.8 | 1.6 |
| LSD (0.05) | | | | 0.8 | 1 | 1.2 | 9 | 0.0 | 12.2 | 4.4 | 0.121 | 0.8 | 0.5 | 0.6 | 0.5 |
| Min | | | | 7.7 | 67 | -0.5 | 225 | 0.5 | -1.4 | -1.2 | 0.925 | 1.6 | 0.9 | 1.0 | 1.1 |
| Max | | | | 11.7 | 75 | 4.2 | 257 | 0.6 | 31.8 | 10.2 | 1.343 | 3.1 | 3.0 | 3.3 | 2.4 |
| NumSignificantSites | | | | 5 | 3 | 1 | 5 | 4 | 1 | 2 | 4 | 4 | 2 | 2 | 3 |
| Error df | | | | 118 | 118 | 118 | 118 | 118 | 117 | 117 | 118 | 117 | 118 | 79 | 118 |
| NumReps | | | | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 3 |
| SitesUsedForComputingMean | | | | 1,2,3,4,5 | 2,4,5 | 4 | 1,2,3,4,5 | 2,3,4,5 | 5 | 4,5 | 2,3,4,5 | 1,3,4,5 | 4,5 | 2,3 | 2,4,5 |

†, GYG, grain yield; AD, Anthesis date; ASI, anthesisi to silking interval; PHT, plant height; EPO, ear position; RL, root lodging; SL, stalk lodging; EPP, ears per plant; ER, ear rot; GLS, grey leaf spot; PS, Puccinia rust; Turcicum leaf blight

**Table 4.3:** Analysis of variance for grain yield (kg $ha^{-1}$) at five locations.

| | **RARS Early**[†] | **KRC** | **CIMMYT** | **RARS Late** | **ART** | **Combined** |
|---|---|---|---|---|---|---|
| Mean | 8.75 | 9.25 | 8.61 | 8.80 | 8.96 | 9.14 |
| *p* | *** | *** | *** | *** | *** | *** |
| LSD (0.05) | 1.73 | 2.63 | 2.21 | 1.80 | 2.09 | 0.85 |
| MSe | 0.80 | 1.85 | 1.28 | 1.21 | 1.71 | 1.37 |
| Min | 5.20 | 4.05 | 4.64 | 6.30 | 6.20 | 7.72 |
| Max | 12.61 | 12.64 | 12.54 | 12.37 | 11.56 | 11.69 |

*, **, ***, ns Indicates significance at 0.05, 0.01, 0.001 and >0.05 probability levels, respectively.

[†] RARS-early, Rattray Arnold Research Station (early planted); KRC, Kadoma Research Centre; CIMMYT, International Maize and Wheat Improvement Centre; RARS-Late, Rattray Arnold Research Station (late planted); ART, Agricultural Research Trust;

For the sites, locations or environments, highly significant ($P<0.001$) differences were observed for all the traits, with GYG and RL being significant at $P<0.01$ (table 4.4).

**Table 4.4**: **Combined analysis of variance and mean square for various agronomic traits.**

| | df | GYG | AD | PHT | RL | df | ASI | EH | SL | ER | EPO |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Env | 4 | 5.7 ** | 209.1 *** | 23413.6 *** | 8.9 ** | 3 | 13.1 *** | 15817.3 *** | 60.3 *** | 40.8 *** | 0.03 *** |
| Entry | 24 | 1.9 *** | 8.4 *** | 226.4 * | 2.4 | 24 | 1.3 | 102.7 *** | 4.0 | 0.4 * | 0.00 ** |
| GCA | 6 | 3.4 *** | 27.8 *** | 491.2 * | 3.9 | 6 | 2.8 | 81.7 | 6.6 | 0.5 | 0.00 |
| SCA | 14 | 1.2 * | 2.5 | 177.6 | 2.4 | 14 | 1.1 | 141.0 *** | 4.1 | 0.4 * | 0.00 *** |
| Env*Entry | 96 | 1.8 *** | 2.9 *** | 135.8 *** | 2.2 | 72 | 0.9 | 30.6 | 3.5 | 0.2 | 0.00 |
| GCA*Env | 24 | 1.7 *** | 3.7 *** | 162.0 *** | 3.3 | 18 | 1.3 | 47.5 | 5.6 | 0.3 | 0.00 |
| SCA*Env | 72 | 1.9 *** | 2.6 ** | 127.1 *** | 1.9 | 54 | 0.8 | 25.0 | 2.8 | 0.2 | 0.00 |
| Residual | 180 | 0.6 | 1.5 | 69.1 | 5.4 | 180 | 0.7 | 47.0 | 5.9 | 1.1 | 0.00 |

*, **, *** Indicates significance at 0.05, 0.01, and 0.001 probability levels, respectively.

† GYG, grain yield; MOI, grain moisture; SD, silking date; PH, plant height; RL, SL and TL, root, stalk and total lodging; NP, number of plants; AD, anthesis date; ASI, anthesis silking interval; EH, ear height; ER, ear rot; ET, *Turcicum* leaf blight; GLS, Grey leaf Spot; MOD, endosperm modification score; GCA, general combining ability; SCA, specific combining ability; Env, environment.

## 4.2 General combining ability (GCA) effects

When the entry mean squares were split into GCA and SCA mean square, the GCA mean squares, were highly significant for grain yield among the QPM lines ($P<0.01$) at both sites at RARS, at KRC and CIMMYT-Harare and were significant ($P<0.05$) at ART-Farm (Table 4.4). Within individual sites, line SC1 and CML511 consistently had the highest positive and negative GCA values respectively at KRC, CIMMYT-Harare and at ART-Farm (Table 4.5).

**Table 4.5**: **General combining ability effects for yield of the QPM lines at five locations and across the locations**

| | RARS† Early | KRC | CIMMYT | RARS Late | ART-Farm | Across Sites |
|---|---|---|---|---|---|---|
| SC10 | -0.63 | -0.56 | 0.24 | -0.77 | 0.12 | -0.32 |
| SC1 | -0.89 | 1.33 | 0.73 | 1.19 | 0.84 | 0.64** |
| SC5 | 0.88 | -0.13 | 0.57 | -0.28 | 0.38 | 0.28 |
| SC2 | 0.41 | -0.61 | 0.05 | -0.20 | -0.81 | -0.23 |
| SC4 | 0.28 | -0.19 | 0.21 | -0.21 | -0.12 | -0.01 |
| WWO1408 | -0.08 | 0.98 | -0.45 | -0.39 | 0.16 | 0.04 |
| CML511 | 0.02 | -0.82 | -1.34 | 0.67 | -0.56 | -0.41 |
| *p* | ** | ** | ** | ** | * | *** |
| SE | 0.26 | 0.40 | 0.33 | 0.26 | 0.31 | 0.25 |

*, **, *** Indicates significance at 0.05, 0.01, and 0.001 probability levels, respectively.

† RARS-early, Rattray Arnold Research Station (early planted); KRC, Kadoma Research Centre; CIMMYT, International Maize and Wheat Improvement Centre; RARS-late, Rattray Arnold Research Station (late planted); ART, Agricultural Research Trust;

**Table 4.6**: General combining ability (GCA) effects for grain yield (t $ha^{-1}$) of the QPM lines across five locations

| Line | Estimate | Probability |
|---|---|---|
| SC10 | -0.32 | 0.20 |
| SC1 | 0.64** | 0.01 |
| SC5 | 0.28 | 0.26 |
| SC2 | -0.23 | 0.35 |
| SC4 | -0.01 | 0.98 |
| WWO1408 | 0.04 | 0.86 |
| CML511 | -0.41 | 0.10 |

SE = 0.248

**, indicates significance at 0.01 probability level.

The GCA and GCA x environment interaction effects across the five sites for grain yield (Table 4.5; Table 4.6; Figure 4.1) were highly significant (P<0.001) with QPM inbred lines 2 (SC1) and 7 (CML511) having the highest and lowest GCA estimates for grain yield and their respective values were 0.64 and -0.41 with the former being significant (P<0.001). Inbred line SC2 had the second highest GCA effects while SC10 and SC4 were the next after the worst and both had negative effects. Besides inbred line SC4 which had the lowest of all the inbred lines with positive GCA effects, the rest belonged to the heterotic group "S" while the second and third lowest negative GCA effects were observed on the lines belonging to the heterotic group "P".

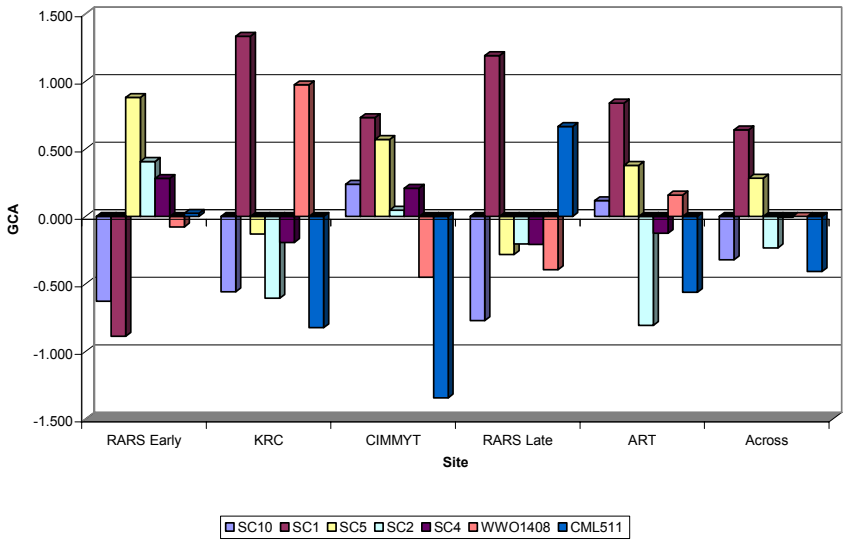


**Figure 4.1: General combining ability (GCA) for yield of the QPM lines across five locations**

† RARS-early, Rattray Arnold Research Station (early planted); KRC, Kadoma Research Centre; CIMMYT, International Maize and Wheat Improvement Centre; RARS-late, Rattray Arnold Research Station (late planted); ART, Agricultural Research Trust;

The top three F1 hybrids in terms of yield all had line 2 (SC1) as a parent while Line 7 (CML511) appeared more frequently as a parent of the lowest yielding hybrids (Table 4.8). The GCA effects were highly significant ($P<0.001$) for AD and significant ($P<0.05$) for PHT. For the other traits, the GCA effects were not significant at $P>0.05$ (Table 4.4).

The GCA effects for endosperm modification score were not significant ($P>0.05$) at RARS-late but were highly significant ($P<0.001$) at ART-Farm where lines 3 and 5 had negative effects (Table 4.7). The combined analysis was therefore not conducted as only one site was significant.

**Table 4.7:** General combining ability (GCA) effects of seven Quality Protein Maize inbred lines for endosperm modification score.

| | Line code | RARS-Late† | ART |
|---|---|---|---|
| 1 | SC10 | -0.14 | 0.06 |
| 2 | SC1 | -0.02 | 0.08 |
| 3 | SC5 | -0.12 | -0.26 |
| 4 | SC2 | 0.12 | 0.06 |
| 5 | SC4 | -0.14 | -0.10 |
| 6 | WWO1408 | 0.28 | 0.14 |
| 7 | CML511 | 0.02 | 0.02 |
| *p* | | **ns** | *** |
| SE | | 0.06 | 0.05 |

***, and ns, Indicates significance at 0.001 and >0.05 probability levels, respectively.

† RARS-late, Rattray Arnold Research Station (late planted); ART, Agricultural Research Trust;

### 4.3 Specific combining ability (SCA) effects

The SCA mean squares for GYG, were significant (P<0.05) at two sites, CIMMYT-Harare and RARS-late (Table 4.8). The combined analysis also revealed significant mean squares for both the SCA and SCA x environment interaction (Table 4.4). The range of the SCA effects was from -1.01 to 0.65 for crosses between line 1 and line 4 (1 x 4) and 1 x 3 respectively. The cross 1 x 4 had the highest negative SCA effects that were significant (-1.01).

**Table 4.8**: The specific combining ability (SCA), mean yield and genetic similarity (GS) of the quality protein maize inbred lines that make up the F1 hybrids across the environments

| ‡ | Parameter Estimate | | Mean Yield tons ha$^{-1}$ | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Hybrid** | **SCA** | **Genetic Similarity** | **RARS† Early** | **KRC** | **CIMMYT** | **RARS Late** | **ART** | **Across** |
| 1x2 | 0.22 | 0.23 | 6.10 | 9.2 | 11.7 | 9.8 | 10.9 | 10.1 |
| 1x3 | 0.65 | 0.25 | 9.03 | 11.3 | 11.3 | 6.7 | 9.6 | 9.8 |
| 1x4 | -1.01* | 0.27 | 5.87 | 7.5 | 8.3 | 7.8 | 7.6 | 8.0 |
| 1x5 | -0.02 | 0.23 | 8.0 | 9.9 | 10.0 | 7.9 | 7.4 | 9.0 |
| 1x6 | 0.01 | 0.26 | 9.6 | 10.5 | 8.0 | 7.3 | 8.8 | 8.5 |
| 1x7 | 0.14 | 0.24 | 9.11 | 6.9 | 8.1 | 9.2 | 8.7 | 8.3 |
| 2x3 | -0.10 | 0.44 | 9.42 | 10.9 | 10.5 | 8.9 | 9.3 | 9.8 |
| 2x4 | 0.00 | 0.37 | 7.46 | 10.8 | 10.6 | 10.6 | 7.4 | 10.1 |
| 2x5 | 0.30 | 0.36 | 7.61 | 10.3 | 10.7 | 10.6 | 10.4 | 10.3 |
| 2x6 | 0.25 | 0.26 | 8.66 | 11.7 | 10.3 | 8.2 | 10.7 | 9.9 |
| 2x7 | -0.67 | 0.36 | 6.62 | 11.8 | 5.9 | 10.5 | 7.8 | 9.1 |
| 3x4 | 0.17 | 0.37 | 9.02 | 8.1 | 10.8 | 8.8 | 9.3 | 9.2 |
| 3x5 | -0.69 | 0.35 | 11.16 | 8.4 | 8.6 | 6.7 | 7.9 | 7.7 |
| 3x6 | 0.16 | 0.23 | 8.46 | 11.1 | 8.5 | 9.5 | 9.7 | 9.3 |
| 3x7 | -0.19 | 0.38 | 7.62 | 7.5 | 9.3 | 10.4 | 8.5 | 9.0 |
| 4x5 | 0.19 | 0.36 | 8.73 | 8.4 | 10.0 | 8.8 | 8.8 | 9.0 |
| 4x6 | 0.02 | 0.24 | 10.05 | 10.5 | 7.8 | 8.3 | 7.3 | 8.3 |
| 4x7 | 0.64 | .29 | 11.21 | 9.7 | 8.9 | 7.2 | 7.9 | 8.3 |
| 5x6 | -0.16 | 0.33 | 7.02 | 10.6 | 10.0 | 8.1 | 8.6 | 9.2 |
| 5x7 | 0.37 | 0.42 | 9.19 | 9.5 | 7.9 | 9.4 | 8.6 | 8.8 |
| 6x7 | -0.28 | 0.31 | 6.67 | 8.5 | 9.3 | 9.2 | 8.0 | 8.4 |
| *p* | ** | | ** | ** | ** | ** | ** | * |
| SE | 0.49 | | 0.87 | 1.32 | 1.11 | 0.90 | 1.04 | 1.37 |

‡Cross between indicated pair of Quality Protein inbred lines

* and **, represents significance probability levels of 0.05 and 0.01, respectively.

† RARS-early, Rattray Arnold Research Station (early planted); KRC, Kadoma Research Centre; CIMMYT, International Maize and Wheat Improvement Centre; RARS-late, Rattray Arnold Research Station (late planted); ART, Agricultural Research Trust;

Across the five sites, the SCA effects for, AD, RL, SL, HC and ASI were not significant.

The interaction between GCA and environment and SCA and environment for GYG, AD, HC and PHT were highly significant (P<0.001). The contribution of the SCA sums of square towards the total sums of square for GYG was lower than that of the GCA (Table 4.9).

**Table 4.9**: Relative importance (%) of GCA and SCA in their contribution to entry sums of square for grain yield across five sites

| | GYG† | AD | PHT | EHT | ER | EPO | PS |
|---|---|---|---|---|---|---|---|
| **GCA %** | 0.55*** | 0.83*** | 0.54** | 0.20 | 0.33 | 0.25 | 0.89* |
| SCA % | 0.45 | 0.17 | 0.46 | 0.80*** | 0.67* | 0.75*** | 0.11 |

*, **, *** Indicates traits with significance combining ability mean squares at 0.05, 0.01, and 0.001 probability levels, respectively.

† GCA, General Combining Ability; GYG, grain yield; AD, anthesis date; PHT, plant height; EHT, ear height; ER , ear rot; EPO , ear position; PS. *Puccinia* rust.

Specific combining ability mean squares were lower than GCA mean squares for GYG, AD, PHT and PS. For ER and EPO, SCA effects were more than GCA for EHT, ER and EPO (Table 4.9).

## 4.4 Relationship between SCA effects of the crosses and molecular genetic distances between the QPM inbred lines

Eleven QPM inbred lines were fingerprinted together with other 33 normal endosperm lines, with a total of 62 SSR markers that are distributed throughout the maize genome. Data below in Table 4.10 summarise statistics per marker. The proportion of missing data for each inbred varied from 0 to 0.24 with an overall value of 0.065, which is acceptable enough to include all individuals and markers in the

study. Heterozygosity of the inbreds ranged from 0 – 0.23 which is within the expected range of 8 % or below for maize inbred lines (Warbuton *et al*., 2002).

**Table 4.10**: Summary statistics per marker

| Marker | SampleSize | No. of obs. | AlleleNo | GeneDiversity | Heterozygosity | PIC† |
|---|---|---|---|---|---|---|
| **umc1196** | 44 | 43 | 5 | 0.72 | 0.19 | 0.66 |
| **nc133** | 44 | 42 | 5 | 0.35 | 0.10 | 0.32 |
| **phi064** | 44 | 39 | 6 | 0.75 | 0.00 | 0.71 |
| **umc1279** | 44 | 42 | 4 | 0.37 | 0.10 | 0.35 |
| **phi062** | 44 | 40 | 2 | 0.29 | 0.00 | 0.25 |
| **phi100175** | 44 | 42 | 3 | 0.36 | 0.00 | 0.33 |
| **umc1304** | 44 | 38 | 2 | 0.23 | 0.00 | 0.20 |
| **phi374118** | 44 | 44 | 4 | 0.67 | 0.05 | 0.61 |
| **phi089** | 44 | 36 | 2 | 0.40 | 0.00 | 0.32 |
| **umc1109** | 44 | 44 | 2 | 0.25 | 0.02 | 0.22 |
| **phi453121** | 44 | 43 | 4 | 0.70 | 0.00 | 0.64 |
| **umc1153** | 44 | 42 | 4 | 0.62 | 0.00 | 0.57 |
| **umc1143** | 44 | 42 | 3 | 0.62 | 0.05 | 0.55 |
| **phi328175** | 44 | 38 | 4 | 0.62 | 0.05 | 0.56 |
| **phi233376** | 44 | 42 | 6 | 0.74 | 0.17 | 0.70 |
| **phi299852** | 44 | 44 | 7 | 0.77 | 0.09 | 0.74 |
| **phi121** | 44 | 43 | 1 | 0.00 | 0.00 | 0.00 |
| **umc1545** | 44 | 44 | 4 | 0.38 | 0.23 | 0.35 |
| **phi423796** | 44 | 43 | 4 | 0.46 | 0.09 | 0.40 |
| **phi031** | 44 | 41 | 4 | 0.49 | 0.00 | 0.46 |
| **phi331888** | 44 | 44 | 4 | 0.54 | 0.00 | 0.44 |
| **umc1061** | 44 | 44 | 5 | 0.57 | 0.00 | 0.53 |
| **phi339017** | 44 | 42 | 4 | 0.43 | 0.00 | 0.39 |
| **phi109642** | 44 | 39 | 3 | 0.65 | 0.05 | 0.58 |
| **phi065** | 44 | 40 | 4 | 0.52 | 0.03 | 0.46 |
| **phi050** | 44 | 40 | 3 | 0.46 | 0.00 | 0.41 |
| **phi032** | 44 | 43 | 3 | 0.51 | 0.00 | 0.40 |
| **phi069** | 44 | 44 | 6 | 0.75 | 0.00 | 0.72 |
| **phi083** | 44 | 42 | 5 | 0.64 | 0.05 | 0.60 |
| **phi448880** | 44 | 42 | 4 | 0.58 | 0.02 | 0.54 |
| **phi108411** | 44 | 38 | 2 | 0.15 | 0.00 | 0.13 |
| **phi213984** | 44 | 42 | 3 | 0.09 | 0.00 | 0.09 |
| **umc1277** | 44 | 35 | 3 | 0.54 | 0.00 | 0.48 |
| **umc1136** | 44 | 42 | 6 | 0.66 | 0.07 | 0.60 |
| **phi452693** | 44 | 44 | 3 | 0.51 | 0.00 | 0.44 |
| **phi102228** | 44 | 37 | 3 | 0.10 | 0.00 | 0.10 |
| **phi015** | 44 | 41 | 3 | 0.39 | 0.15 | 0.34 |
| **phi079** | 44 | 43 | 4 | 0.47 | 0.00 | 0.43 |
| **phi96342** | 44 | 38 | 5 | 0.24 | 0.08 | 0.23 |
| **phi308707** | 44 | 44 | 5 | 0.65 | 0.14 | 0.59 |
| **umc1152** | 44 | 43 | 7 | 0.75 | 0.12 | 0.71 |
| **nc130** | 44 | 41 | 4 | 0.52 | 0.07 | 0.42 |
| **phi002** | 44 | 44 | 2 | 0.13 | 0.00 | 0.12 |
| **phi006** | 44 | 43 | 2 | 0.21 | 0.00 | 0.18 |
| **phi014** | 44 | 43 | 4 | 0.53 | 0.00 | 0.43 |
| **phi024** | 44 | 40 | 4 | 0.65 | 0.00 | 0.59 |
| **phi059** | 44 | 35 | 6 | 0.76 | 0.11 | 0.72 |
| **phi076** | 44 | 28 | 2 | 0.13 | 0.00 | 0.12 |
| **phi084** | 44 | 42 | 4 | 0.52 | 0.00 | 0.46 |
| **phi093** | 44 | 43 | 3 | 0.65 | 0.00 | 0.58 |
| **phi029** | 44 | 39 | 5 | 0.67 | 0.05 | 0.62 |
| **zcaa391** | 44 | 43 | 10 | 0.88 | 0.14 | 0.86 |
| **phi011** | 44 | 36 | 5 | 0.59 | 0.11 | 0.51 |
| **phi034** | 44 | 44 | 6 | 0.77 | 0.23 | 0.73 |
| **phi046** | 44 | 42 | 2 | 0.48 | 0.00 | 0.37 |
| **phi056** | 44 | 43 | 8 | 0.74 | 0.09 | 0.71 |
| **phi123** | 44 | 42 | 2 | 0.46 | 0.00 | 0.35 |
| **phi96100** | 44 | 40 | 5 | 0.75 | 0.00 | 0.70 |
| **phi104127** | 44 | 42 | 3 | 0.53 | 0.00 | 0.43 |
| **phi227562** | 44 | 42 | 6 | 0.62 | 0.00 | 0.58 |
| **umc1399** | 44 | 42 | 4 | 0.65 | 0.07 | 0.60 |
| **phi111111** | 44 | 40 | 5 | 0.60 | 0.03 | 0.56 |
| **Mean** | 44 | 41 | 4 | 0.51 | 0.04 | 0.46 |

**†PIC, polymorphism information content**

Jaccard's genetic similarity coefficients were calculated for all pairs of lines in the study and can be found in Table 4.11. The genetic similarities were very variable, and ranged from the most genetically similar pair of lines (SC6 and SC8) with a similarity of 0.539 to the most distant pair (SC7 and SC10) with a similarity of only 0.159.

An Unpaired Group Method using Arithmetic Averages (UPGMA) dendrogram was generated (without bootstrapping) using these similarity coefficients (Figure 4.2). The dendogram exhibited five fairly rough clusters at a cut-off similarity of 0.35, (cluster 1 = SC9 and SC7; cluster 2 = SC5, SC1 and SC3; cluster 3 = SC8, SC6, CML175 and SC2; cluster 4 = SC4 and cluster 5 = SC10. Each cluster contains much within-cluster variation, and a relatively short distance between clusters. In addition, a single outlier (lines not belonging to any cluster) was observed (SC10). However, this outlier was not much more distant to the clusters than were the clusters to each other.

**Table 4.11**: **Jaccard's genetic similarity coefficients**

| | **Parents** | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Parents** | **SC9** | **SC8** | **CML175** | **SC7** | **SC4** | **SC6** | **SC5** | **SC10** | **SC1** | **SC3** | **SC2** |
| **SC9** | 1.00 | | | | | | | | | | |
| **SC8** | 0.26 | 1.00 | | | | | | | | | |
| **CML175** | 0.36 | 0.41 | 1.00 | | | | | | | | |
| **SC7** | 0.40 | 0.25 | 0.29 | 1.00 | | | | | | | |
| **SC4** | 0.32 | 0.36 | 0.36 | 0.29 | 1.00 | | | | | | |
| **SC6** | 0.38 | 0.54 | 0.34 | 0.38 | 0.30 | 1.00 | | | | | |
| **SC5** | **0**.37 | 0.20 | 0.25 | 0.27 | 0.35 | 0.25 | 1.00 | | | | |
| **SC10** | 0.30 | 0.34 | 0.33 | 0.16 | 0.23 | 0.24 | 0.25 | 1.00 | | | |
| **SC1** | 0.43 | 0.26 | 0.29 | 0.33 | 0.36 | 0.36 | 0.44 | 0.23 | 1.00 | | |
| **SC3** | 0.30 | 0.24 | 0.32 | 0.29 | 0.25 | 0.33 | 0.39 | 0.23 | 0.40 | 1.00 | |
| **SC2** | 0.32 | 0.42 | 0.48 | 0.28 | 0.36 | 0.37 | 0.37 | 0.27 | 0.37 | 0.36 | 1.00 |

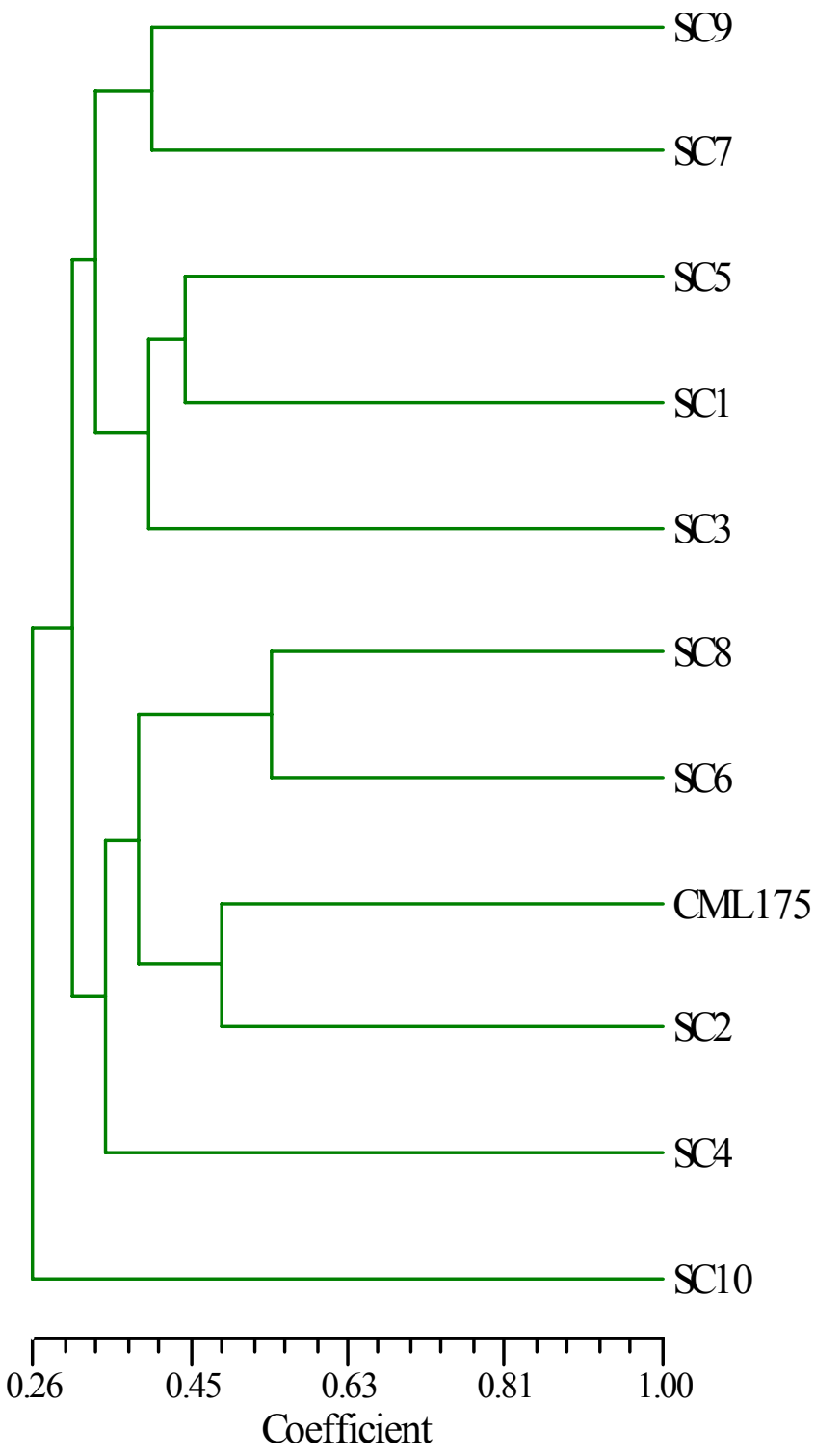


**Figure 4.2 :** An Unpaired Group Method using Arithmetic Averages (UPGMA) dendrogram (without bootstrapping) for the Jaccard's genetic similarity coefficients

## 4.5 The correlation between SCA effects, genetic distance and yield parameters

The correlation between the combined SCA and the genetic similarity (GS) was moderate and not significant (0.34), while that of GS and individual site SCA was positive and not significant for all other sites but RARS-late where it was negative and not significant (Table 4.12; Figure 4.3).

Genetic similarity and yield had a low and non significant correlation for individual sites, with KRC and ART having negative values. The correlations between GS and yield *per se* ranged from -0.17 for RARS-late to 0.60 for RARS-early. Table 4.12 also shows the correlation between GS and the combined yield which was equally low and not significant ($r$=0.25).

The correlation between the combined SCA and yield of individual sites was positive and high (greater than 0.72) although it revealed no significant differences (Table 4.12). The correlation between the combined SCA and combined yield was significant ($P<0.01$) and high (0.78).

High positive correlation between individual site SCA and individual site mean yield were observed at all the five sites (Table 4.12).

The regression of SCA and GS (Figure 4.3) was not significant and was low ($r^2$ = 0.11).

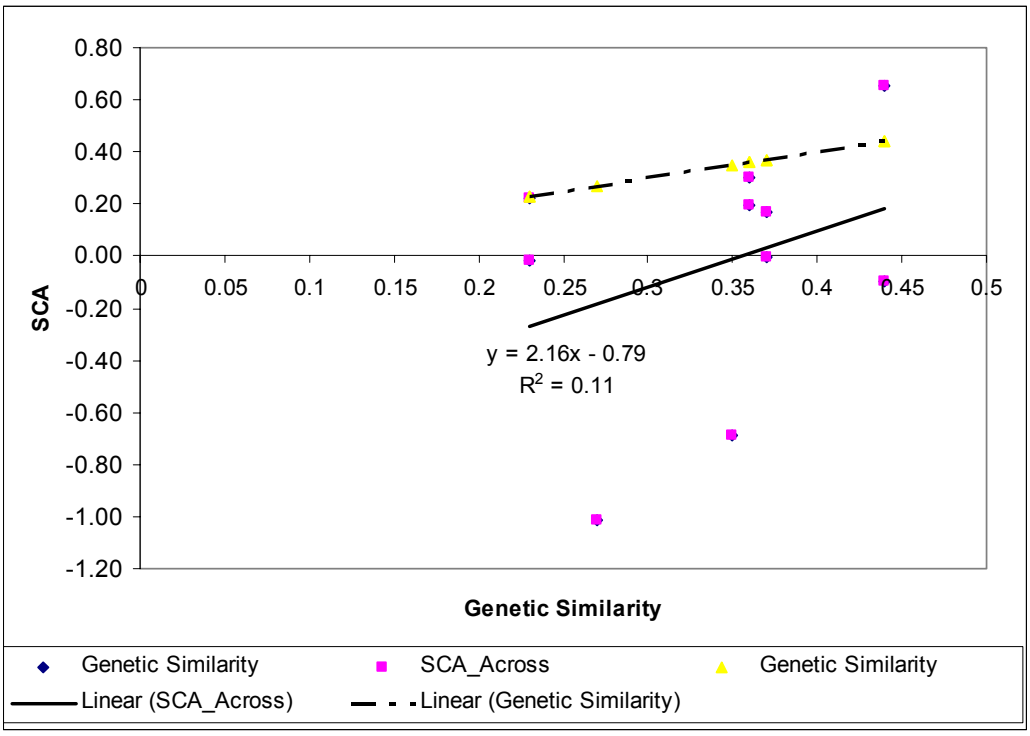


**Figure 4.3**: Regression of genetic similarity (GS) on specific combining ability (SCA)

**Table 4.12**: Correlations coefficients between specific combining ability (SCA) and yield, genetic similarity (GS) and yield, and GS and SCA

| | SCA & yield | GS and Yield | GS & SCA |
|---|---|---|---|
| **Across** | 0.78** | 0.25 | 0.34 |
| **RARS-Early** | 0.86 | 0.60 | 0.51 |
| **KRC** | 0.72 | 0.47 | 0.39 |
| **CIMMYT** | 0.79 | 0.22 | 0.10 |
| **RARS-Late** | 0.74 | -0.07 | -0.36 |
| **ART** | 0.77 | 0.15 | 0.05 |
| p | ns | ns | ns |

**, and ns, Indicates significance at 0.01 and >0.05 probability levels, respectively.

†, RARS-early, Rattray Arnold Research Station (early planted); KRC, Kadoma Research Centre; CIMMYT, International Maize and Wheat Improvement Centre; RARS-late, Rattray Arnold Research Station (late planted); ART, Agricultural Research Trust;

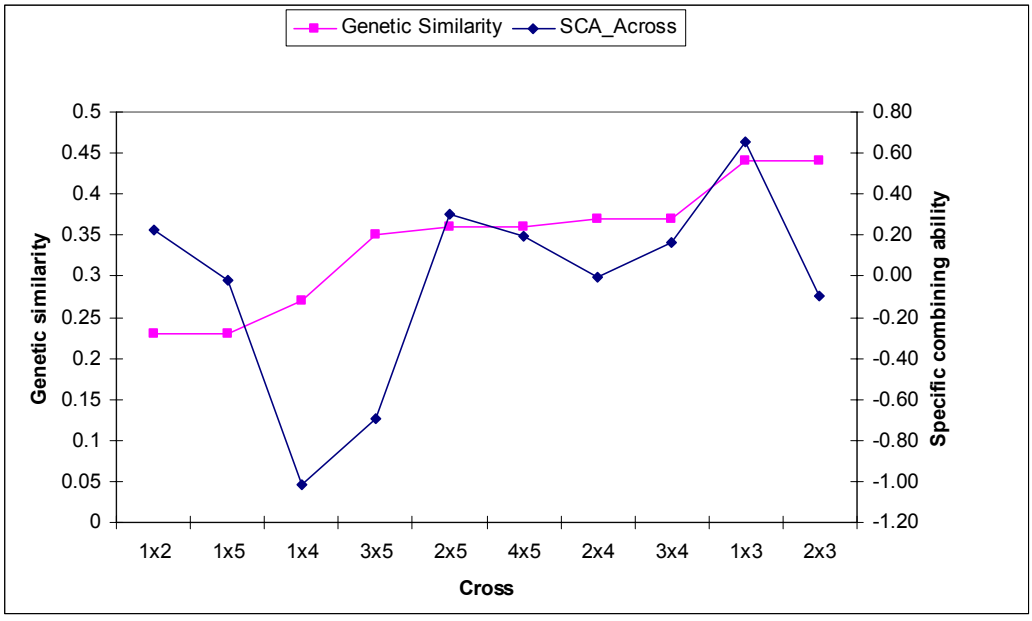


**Figure 4.4**: Relationship between genetic similarity (GS) and specific combining ability (SCA)

The lowest of the hybrids in terms of yield, was a product of SC4 and SC5 with one of the highest GS of 0.35, while the highest yielding two hybrids are products of SC1 and SC10, and SC4 and SC1 both with a low GS of 0.23 (Figure 4.4). The trend lines for the SCA and GD were variable with no apparent trend.

# Chapter 5

# DISCUSSION

## 5.1 Performance *Per Se* of 11 Inbreds and the F1 Hybrids for QPM Trait

Although the whole set of QPM inbred lines used were regarded as QPM germplasm on the basis of their pedigrees, the quality data collected to provide a general idea on the quality of lines from the single replication indicated that quality levels of these lines fell short of the acceptable level. Most of the experimental QPM inbred lines fell short of desired quality in terms of tryptophan and nitrogen content indicating that further improvement is required. However, despite the observation that inbred lines SC10 and the later rejected SC7 had the highest protein content, the quality index was low due to low tryptophan levels. Irrespective of that, there were a few lines that could be regarded as true QPM which would find utility in the QPM hybrid development. Inbred lines, WWO1408, CML159 and CML175 released as QPM lines, exhibited high level of quality compared to the experimental lines, although experimental lines SC5 and SC9 were of comparatively good quality with quality index values above that described as being QPM.

Comparison of the quality data from the parental lines and that of the F1 hybrids revealed that the crosses between QPM inbred lines with good quality, SC5 and WW1408 and between inbred lines WW1408 and SC5, had high tryptophan (0.073 % and 0.066 %) and quality index (0.81 and 0.74), respectively. In Contrast, hybrids made up of inbred lines with low quality such as SC10 X SC1 and SC2 X SC4 had low quality index values (0.45 in both cases of this example). The QPM hybrids with high tryptophan and protein levels, were products of QPM inbred lines that in turn had good quality values. This indicates that additive gene action is playing a major role in conferring high protein quality alleles in QPM. It therefore suggests that the quality of the hybrids can be predicted if the quality of the inbred lines used is known.

Additive gene action has been reported to be more important than non-additive effects such as dominance for percent protein in grain, and percent tryptophan in protein (Singh *et* al., 1977; Motto *et* al. 1978; Wessel-Beaveret *et al*., 1985). The additive gene effects for lysine and tryptophan have been seen to be important by Sreeramulu and Bauman (1970) and Bjarnason *et al*. (1976). In the study by Pixley and Bjarnason (1993), most of the genetic variability for tryptophan concentration in protein among QPM hybrids was additive and interactions of genotype by location effects (G x E) were small. Of the four diallel studies conducted by Pixley and Bjarnason (1993), two of them had significant GCA effects for tryptophan concentration in grain and none exhibited significant differences for SCA effects. The interaction for genotype by location was significant for all the diallel studies.

This importance of additive gene action was further illustrated in terms of heritability by various studies which ranged from 17 % to 72 % (Dudley *et al*.,1971); 7 % to 47

%, (Dudley *et al*., 1975) and 76 % (Wessel-Beaveret *et al*., 1985). The heritability that was reported for percent tryptophan in protein was 27 % (Motto, 1979). The heritability estimates, particularly narrow sense heritability, suggest that additive gene effects are more important than non-additive gene effects, which is in congruence with studies in which significant GCA effects and their relative magnitudes were associated with additive gene action being more important than non-additive effects as estimated by SCA (Betran *et al*., 2003; Long *et al*., 2004, Makumbi, 2005). This agrees with the observations of the quality of the lines against the quality of the hybrids made in various combinations.

## 5.2 Yield Performance *per se* of Hybrids

The results for the other agronomic traits were variable from site to site and from trait to trait. The results revealed that, the best entries were commercial checks in terms of grain yield, although some experimental QPM, F1 hybrids were statistically at par with some of the commercial checks. The commercial check hybrid SC633 was the highest at RARS-early, KRC and ART-farm. In two diallel trials conducted by Bhatnagar *et al*. (2004), commercial hybrids out yielded the QPM single crosses while Pixley and Bjarnason, 2002 reported the availability of some QPM products in different parts of the world that are as good or even better than commercial products.

Some of the hybrids consistently yielded highly across all the environments which is indicative of a possibility of producing stable QPM hybrid that are comparable with the hybrid obtained from crossing lines SC1 x SC4.

As there were significant differences among the environments for all the traits including endosperm modification, it implies that selection for these traits at any of the five locations was not as good as at any other site as the environments were different. The hybrid performance in terms of grain yield was inconsistent across the environments since entry x environment interaction was highly significant.

Since the mean square error for most of the traits including yield varied significantly, it suggests that the germplasm used was variable enough to continue with the genetic studies that were being pursued, as a result, the analysis for combining ability was therefore performed, (Hallauer and Miranda, 1988).

## 5.3 Gene Action for Yield and QPM Traits

### 5.3.1 Endosperm Modification

The analysis of the endosperm modification had mixed results from the sites that were evaluated. As a result, no clear cut conclusions could be made on the type of gene action involved basing on combined data. The significant endosperm modification score at the sites where it was recorded shows that there is enough variability within the germplasm used. The environmental effects were greater as indicated by different results from both environments. As Lopes and Larkins (1996) confirmed findings by Bauman (1975) that the frequency of vitreous types differs from one population to the other, as they disputed previous reports that the vitreouseness is influenced by the environment. Pixley and Bjarnason (2002) reported that endosperm modification was not significant among the single cross and three way hybrids. This is not surprising as the whole effort in the QPM breeding program is to develop products carrying high quality but with well modified kernel.

Two or few genes could be controlling vitreouseness even though multiple genes have been associated with vitreouseness. Bauman (1975), and Lopes and Larkins (1995) observed that 1/16 of the progenies from selfing the F2 had a phenotype similar to that of the parents, which confirms that two genes were involved, hence non additive gene action.

The magnitude of the GCA effects when compared to the SCA effects as observed from the ANOVA tables further suggests that additive gene action is more important than non-additive in conferring genes conferring vitrouseness or modification of the endosperm.

The inheritance of the gene controlling endosperm vitrouseness have been said to be complex (Bjarnason and Vasal, 1992; Larkins, 1995; Pixley and Bjarnason, 2002). The results from Art-Farm, the only site that was significant ($P<0.05$), suggest that additive gene action is more important than non-additive in conferring high modification of the endosperm. This implies that selection of lines characterized as good in terms of vitrouseness will consistently display the desired endosperm and pass this trait on to their offspring, the phenotype of which can be predicted if the parent performance is known.

### 5.3.2 Combining Ability Effects of Lines for Yield, other Agronomic and QPM Traits

The GCA effects for yield were found to be significant suggesting the importance of additive gene action. It can therefore be inferred that additive gene effects played an important role in conferring high yielding alleles. Inbred line SC1 had the highest and

positive GCA effects while CML511 had the highest negative effects across all the environments. The GCA effects of inbred lines belonging to the "SC" heterotic group had positive values while those from the "P" had negative values. Positive GCA suggests that the lines contributed favourable alleles for yield. This is in agreement with several other studies elsewhere involving normal endosperm maize, including Betran *et al*. (2003) who found GCA effects to be important even in drought environments while under low nitrogen, dominance effects were important. These trials were however conducted under optimum conditions at all the five sites, and these results agree with those of Pixley and Bjarnason (1993) who found significant GCA effects for grain yield of QPM hybrids in three of the four trials they conducted.

The QPM inbred line SC1 which had the highest effects, appeared more frequently among the high yielding hybrids. This is suggestive that it can be chosen as the tester for the QPM breeding program, if the quality was acceptable. However, the quality of the line was low. Where GCA effects are more important, the inbred lines involved need to be evaluated for yield potential. The inbred lines showing high yield potential under various environments, are possible candidates for a good performing hybrid in terms of yield. With significant GCA effects and not significant SCA effects, it can be inferred that additive gene effects are more important than non-additive effects hence, selection based on the parent performance is effective. The implication of this is that selection of lines characterized as good for yield will give rise to products that exhibit that trait or the offspring can be predicted if the parent performance is known. With the better understanding of the GCA and SCA effects of these inbreds and hybrids, respectively, for agronomic traits such as yield, maize breeders in the region would possibly be more efficient in generating QPM hybrids and even open-pollinated

varieties exhibiting acceptable grain and agronomic characteristics (Long *et al*., 2004). Perhaps, the small to large GxE effects observed and reported by Motto (1979) suggests that performance of QPM hybrids might not be stable, and that hybrid ranking might change in each environment.

### 5.3.3 Specific Combining Ability

Determination of the significant effects of SCA is of paramount importance in a maize breeding program where the hybrid is the ultimate product. Significant SCA effects denote the importance of non-additive effects such as dominance. The SCA effects for yield were significant ($P<0.05$) in the combined analysis and at only two sites of the five sites. The cross between line 1 and line 4 which had the highest negative and significant SCA effects (undesirable as it signifies low yield potential) is a product of lines SC10 and SC4, both belonging to the heterotic group "P", hence the lowest yielding hybrid. Conversely, the cross between line 1 line 3 had the highest positive SCA effects and is a product of SC10 and SC5 which belong to heterotic groups "P" and "S" respectively. It therefore can be used to measure the magnitude of relationship of the lines (Hallauer and Miranda, 1988). The crosses with lines belonging to the same heterotic group had negative SCA effects while those that came from different heterotic groups had positive effects, an observation also made by Betran *et al*. (2003) with normal endosperm maize.

Contrasting reports have been made about the significance of SCA effects in various studies. Of the four trials conducted by Pixley and Bjarnason (1993), significant GCA effects for grain yield were observed in three trials and SCA effects were only significant in one trial. In an evaluation of seven tropical white maize populations in a

diallel mating design by Vasal *et al*. (1992), the SCA effects for grain yield of normal endosperm maize was not significant. Contrary to these reports, Betran *et al*. (2003) observed and reported significant SCA effects but there were no significant SCA x environment interaction effects for grain yield of the normal endosperm maize while Makumbi (2005) reported no significant SCA effects across well watered sites, across drought sites and across low nitrogen sites, but the effects were significant in the combined analysis. Bhatnagar *et al*., 2004 who observed significant SCA and non significant GCA, attributed such differences to type of germplasm used in terms of whether it was tropical, subtropical or temperate. The germplasm used in this study were basically subtropical.

### 5.3.4 Relative Importance of GCA and SCA

Although both the GCA and SCA effects were significant in the combined analysis, the SCA effects were relatively of less importance as their mean squares were less than those of GCA effects. This would imply that, additive effects were more important in contributing alleles that enhance yield of QPM products if such magnitudes equate to a ratio between the GCA variance and SCA variance. Irrespective of that, it is still important for certain crosses that are unique (Hallauer and Miranda, 1988).

The observation that the interaction between the GCA and environment, and SCA and the environment were highly significant, suggests that the GCA and SCA variance varied between environments. The magnitude of the GCA mean square was greater than that of the interaction. It therefore means that, the interaction effects were of less importance as compared to the GCA effects. This is in harmony with an observation

that the small to large G x E effects, as reported by Motto (1979), suggests that performance of QPM hybrids might not be stable, and that hybrid ranking might change in each environment. This is also in line with conclusions by Bhatnagar *et al*. (2004) that the QPM germplasm tend to exhibit different characteristics in different environments depending on the origin of the materials as non-additive gene action was reported to be important in improved tropical white endosperm populations. Additive gene action has been associated with grain yield in CIMMYT lowland tropical late, subtropical QPM germplasm and temperate intermediate maturing germplasm (Bhatnagar *et al*., 2004).

## 5.4 Estimation of genetic distance using Simple Sequence Repeat markers

### 5.4.1 Cluster analysis

The cluster analysis revealed that five distinct groupings existed within the lines and this was confirmed by the pedigree data. Several observations that pedigree information agrees with molecular markers have been reported by Melchinger *et al.* (1990); Dudley *et al.* (1991); Boppenmaiaer, Melchinger, Seitz, Geiger and Herrmann (1993); Lu and Bernardo, (2001); Warburton *et al*. (2002), Betran *et al*. (2003) and Bhatnagar *et al*. (2004), using both molecular tools and agronomic data analysed by tools such as biplots or principle components.

The range of the genetic similarity obtained in this study were within the ranges reported by Senior *et al*. (1998) in a set of temperate inbred lines (0.17 to 0.92 with an average of 0.59). In tropical and subtropical maize, Xia *et al*. (2005) reported a range

of genetic similarities of 0.16 to 0.88 with an average of 0.64, and Reif *et al.* (2003) reported a range of 0.50 to 0.58 with an average of 0.60 for 83 SSR markers.

However, the clusters had variation within each cluster indicating that the lines within each cluster are only slightly more similar than lines between different clusters, and that there is considerable variation in this germplasm set as a whole. This is not surprising as the pedigree of a certain heterotic group (the cluster) could be having other lines introgressed as the source of the *opaque*-2 which does not fit precisely into the local heterotic groups. The first cluster (SC9 and SC7) has lines that basically have the heterotic group "W" germplasm while the second cluster (SC5, SC1 and SC3) is made up of the "SC" heterotic grouping. Cluster 3 with SC8, SC6, CML175 and SC2 has one thing in common in that, besides CML175, the other lines originate from South African germplasm, which is basically the M37W, Portchefstroom Pearl and the Pride of Saline (Rupende, personal communication). The *opaque-2* donor for all other lines other than SC8, are released CIMMYT maize lines (CML), which, Bantte and Prasanna (2003) found to be distinctively different from the other germplasm used in their study where other sources of germplasm was used, although Warburton (2002) found conformity with pedigree information of the CIMMYT lines.

### 5.4.2 Relationship of genetic distance and *per se* hybrid performance

The hybrids that were made up of lines coming from divergent sources, as indicated by the molecular marker may agree with the yield data in determining the relative relationship of the donor lines to the parents of the hybrid. If the hybrid is made up of lines that are not significantly distant apart or are intermediate, the level of agreement between marker and yield information may be stronger. This could be the case in this

study where QPM programs are relying on the same source of the *opaque-2* gene. The correlation of molecular marker genetic distance (which is the inverse of genetic similarity), with specific combining ability was negative and low suggesting that the predictive value was not strong.

The correlation between GS and combined yield was low. Melchinger *et al*. (1990) found a positive but small correlation, Boppenmaier *et al*. (1993) found it to be significantly positive in the flint x flint crosses and not in flint x dent nor dent x dent while Betran *et al*. (2003), found a positive correlation. In 2002, Gutierrez *et al*. (2002) also concluded that GD estimates based on markers were of no value in predicting the performance *per se* in cotton where they found that the direction of the correlations often changed among crosses and environments.

As one of the lowest yielding hybrid, the F1 of the cross between inbred lines 3 and 5 from inbred lines SC4 and SC5 with one of the highest GS, in other words, the least distant apart, while the highest yielding hybrid (line 1 x line 2) is a product of SC10 and SC1 with a low GS, loosely confirms the hypothesis that the more distant apart or the least similar the lines are, the higher the heterosis. This is not strong as there are some lines that had a relatively high GS but with higher heterosis (line 1 x and line 3) and this was also observed by Dudley *et al.* (1991). Melchinger *et al.* (1990) found similar results where a few entries of molecular data confirmed with pedigree data, with a few deviations occurring while Boppenmaier *et al*. (1993) found a positive correlation between lines from the same heterotic group (flint x flint) and not from different texture lines (flint x dent) nor dent x dent, another same texture.

## 5.5 Relationship of SCA Effects and Genetic Distance, and SCA Effects and *per se* Hybrid Performance

Since the correlation between the combined SCA and the genetic similarity (GS), were positive and moderate in line with reports by Melchinger *et al*. (1990) and Betran *et al*. (2003), the implication is that there was an intermediate relationship. The low values are in agreement with Dudley *et al.* (1991) who used MRD, where the correlation of MRD with SCA for yield was very low, although it was significant. Reif *et al*. (2003) found a high, positive and significant ($P<0.01$) correlation between PMPH and $MRD^2$ but a negative, significant ($P<0.05$) and low correlation coefficients with SD and PHT.

Distortion of the correlation can be attributed to inclusion of all crosses including those with very high and very low similarity coefficients which have been reported by Betran *et al*. (2003) that GD was correlated with the performance of the F1 of lines coming from the same heterotic group. Reif *et al*. (2003), attributed reduced PMPH that is common with lines with wide genetic background, to lack of co-adaptability between allelic and non-allelic combination of genes from parent genomes that give rise to less or negative dominance and negative epistatic effects respectively. In this study, the lines used were well adapted which could explain the reasons why there was no optimum relationship between GS and SCA, and GS and yield.

The regression analysis of GS on SCA also revealed lack of relationship between these two attributes. However, the trend lines seemed to converge as the GS increased. This supports earlier findings that the molecular tool could be more

important in determining closely related lines that in general, reveal negative heterosis. As such, it is useful in eliminating or avoiding such crosses.

Use of marker genotype information in this case, cannot be used reliably to predict relative hybrid performance, unless extremes have been excluded to include those with GD smaller than a certain threshold for that particular germplasm. Boppenmaier *et al*. (1993) confirmed earlier observations that the predictive power of RFLP data is restricted to crosses between inbred lines from the same heterotic group which could not be extended to the lines coming from divergent heterotic groups.

A high positive correlation between the SCA effects and yield of both individual sites and across the environments indicates that SCA can be used to predict the performance of the hybrid. A similar result was obtained by Betran *et al*. (2003) with normal endosperm maize where it was found that it is more reliable than using various heterosis values, such as the PMPH.

# Chapter 6

## CONCLUSIONS AND RECOMMENDATIONS

### 6.1 Conclusions

Although both the GCA effects and SCA effects for yield were significant, the GCA effects were relatively more important than the SCA effects, it can therefore be concluded that the GCA effects were slightly more important in conferring high yielding alleles than the SCA effects. As such, additive gene action is associated with the conferring of high yield alleles in these QPM inbred lines.

This implied that additive gene effects were more important than non-additive effects in the inheritance of yield and endosperm modification in the southern African and CIMMYT QPM inbred lines under study.

The hypothesis that there is a significant and positive association between SCA effects of the hybrids for grain yield and the molecular genetic distances among the QPM inbred lines under study is rejected. This is due to the observation that there was no clear cut relationship particularly beyond a certain stage of GS. At higher level of GS, the relationship is strong.

There was no apparent relationship observed between the SCA and the genetic distance, and GD and yield. Use of marker genotype information, therefore, could not be used reliably to predict relative hybrid performance. The high level of variation observed to exist among these lines will aid in future selection and will allow many potentially productive hybrids to be made. However, there is little partitioning of this

variation into homogenous groups, such as would have formed good *a priori* (selection method based on the *per se* parent performance) heterotic groupings.

The SSR markers were able to classify the QPM inbred lines into the heterotic groups known according to the pedigree information of the recurrent normal endosperm parent.

## 6.2 Recommendations

Since the general combining ability for yield and endosperm modification of the QPM lines were more important, it is important that the lines making up a hybrid should have desirable alleles for high yield in order for the resultant hybrid to carry that desirable trait and *per se* desirable characteristics including high yield potential for the lines to be of use in a breeding program.

The more distant apart the germplasm is, as expressed by the genetic similarity, there is hardly any association between GD and F1 grain yield. This suggests that using only genetically distant relatives reduce the predictive value for the level of heterosis. By the same token, the tool is quite effective for lines within the same heterotic group. Therefore, for this tool to be effective, it is important to determine the threshold levels of GD. Hence use of genetic distance at molecular level could not be used in isolation but should be complemented by phenotypic data from field trials in the prediction of hybrid performance.

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# APPENDICES

**Appendix 4.1**: Quality of the Quality Protein Maize F1 hybrids

| Parent 1 i† | Parent 2 j | Tryptophan % | Nitrogen % | Protein % | Quality Index |
|---|---|---|---|---|---|
| SC10 | SC1 | 0.038 | 1.35 | 8.45 | 0.45 |
| SC10 | SC5 | 0.043 | 1.29 | 8.09 | 0.53 |
| SC10 | SC2 | 0.039 | 1.28 | 8.01 | 0.49 |
| SC10 | SC4 | 0.057 | 1.66 | 10.35 | 0.55 |
| SC10 | WWO1408 | 0.047 | 1.34 | 8.39 | 0.56 |
| SC10 | CML511 | 0.035 | 1.16 | 7.28 | 0.48 |
| SC1 | SC5 | 0.046 | 1.52 | 9.52 | 0.48 |
| SC1 | SC2 | 0.041 | 1.44 | 9.02 | 0.45 |
| SC1 | SC4 | 0.052 | 1.65 | 10.32 | 0.50 |
| SC1 | WWO1408 | 0.048 | 1.35 | 8.45 | 0.56 |
| SC1 | CML511 | 0.050 | 1.76 | 11.02 | 0.45 |
| SC5 | SC2 | 0.040 | 1.52 | 9.48 | 0.42 |
| SC5 | SC4 | 0.039 | 1.60 | 10.00 | 0.39 |
| SC5 | WWO1408 | 0.066 | 1.42 | 8.88 | 0.74[a] |
| SC5 | CML511 | 0.046 | 1.57 | 9.83 | 0.47 |
| SC2 | SC4 | 0.035 | 1.59 | 9.96 | 0.36 |
| SC2 | WWO1408 | 0.055 | 1.53 | 9.54 | 0.58 |
| SC2 | CML511 | 0.048 | 1.63 | 10.20 | 0.47 |
| SC4 | WWO1408 | 0.041 | 1.33 | 8.31 | 0.50 |
| SC4 | CML511 | 0.046 | 1.44 | 8.99 | 0.51 |
| WWO1408 | CML511 | 0.073 | 1.44 | 8.99 | 0.81[a] |

†i, inbred parent 1; j, inbred parent 2

[a], Quality index acceptable

**Appendix 4.2:** Analysis of variance for grain yield (GYG) across five sites in Zimbabwe

| **Source** | **df** | **SS** | **MS** | **F** | ***p*** |
|---|---|---|---|---|---|
| Env | 4 | 22.65 | 5.66 | 3.16 | 0.02 |
| Entry | 20 | 37.40 | 1.87 | 3.19 | 0.00 |
| GCA | 6 | 20.43 | 3.41 | 5.81 | 0.00 |
| SCA | 14 | 16.97 | 1.21 | 2.07 | 0.03 |
| Env*Entry | 80 | 143.41 | 1.79 | 3.06 | 0.00 |
| GCA*Env | 24 | 39.97 | 1.67 | 2.84 | 0.00 |
| SCA*Env | 56 | 103.44 | 1.85 | 3.15 | 0.00 |
| Residual | 180 | | 0.59 | | |
| GCA SE | 0.24 | | | | |
| SCA SE | 0.50 | | | | |

**Appendix 4.3:** ANOVA for modification score at RARS late

| **Source** | **df** | **SS** | **MS** | **F** | ***P*** |
|---|---|---|---|---|---|
| **Entry** | 20 | 1.03 | 0.05 | 2.57 | 0.0068 |
| **GCA** | 6 | 0.74 | 0.12 | 6.13 | 0.0002 |
| **SCA** | 14 | 0.29 | 0.02 | 1.04 | 0.4413 |
| **Residual** | 4 | 36 | | 0.02 | |
| GCA SE = 0.06 | | | | | |
| SCA SE = 0.12 | | | | | |

**Appendix 4.4:** ANOVA for modification score at ART

| Source | df | SS | MS | F | *P* |
|---|---|---|---|---|---|
| **Entry** | 20 | 1.02 | 0.05 | 3.14 | 0.0014 |
| **GCA** | 6 | 0.56 | 0.09 | 5.71 | 0.0003 |
| **SCA** | 14 | 0.46 | 0.03 | 2.04 | 0.0425 |
| **Residual** | | 36 | | 0.02 | |

GCA SE = 0.05

SCA SE = 0.10