Quantitative genetic analysis of agronomic and kernel endosperm traits in quality protein maize (QPM) and investigations of the putative nutritional value of contaminated QPM crops

by

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Thesis Abstract

The importance of maize in sub-Saharan Africa and the potential of quality protein maize (QPM) to alleviate the nutritional gap caused by lack of access to adequate protein rich foods were highlighted. Frustrations from complex inheritance systems of the QPM trait leading to calls for more information on the inheritance and stability of the QPM trait, fear of total loss of the QPM trait due to the recessive nature of the opaque-2 gene to the wild type gene in normal endosperm maize when QPM and normal endosperm maize coexist, lack of information on the nutritional value of contaminated QPM grain, and poor linkages with the smallholder farmers were all cited as drawbacks in the promotion and adoption of QPM. Therefore the objectives of the study were:

1) To solicit the participation of smallholder farmers in the development and setting up of QPM breeding goals, objectives and dissemination strategies;
2) To estimate general combining ability (GCA), specific combining ability (SCA) and reciprocal cross effects on anthesis days, quality traits and grain yield among the publicly available elite QPM inbred lines;
3) To compare experimental QPM hybrids with selected check cultivars, and normal endosperm maize hybrids for grain yield performance and kernel endosperm modification scores;
4) To evaluate QPM hybrids for grain yield and kernel endosperm modification scores in selected sub-Saharan Africa target environments.
5) To determine the level of normal endosperm maize pollen contamination that can occur in quality protein maize without loss of nutritional superiority;
6) To estimate the average levels and the patterns of foreign maize pollen contamination in QPM crops coexisting with normal endosperm maize varieties.

The contribution of smallholder farmers in setting breeding goals and dissemination strategies for QPM was solicited. One major finding was that the kernel endosperm qualities of landrace “Hickory King” need to be incorporated into new QPM varieties so as to encourage adoption. Farmers preferred getting information on QPM varieties through their local Agricultural Research and Extension (AREX) officers.

A diallel study of 36 F_1 QPM hybrids and their reciprocals was conducted across seven environments for agronomic traits and three environments for nutritional value traits. There were significant differences for all traits analysed using Griffing Method 3 model 1. General combing ability effects were significant and important in the control of anthesis days, kernel endosperm modification, protein content, tryptophan content, and Quality Index (QI). Specific combining ability effects were highly significant and important in the control of grain yield. There were significant SCA effects for anthesis days and QI but the proportions were lesser than the corresponding GCA effects in both traits. Kernel endosperm modification had significant GCA effects and nonsignificant SCA effects.

Reciprocal-cross differences were significant for anthesis days, tryptophan content and QI. Nonmaternal effects were significant for tryptophan content whilst both maternal and nonmaternal effects were significant for QI and anthesis days. Nonmaternal effects were relatively more important than maternal effects in all the cases where there were significant reciprocal-cross differences. The cross with the highest SCA effects for grain yield was CZL03016/CML144. The most desirable cross with the lowest anthesis days was CZL03016/CML144 whilst the most desirable inbred line with the lowest anthesis GCA effects was CZL03016. The inbred line with the most desirable GCA effects for protein content, tryptophan content and QI was CML264Q. Inbred line CML264Q crossed to CZL03016 had significant SCA effects for QI. The most desirable GCA effects for kernel endosperm modification were associated with inbred line CZL03016 followed by CZL01006. Maternal effects for both tryptophan content and QI were associated with inbred line CML264Q.

Genotype by environment interaction effects across all the seven environments were significant for grain yield and kernel endosperm modification. Check hybrids performed better than experimental hybrids for grain yield but were not different for kernel endosperm modification. The normal endosperm maize hybrids were significantly better for both grain yield and kernel endosperm modification. However, in all the comparisons the best check or normal endosperm maize hybrid was not significantly better than the best experimental or QPM hybrid, respectively. The most desirable score for kernel endosperm modification was from the cross of CZL01006 to CZL03016 though not significantly different from the check hybrid with the best score. AMMI1 was the best model for kernel endosperm modification scores and AMMI2 was suitable for grain yield. Both environments and hybrids were diverse.

Grain yield of most hybrids was not stable with specific adaptation to environments. The most stable hybrid with no specific adaptation was CML176/CML181f with a mean yield of 6.51t ha^{-1}.

The putative nutritional superiority of normal endosperm maize pollen contaminated QPM as measured by the QI depended on the environmental conditions. The moisture stressed environment (CIMMYT Harare) had a lower QI value (0.858) and a lower tolerance to pollen contamination of 15.3% whereas the grain produced under near to optimum growing conditions (ART farm) had a higher QI value (0.915) and a higher tolerance to pollen contamination of 31.9% before total loss of nutritional superiority. Thus contaminated QPM grain had nutritional superiority up to a certain point before total loss of nutritional superiority.

Geostatistical analysis was used to determine the levels and patterns of pollen contamination that occur when QPM and normal endosperm maize crops coexist under conditions minimising both temporal and geographical isolation to the lowest possible levels for the two independent crops. Higher pollen contamination levels were restricted mostly to the sections of the QPM crop proximal to the rows of normal endosperm maize crop, with the central parts of the QPM crops experiencing relatively low levels of contamination. For the four experiments (QCS200711, QCS200712, QCS200721 and QCS200722) in which the thresholds to nutritional superiority were determined, 87.9%, 94.8%, 62.2% and 65.6% of the crop areas passed for superior QPM grain, respectively. Estimates for average contamination levels of homogenous mixtures of grain from each of the nine experiments were below 20% contamination. The contamination levels were far less than previously thought.

“Hickory King” kernel quality attributes were important in breeding QPM varieties for the smallholder farmers. Parents of the 72 hybrids were diverse for the agronomic characters studied and three of the experimental hybrids were found to be adapted and comparable to the check varieties. Quality protein maize tolerance to foreign pollen contamination without loss in nutritional superiority depended on growing conditions. The coexistence of QPM and normal endosperm maize without total loss of QPM nutritional superiority was feasible.

FACULTY OF SCIENCE AND AGRICULTURE

Declaration

I, Lewis Machida, declare that:

1. The research reported in this thesis, except where otherwise indicated, is my original research.

2. This thesis has not been submitted for any degree or examination at any other university.

3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.

4. This thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:
   a. Their words have been re-written but the general information attributed to them has been referenced
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Signed

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

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Dr John Derera (Supervisor)

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Professor Pangirayi Tongoona (Co-Supervisor)

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Dedication

This thesis is dedicated to my loving wife (Apollonia), children (Tinotenda, Tinaishe and Tinemi), my father (Canias) and mother (Virginia).

Contents

List of tables

List of figures

List of abbreviations

AD	Anthesis days
AMMI	Additive main effects and multiplicative interaction
AMMI0	AMMI without PCA analysis
AMMI1	AMMI with one significant IPCA
AMMI2	AMMI with two significant IPCAs
AMMIF	AMMI full model with all significant IPCAs
ANOVA	Analysis of variance
ART	Agricultural Research Trust
CH	CIMMYT Harare
CIMMYT	International Centre for Maize and Wheat Improvement
CML	CIMMYT Maize Line
CV	Coefficient of Variation
CZL	CIMMYT Zimbabwe Line
DF	Degrees of freedom
E	Environment
Envnts	Environments
F	F value
GCA	General combining ability
GE	Genotype x environment interaction
IPCA	Interaction Principal Component Axis
IPCA1	Interaction Principal Component Axis one
IPCA2	Interaction Principal Component Axis two
K	Potassium
Kmod	Kernel endosperm modification
KRC	Kadoma Research Centre
LSD	Least significant difference
M	Maternal effects
N	Nitrogen
NM	Nonmaternal effects
P	Phosphorous
PRA	Participatory Rural Appraisal
Prob	Probability
Prot	Protein
QI	Quality Index
QPM	Quality protein maize

QCS	Quality protein maize contamination study
RARS	Rattray Arnold Research Station
REC	Reciprocal effects
Reps	Replicate
RMSE	Root mean square error
SC	Seed Co Ltd
SCA	Specific combining ability
SS	Sum of squares
SSA	Sub-Saharan Africa
SVD	Singular value decomposition
Try	Tryptophan

Introduction to thesis

Maize is one of the staple crops consumed in both large quantities and high frequencies by the inhabitants of sub-Saharan Africa and is produced on more than 22 million hectares (FAOSTAT, 2004). However, normal maize is deficient in two essential amino acids (lysine and tryptophan) that cannot be synthesised by the human body (Bressani, 1992) and consumption of normal maize without complementary protein rich foods predisposes the consumers to protein malnutrition which in worst cases manifests as kwashiorkor[1]. Those affected by protein-energy malnutrition mostly are the poor who cannot afford both leguminous and animal product sources of proteins. Most children from poverty stricken families are weaned-off to a diet based mostly on maize porridge without complementary sources of the much needed protein for healthy growth and development during childhood.

Anthropometric measures like child weight, stunting, and wasting are used to indicate levels of protein-energy malnutrition. In Zimbabwe 16.6% of the children under five were found to be underweight, 29.4% were chronically malnourished (stunted), and 6.4% were acutely wasted (UNICEF, 2006). This is despite the development of quality protein maize (QPM) whose biological value was reported by Bressani (1992) to be 80% of the equivalent of casein found in milk. The protein quality of various food crops is presented in Table 1.

The potential nutritional benefits of QPM indicated in Table 1 were also demonstrated by Graham et al. (1989), Graham et al. (1990), and Graham (1993). Previously the potential benefits of opaque-2 maize (also listed in Table 1) had been reported by Bressani (1966) and Graham et al. (1980) in children, and Clark (1966) in adult humans. On the African continent, protein energy deficiency is the major nutritional problem affecting children (Kinabo, 2001). Although moderate and severe protein-energy malnutrition in children can be clinically managed the efforts are still ineffective in many parts of the world (WHO/NDH, 2004). Curative approaches to management of protein energy deficiency tend to be very difficult and, therefore, preventive efforts should be promoted. This makes the breeding and promotion of crops with improved protein-energy nutritional value one of the sustainable approaches in solving malnutrition problems of the poor.

[1] **Kwashiorkor** is protein calorie malnutrition which can lead to infant morbidity and mortality. It disables the immune system such that the child is susceptible to a host of infectious diseases. The term is from Ivory Coast where it means the deposed child (weaned off). Source: MedicineNet.com. 1998. Webster's New World Medical Dictionary [Online]. Available by MedicineNet.com http://www.medterms.com/script/main/art.asp?articlekey=4124 (posted 12 October 2008).

Table 1 Protein quality of maize and other cereal grains

Cereal	Protein Quality (% casein)
Common maize	32.1
Opaque-2 maize	96.8
QPM	82.1
Rice	79.3
Wheat	38.7
Oats	59.0
Sorghum	32.5
Barley	58.0
Pearl millet	46.4
Finger millet	35.7
Teff	56.2
Rye	64.8

Source: FAO (1992)

There are other alternative strategic options for protein nutrition improvement which are fortification and processing but unfortunately they are both not suitable in the smallholder farming sector. Fortification is feasible in maize but it works well in situations where the affected consumers purchase the staple food all the time and unfortunately in Africa the people grow their own food (Bouis et al., 1999). Processing to improve nutritional value can be a problem because processing tends to conflict with customs and traditions in many cases (Andersen, 2003). This makes genetic manipulation of the staple crop one of the sustainable options (Andersen, 2003; Bouis et al., 1999). Therefore, QPM, a lysine and tryptophan rich product of genetic-fortification of normal endosperm maize becomes one of the sustainable options for providing nutritional security to millions of nutritionally insecure inhabitants of maize consuming regions in sub-Saharan Africa.

Quality protein maize development

The development of consumer acceptable high lysine maize was not easy and the initial product before QPM was not successfully adopted due to inferior agronomic and kernel quality characteristics. The opaque-2 maize that preceded QPM was more vulnerable to field pests, lower yielding than normal endosperm maize, more susceptible to ear rots, characterised by slow drying of dull non vitreous soft kernels which the consumers were not accustomed to (Mertz, 1994; Villegas, 1994). These drawbacks were gradually overcome almost twenty years ago (Vasal, 2002a) by the persistent and tenacious efforts of mainly three breeding programmes, 1) the International Centre for Maize and Wheat Improvement (CIMMYT) at El

Batan in Mexico, 2) Crow's Hybrid Corn Company, Milford, Illinois, USA, and 3) the Grain Crops Institute, University of Natal Pietermaritzburg, South Africa (under Helmut Otto Gevers). The work that is well documented and accounted for is that of the CIMMYT programme under the guidance of geneticist and breeder Surinder K.Vasal, and biochemist Evangelina Villegas (Crow and Kermicle, 2002; Mertz, 1994; Vasal, 2002b; Vietmeyer, 2000). Despite the overcoming of hurdles involved in the development of an acceptable high lysine maize product in the form of QPM, the requirements of the QPM breeding process remain complex and difficult to attain for most maize breeding programmes in Africa (Vivek et al., 2008). This has a negative bearing on the availability of locally adapted QPM breeding materials for most countries with poorly funded maize breeding programmes.

Thus, although QPM technology has been in existence for almost three decades, its adoption and utilisation in most of the nutritionally challenged parts of Africa is very low. Total maize area for sub-Saharan Africa was estimated as 22 million hectares by FAO (FAOSTAT, 2004) whilst the 2005 estimate for the area under QPM was 200 000 hectares (Krivanek et al., 2007). Thus about one percent of the annual maize area in sub-Saharan Africa is planted to QPM varieties. This is mainly because of challenges that are confronted in the areas of QPM breeding, QPM varietal maintenance, and QPM varieties dissemination.

Quality protein maize genetics and breeding

Despite the work and findings of numerous researchers who include Sriwatanapongse et al. (1974), Bjarnason et al. (1976), Bjarnason et al. (1977), Vasal et al. (1980), Wessel-Beaver and Lambert (1982), Vasal et al. (1984), and Hohls et al. (1996) on the elucidation of the genetics and inheritance of QPM kernel endosperm modifiers, Vasal (2000) lamented the need for more work to be carried out on the mechanism of inheritance of endosperm modifiers. Considering the investment requirements, difficulties, complexities and frustrations involved in QPM germplasm development versus the resource capacities and or level of expertise, Vasal (2000) proposed a strategy where QPM breeding programmes would make use of information from evaluation of CIMMYT QPM inbred lines to identify and select materials for release in their areas. The genetic evaluation of locally available QPM germpalsm would be a great step towards the generation of more information in the genetics and breeding potential of QPM germplasm.

Skepticisms and myths associated with QPM

There is skepticism about the stability and consistence of performance of QPM varieties under diverse growing conditions that include drought conditions, poor fertility soils, or poor isolation from normal endosperm maize crops at flowering (Vivek et al., 2008). Twumasi-Afriye et al. (1996) cited the myths in Ghana as the following: a) QPM varieties yield lower than normal endosperm maize; b) lysine and tryptophan are heat labile and would be destroyed during cooking; c) In all circumstances QPM crops lose all their nutritional superiority when coexisting with normal endosperm maize varieties due to the high levels of cross pollination that can occur considering the relatively small size of plots in the smallholder farming areas. These myths were researched and found to be not true (Vivek et al., 2008). However the adoption and interest in QPM is still low in most parts of Africa and some of the myths and skepticism still prevail. Therefore it would be appropriate to demystify some of the myths with research work done under local conditions and involving the intended beneficiaries as witnesses.

Rationale for research

The research work conducted in this thesis was implemented in an effort to address some of the challenges and constraints that are expected to be experienced in the development and promotion of QPM varieties in the smallholder farming areas in Africa.

Farmer participatory research in QPM development

The interaction of the smallholder farmers and novel technologies has always been a source of both amazement and frustrations to agricultural research scientists. It seems African farmers are quick to adopt new technologies in other spheres (e.g. cellphones) but slow or hesitant to adopt new technology in agriculture. DeVries and Toenniessen (2001) emphasised the need for constant involvement and consultations between farmers and researchers to guarantee that the expectations of the farmers are addressed in the development of new technology for the agricultural sector. Currently there are no known reports on successes of Participatory Rural Appraisal (PRA) in QPM development activities but in other developmental projects the use of the PRA approach yielded positive results. The success of development projects in irrigation, livestock, water, and agriculture was found to be highly dependent on participation by the local people (Guijt, 1991; Pretty and Voudouhue, 1996). In a study of 121 rural water supply projects in 49 countries of Africa, Asia and Latin America the best project results were when the intended beneficiaries were involved in decision making during all stages and the poorer results were when the intended beneficiaries were just involved in information sharing and consultations (Narayan, 1993).

In a study of the relationship between participation and rural water supply project outcomes, Prokopy (2005) found that in projects where there was community participation in the form of either decision making at the household level or capital contribution there were better project outcomes. It is expected that by involving the intended beneficiaries in the development and dissemination of QPM technology through the PRA approach there will be better project outcomes. Therefore, in order to pre-empt any possibility of strong rejection of QPM technology in the smallholder farming sector, smallholder farmers were involved in part of the work leading to the writing of this thesis. An attempt to identify the issues that QPM researchers and extension agents need to consider and address in order to align their projects with the expectations, aspirations and hopes of the smallholder farmers regarding QPM varieties development was made. Thus a PRA project on the subject of QPM breeding and dissemination was conducted in four villages situated in three districts of Zimbabwe.

Agronomic potential of QPM genotypes

Several studies were conducted on the agronomic potential of QPM varieties and QPM varieties were found to be as competitive as normal endosperm maize hybrids and in the study of Pixley and Bjarnason (1993) the best QPM materials were found to yield at least 14% better than normal endosperm maize checks on average. Although Pixley and Bjarnason (1993) reported that QPM yield level potentials were now of the same level as normal endosperm maize, in a later study, using different germplasm and different test environments Bhatnagar et al. (2004) found normal maize checks yielding higher than QPM materials but the yield differences were not significant. Krivanek et al. (2007) reported the occurrence of QPM varieties yielding 5% less than normal endosperm maize varieties in some genetic backgrounds and encouraged the need to investigate the cause. It was in that context that the breeding potential of QPM inbred lines was evaluated under local conditions.

Stability of agronomic performance of QPM varieties

There has been concern over the stability of the performance of QPM genotypes across environments (Vivek et al., 2008). The expression of the kernel endosperm traits which include modification, protein content, tryptophan content and quality index across environments has been a subject of interest among QPM researchers. Pixley and Bjarnason (2002) reported stable performance of QPM cultivars for protein content, tryptophan content, concentration of tryptophan in protein and kernel endosperm modification. Differential decline in the protein quality with some genetic backgrounds despite the presence of the opaque-2 gene was

observed (Vasal, 2002a). Few studies on stability of agronomic performance of such an important type of maize have been done. Considering this, together with the information on scepticism and myths about QPM there is, therefore, great need to assess the agronomic performance of the available QPM genotypes across several environments for the benefit of both QPM breeders and potential QPM consumers.

Challenges in implementing QPM technology in the smallholder farming sector

Farmers grow more than one variety in a season and when QPM varieties get deployed they will be coexisting with normal endosperm maize varieties potentially leading to the loss of the QPM trait due to the recessive nature of the opaque-2 gene to the wild type allele in normal endosperm maize. The loss of the QPM trait when the flowering of both normal endosperm maize and QPM crops that are in coexistence overlaps has been a source of skepticism in QPM advocacy and recommendation among some researchers and extension agents (Twumasi-Afriye et al., 1996). This is because theoretically the nutritional value of a QPM crop whose silks are contaminated by normal endosperm maize pollen grains is not different from that of a normal endosperm maize crop. However, in practice when the events of cross pollination are considered, not all the QPM silks get pollinated by the normal endosperm maize pollen grains and so should the whole mixture of grain from the normal endosperm maize pollen contaminated QPM crop be condemned just because some of the kernels reverted to normal? If not then at what point should we consider the QPM crop to be not superior to normal endosperm maize grain? This is a very pertinent question related to QPM dissemination and had to be answered in the context of local conditions in the interest of helping local farmers.

Cordova (2000) pointed out that the feared levels of contamination in QPM crops under smallholder growing conditions could be much lower than originally thought. This idea is supported by the assertion from Burris (2001) that contamination under conditions where the female plants in both crops are potential pollinators (and the QPM plants are not emasculated) is different from contamination studies where female plants are emasculated because when both crops are potential pollinators there would be copious amounts of desirable QPM pollen competing for silks with foreign pollen. This therefore requires the conducting a study to ascertain the levels of pollen contamination that can occur instead of applying recommendations from seed production.

The next step in addressing this issue was to get the levels of contamination that occurred under real field situations so that the information on the nutritional superiority of contaminated

QPM grain can be related to real life situations. The known study on contamination of QPM by normal endosperm maize was reported by Twumasi-Afriye et al. (1996) and was conducted in Ghana. In the present study ordinary kriging method was used. Kriging is an exact spatial interpolation technique (Isaaks and Srivastava, 1989; Webster and Oliver, 2001). Kriging has been in use in agriculture (Webster and Oliver, 1990), but this was the first time that kriging was used to study levels and patterns of cross pollination. The study in Ghana was successful but pollen contamination extents and patterns tend to be highly influenced by local climatic conditions and, therefore, it is pertinent that the study be done under local conditions and using a more robust technique to estimate contamination levels at unsampled points in the process of producing a prediction map for the contamination pattern. The two issues of levels of pollen contamination when QPM crops coexist with normal maize varieties crops, and the pollen contamination level at which QPM grain loses nutritional superiority are very important questions in the dissemination of QPM varieties. This is because smallholder farmers will always grow more than one variety in an attempt to manage risk of crop failure.

Research objectives

The specific objectives of the thesis were as follows:

1) To solicit the participation of smallholder farmers in the development and setting up of QPM breeding goals, objectives and dissemination strategies;
2) To estimate GCA, SCA and reciprocal effects on anthesis days, quality traits and grain yield among the publicly available elite QPM inbred lines;
3) To compare the performance of experimental QPM genotypes against selected check cultivars, and also compare the performance of QPM hybrids against normal endosperm maize hybrids for both grain yield levels and kernel endosperm modification scores;
4) To investigate the adaptation of QPM genotypes for grain yield and kernel endosperm modification in sub-Saharan Africa target environments using the AMMI technique;
5) To determine the level of normal endosperm maize pollen contamination that can occur in quality protein maize without loss of nutritional superiority;
6) To estimate the average levels and visualise the patterns of foreign maize pollen contamination in QPM crops coexisting with normal endosperm maize varieties under conditions simulating the worst-case scenario for crosspollination.

Research hypotheses

There were five main hypotheses investigated in this study and these were:

1) Smallholder farmers have preferences and perceptions on maize varieties and these have implications for QPM varieties breeding and dissemination
2) The available set of QPM inbred lines exhibit genetic variability for grain yield, anthesis days and kernel quality traits.
3) High yielding QPM crosses can be found among crosses of elite CIMMYT QPM inbred lines;
4) Adapted QPM crosses can be found among crosses of elite CIMMYT QPM inbred lines;
5) The contamination of QPM by normal endosperm maize pollen is far less than previously thought;
6) The nutritional value of QPM grain from a normal endosperm maize pollen contaminated QPM crop is better than that of normal endosperm maize grain.

Thesis structure

The structure and layout of the thesis is as follows:

1) Chapter 1: Review of the literature.
2) Chapter 2: Farmers' preferences and perceptions of maize varieties and their implications on development and dissemination of quality protein maize varieties in Zimbabwe.
3) Chapter 3: Combining ability and reciprocal effects analysis of elite QPM inbred lines in subtropical environment.
4) Chapter 4: Evaluation of quality protein maize (QPM) F_1 hybrid genotypes for grain yield and kernel endosperm modification in subtropical environments.
5) Chapter 5: Determination of normal endosperm maize pollen contamination levels that can occur in quality protein maize (QPM) without loss of nutritional superiority.
6) Chapter 6: Geostatistical analysis of quality protein maize (QPM) crops contamination with pollen from adjacent normal endosperm maize fields.
7) Chapter 7: Overview.

The thesis is written in a composite format and as such there is overlap of both information and references across chapters.

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1 Literature Review

1.1 Introduction

The chapter reviewed literature on the theory, gene action, breeding potential and combining ability studies of quality protein maize (QPM) for different important agronomic traits, the concern about the stability of both agronomic and nutritional performance of QPM considering the potentially high fluctuations in environmental conditions that characterise the target environments, the nutritional value of contaminated QPM and the coexistence of QPM and normal maize cultivars. The challenges involved in the coexistence of QPM and normal maize and the available options to the farmers were also reviewed. The concerns and uncertainty about the nutritional value of QPM produced under poor conditions of isolation from normal maize pollen at flowering were reviewed as well.

1.2 Genetic studies of QPM genotypes

In order to address the constraints and hurdles emanating from the nature of genetics involved in the breeding of QPM materials, QPM geneticists and breeders need to explore and diagnose the genetic makeup of QPM materials (populations or varieties) if there is a definable population or the genetic effects of various QPM breeding lines if there is no definable population such as in Bhatnagar et al. (2004) study. The successful diagnosis of the genetic architecture of a set of QPM genotypes for a specific genetic trait can help establish the nature of inheritance for the genetic trait and or determine the potential usefulness of the set of genotypes in the breeding and improvement of the trait (Dabholkar, 1999). The availability of this information enables the development and implementation of an appropriate breeding strategy.

1.3 Study of gene action

There are several mating designs that can be utilised in studying the genetic architecture of a set of genotypes and these include bi-parental progenies, parent offspring regression, North Carolina (NC) design I, NC design II, NC design III, Triple test cross, and diallel mating design (Dabholkar, 1999; Hallauer and Miranda Filho, 1988; Mather and Jinks, 1982). However among all the mating designs the diallel mating design is the most used and abused design (Hallauer and Miranda Filho, 1988). This is because it is more robust than the other designs. The diallel mating design is when all possible matings among a set of genotypes are conducted and the progenies grown for genetic analysis. The fact that all possible crosses among a set of genotypes has to be made limits the number of parents that can be studied in a diallel mating design to a few. Detailed reviews and accounts on aspects and various forms of diallel design

were conducted by several researchers who include Dhillon and Singh (1978), Baker (1978), and Christie and Shattuck (1992), and most of the abuses of the diallel mating design emanate from misinterpretation of the findings (Arunachalam, 1976; Baker, 1978; Christie and Shattuck, 1992).

In past studies on the genetic architecture of QPM kernel endosperm factors and other agronomic and quality attributes in QPM materials, the diallel mating design has been used in most cases. Notable examples include Sriwatanapongse et al. (1974), Vasal (1975), Bjarnason et al. (1976), Bjarnason et al. (1977), Vasal et al. (1980), Hohls et al. (1996), Pixley and Bjarnason (1993), Vasal et al. (1993b), Vasal et al. (1993a), Fan et al. (2004), Bhatnagar et al. (2004), and Jompuk et al. (2007). The type of plant material available, and interest and objectives of the researcher(s) determined how the various forms of the diallel designs and analyses reviewed by Christie and Shattuck (1992) were employed in the generation of information on the subject of QPM genetics and breeding.

The past genetic and breeding studies on QPM germplasm can be broadly and generally divided into two main groups. The first group belongs to studies that focused on the inheritance and genetic control of QPM traits (mostly kernel endosperm modification traits) with findings expressed in the form of additive and non-additive genetic variance components. The second group belongs to studies on the genetic effects and relationships among the germplasm materials expressed in the form of general combining abilities (GCA) and specific combining abilities (SCA). In Griffing (1956) diallel mating designs the primary analyses of variance that give rise to the two different interpretations are the same and this is what causes abuse if the correct interpretation is not done.

Although analysis of the diallel mating design has various forms which are 1) Griffing's analysis (Griffing, 1956), 2) Hayman and Jinks analysis (Hayman, 1954a; Hayman, 1954b; Jinks, 1956; Jinks, 1954; Jinks and Hayman, 1953); 3) Gardner and Eberhart's analysis (Eberhart and Gardner, 1966; Gardner and Eberhart, 1966), and 4) partial diallels (Gilbert, 1958.; Kempthorne and Curnow, 1961), in maize breeding the mostly used forms of analyses are Griffing's analyses and Gardner and Eberhart's analysis. This is because the nature of inheritance of most traits of economic importance in maize is quantitative such that the prerequisite assumptions for Hayman and Jinks analysis (Dabholkar, 1999) are violated by the genetic nature of most traits, and partial diallels would be convenient but the mechanics of statistically handling partial diallels

are relatively difficult for most ordinary breeders (Christie and Shattuck, 1992). The Gardner and Eberhart analysis is suitable for analyzing varieties and populations and it is used less relative to Griffing's analysis because most maize breeding programmes are oriented towards development and release of hybrid varieties and hence their interests are in the analysis of inbred lines and their crosses.

Unlike the Hayman Jinks analysis which is suitable for most self pollinating species, Griffing's analysis is amenable to interpretations that produce estimates of additive and non-additive genetic components of variance, and general and specific combining abilities depending on whether the model is fixed or random (Christie and Shattuck, 1992). The random model is where the parents represent a definable population and leads to estimation of additive and non-additive genetic variance components from the mean square values obtained in analyses of variance tables. The fixed model is where the parents are products of different pedigree breeding projects and do not represent a definable population and the appropriate interpretation is where the mean square values in the analysis of variance table are measures of general combining abilities and specific combining abilities. Some researchers often interchange either the utilisation of the fixed model for the random model and or the interpretations leading to abuse of the diallel design.

The concept of general combining abilities and specific combining abilities was proposed by Sprague and Tatum (1942) and adapted for the diallel mating design by Griffing (1956) and Gardner and Eberhart (1966) and Eberhart and Gardner (1966). Griffing (1956) proposed four variations of the Griffing's diallel analysis, known as Methods I to 4, each with both random and fixed model interpretations. The mostly used design in maize is Method 4 mainly because of the absence of reciprocal differences for most traits of economic importance in maize and the need to reduce the sampling error by including a reasonably high number of different parents in the analysis. In cases where reciprocal differences between crosses are suspected it is prudent to use Griffing Method 3.

1.4 Germplasm influences on diallel mating design

The germplasm available has an influence on the mating design and the analyses that can be conducted. In species where sexual mating is difficult it is not possible to have diallel mating designs and also in species where selfing is not possible Hayman and Jinks, and Gardner and Eberhart analyses can not be conducted (Christie and Shattuck, 1992). Contextually the availability of inbred lines from the same population or initial cross, and their respective F_1

crosses and reciprocals allows for the conduction of several forms (if not all) of diallel designs and the statistics that can be derived are components of genetic variance (Eberhart and Gardner, 1966; Gardner and Eberhart, 1966; Griffing, 1956; Hayman, 1954b; Jinks and Hayman, 1953). However when the inbred parents originated from genetically different sources through various pedigree technique manipulations several diallels can be performed but the obtainable statistics are general combining abilities and specific combining abilities mean squares which cannot be extended to variance components (Baker, 1978). Most of the locally and publicly available QPM germplasm in sub-Saharan Africa originated from different populations through the pedigree breeding technique and as such the studies that can be conducted are theoretically limited to the determination of general and specific combining abilities, and estimation of reciprocal-cross effects if desired.

1.5 Gene action of kernel endosperm factors

Several researchers reported on genetic studies that elucidated the role of kernel endosperm factors in the development of hard vitreous *opaque-2* maize and these include (Bjarnason et al., 1976; Bjarnason et al., 1977; Hohls et al., 1996; Sriwatanapongse et al., 1974; Vasal, 1975; Vasal et al., 1980; Wessel-Beaver and Lambert, 1982). The diallel mating design was employed in most of the studies except that of Wessel-Beaver and Lambert (1982) who used the concept of generation means and scaling tests. Most research findings reported that in the inheritance of endosperm modifying factors additive variance was more important than non-additive variance (Bjarnason et al., 1976; Hohls et al., 1996; Vasal, 1975; Vasal et al., 1980), whilst Wessel-Beaver and Lambert (1982) reported that no simple model fitted the modifying gene action and that the inheritance for endosperm modifiers was complex. Tthis could be the reason why in addition to the major gene action being found to be of the additive type (Bjarnason et al., 1976; Hohls et al., 1996; Vasal, 1975; Vasal et al., 1980); partial dominance was also reported by Vasal (1975), Bjarnason (1976), Vasal et al. (1980), (Wessel-Beaver and Lambert (1982) and Hohls et al. (1996). The implications of the endosperm modification trait being under the control of additive genes means that the inheritance cannot be explained by simple Mendelian models but through quantitative genetic models.

1.6 Reciprocal cross differences

The advantage of QPM over normal endosperm maize is high kernel endosperm lysine and tryptophan content. This makes QPM kernel endosperm properties the focus of the attention when breeding QPM in addition to other agronomic traits. However, in some situations the expression of kernel endosperm traits is under the influence of either maternal or nonmaternal

effects potentially leading to reciprocal differences between crosses. Failure to account for reciprocal effects, when they are present, risks biasing the estimates for general combining ability or additive genetic variance and specific combining ability or nonadditive genetic variance and heritability estimates (Crusio, 1987; Roach and Wulff, 1987). The earliest known study attributing reciprocal differences between F_1 crosses and their reciprocals to maternal effects and or nonmaternal effects was that of Cockerham (1963). However due to the instability and inconsistencies and the relative magnitude of reciprocal-cross effects in previous studies on various traits in maize (Hallauer and Martinson, 1975; Mann et al., 1981; Pollmer et al., 1979; Widstrom, 1972) there does not seem to be much interest in the study of reciprocal-cross differences. The importance of maternal effects in kernel endosperm modification was confirmed by Vasal (1975) and Vasal et al. (1980) whilst Bjarnason et al. (1976) and Wessel-Beaver and Lambert (1982) reported that maternal effects were unimportant. The Hayman and Jinks analysis can handle reciprocal cross differences whilst Methods (1 and 3) of Griffing (1956) analysis can detect and measure reciprocal cross differences.

Reciprocal cross differences can be further partitioned into maternal and nonmaternal effects (Zhang and Kang, 1997). Maternal effects are the expression of the maternal genotype in the phenotype of an individual rather than its genotype (Roach and Wulff, 1987). Nonmaternal effects are unexplained differences attributed to the interaction of cytoplasmic factors and nuclei genes (Lopez et al., 2003). The partitioning helps in deciding whether maternal or extranuclear factors are involved in the expression of a trait. In the case where maternal effects are significant it would be important to note that the phenotype expressed by the F_1 individual is not necessarily its genotype but represents the genotype of the female parent. The knowledge of maternal effects helps in deciding the parents through which to introduce desired alleles (Kollipara et al., 2002) or the direction in which crosses should be deployed in the field (Lopez et al., 2003). It is important to note that there are three types of maternal effects; nuclear genetic, cytoplasmic genetic and environmental maternal effects (Roach and Wulff, 1987). The effect of both cytoplasmic and genetic maternal effects when present is to inflate the detectable amount of genetic variance, and the response to selection is slowed if the trait is completely under maternal control (Roach and Wulff, 1987). On the other hand if environmental maternal effects are involved then environmental noise in the experiment is inflated and as a result response to selection will be slowed as well (Roach and Wulff, 1987). The inconsistence and instability of reciprocal cross effects has been reported by Pollmer et al. (1979), Mann et al. (1981) and

Melchinger et al. (1985). Based on this, Melchinger et al. (1985) concluded that testing for reciprocal differences is not worth while in practical maize breeding.

In maize, it is recommended that the study of kernel endosperm traits be conducted with Griffing Method 3 so that reciprocal cross effects can be investigated (Zhang and Kang, 1997). It would be prudent to use a model that can detect and measure reciprocal cross effects in the study of QPM kernel endosperm traits when present. Before deciding on exploiting reciprocal cross differences, Melchinger et al. (1985) suggested that their presence should be tested for across several years and several locations but considers the exercise not worth while in practical maize breeding. However in order to precisely estimate general and specific combining abilities that are free from the confounding effect of maternal differences, then reciprocal cross effects need to be considered even though they are known to be inconsistent and unstable.

1.7 General and specific combining ability effects

Literature on the determination of combining abilities in QPM has been limited to a few publications. The earliest reported work on combining abilities of QPM germplasm was by Vasal et al. (1993a and 1993b). This was immediately followed by the report of Pixley and Bjarnason (1993). The two studies by Vasal et al. (1993a and b) were on CIMMYT QPM populations. The experiments in the first study were all grown in Mexico whilst those for the second study were grown at locations in both Mexico and United States of America (Tennessee, Mississippi, and Indiana States). In the study of Pixley and Bjarnason (1993) QPM inbred lines were studied in four different diallel experiments. The experiments were grown at locations in Colombia, Honduras, Costa Rica and Mexico. The parents involved were the first generation of inbred lines from the CIMMYT QPM breeding programme.

Fan et al. (2004) reported the combining abilities among five yellow endosperm tropical and subtropical QPM lines from CIMMYT, and five yellow endosperm Chinese QPM lines. Combining abilities of two separate experiments on QPM inbred lines were also reported by Bhatnagar et al. (2004). The inbred lines were from three different programmes, Texas A&M University (TAMU), CIMMYT, and South Africa (Hans Gevers' breeding programme). The environments for the study were all in the southern part of USA, Texas. The study of Jompuk et al (2007) was based on inbred lines extracted from three CIMMYT populations. The important role of CIMMYT QPM germplasm in different breeding programmes is evident in its wider usage. This is because QPM breeding is not easy and is expensive. This makes the study of locally available QPM germplasm more relevant for the benefit of local farmers. Little work has

been done in the form of combining ability studies in QPM on the African continent apart from the work of Hohls et al. (1996) which was conducted in South Africa, Hohls et al. (1996) found that both kernel vitreousness and kernel hardness were determined by partially dominant genes whilst additive gene action was responsible for kernel modification. Combining abilities tend to be influenced by the environment and the germpalsm used in the study (Falconer and Mackay, 1996). Therefore it would be important to investigate the GCA and SCA relationships among the set of publicly available QPM inbred lines in the sub-Saharan Africa region.

Whilst Vasal et al. (1993a and b) and Pixley and Bjarnason (1993) reported significant GCA effects for grain yield across environments, Bhatnagar et al. (2004) experiment found nonsignificant GCA effects for grain yield across environments but highly significant GCA for other agronomic and quality traits. The SCA effects for grain yield across locations were not significant in the studies of Vasal et al. (1993a and b) and Pixley and Bjarnason (1993) but were significant in Bhatnagar et al. (2004) study. One of the reasons for lack of significant SCA effects for grain yield in earlier CIMMYT germpalsm is that it had been developed without due regard for heterotic groups. The existence of significant SCA effects for grain yield in QPM maize would allow the development of high yielding QPM hybrids from inbred lines. The currently acknowledged high yields in normal endosperm maize are a result of the existence of significant SCA effects in the sets of germplasm used by the different hybrid maize breeding programmes. For QPM maize to be able to compete with normal endosperm maize cultivars for grain yield and other agronomic traits it would be important for the available QPM germplasm in any region to exhibit significant SCA effects that can be exploited in the form of heterosis.

In Bhatnagar et al. (2004) study, SCA effects across environments were not significant for all quality traits except test weight whilst in Vasal (1993a and b) and Pixley and Bjarnason (1993) studies, SCA for all quality traits across environments were also nonsignificant. In the study of Vasal et al. (1993b), yield potential was found to be influenced by the type of endosperms involved in a cross, with both dent x dent, and dent x flint crosses yielding higher than flint x flint crosses. However the kernel endosperm modification for flint x flint was the most desirable followed by dent x flint and then dent x dent. It would be interesting to find out whether this is also reflected in the available QPM germpalsm in Southern Africa.

1.8 Agronomic performance of QPM genotypes

In the studies of Pixley and Bjarnason (1993), Jompuk et al. (2007) and Bhatnagar et al. (2004) normal endosperm check varieties were included and it was only in Pixley and Bjarnason (1993)

study where QPM hybrids clearly outperformed normal endosperm hybrids for grain yield potential by at least 14% on average. In the study of Bhatnagar et al. (2004) the contrast of normal endosperm check varieties against QPM F_1 hybrids was not significant ($p > 0.05$) but the mean of normal endosperm checks was at least 0.5t ha^{-1} higher than that for the QPM F_1 hybrids. Similarly, in Jompuk et al. (2007) study there were no significant differences for grain yield between the normal endosperm hybrid check varieties and the QPM F_1 hybrids. However, Jompuk et al. (2007) report was based on one environment. There were differences in genetic backgrounds between the QPM materials and the normal endosperm maize checks that were used in the studies of both Jompuk et al. (2007) and Bhatnagar et al. (2004) with the normal endosperm check entries being well adapted to the test locations better than the QPM F_1 hybrids. In the study of Pixley and Bjarnason (1993) where the QPM entries performed better than normal endosperm maize entries, the normal endosperm checks used were supposedly adapted to the environments. The reason for implementation of such a seemingly unfair comparison between QPM hybrids and normal endosperm check entries is because in most environments globally, the development of well adapted QPM germplasm is still in its infancy. Therefore, there is need to conduct more work on both the development and evaluation of the breeding potential of QPM germplasm. Some of this work entails the regular and periodic generation of information on general and specific combining abilities of QPM breeding lines.

The lack of overwhelming yield superiority of QPM cultivars over normal endosperm cultivars was also reported in the review by Krivanek et al. (2007) who proposed that more research effort was needed for some QPM genetic backgrounds. Arguably much less effort has been devoted to the improvement of QPM germplasm and varieties compared to the effort spent on normal endosperm maize varieties. This has been the trend despite that at least 20% of the children under five in Zimbabwe suffer from protein energy malnutrition (UNICEF, 2006) a situation which can potentially be alleviated with the growing and consumption of QPM grain among other measures. Therefore, it is prudent to investigate the agronomic potential of QPM cultivars under local conditions.

Vasal et al. (1984) reported that the development of QPM germplasm required the manipulation of two genetic systems. This information however did not make it easier for ordinary maize breeders to develop QPM cultivars because maize breeders continued to experience frustrating inconsistencies in their attempts to develop QPM versions of elite adapted normal endosperm maize varieties (Gevers, personal communication, 2005). This is in agreement with the

experience at CIMMYT where although previous work had indicated that kernel endosperm modification was mainly controlled by additive components of variance , Vasal et al. (1984) and Vasal (2002) referred to the inheritance of kernel endosperm modification as complex. Vasal (2002) advocated for the elucidation of mechanism(s) controlling kernel endosperm modification, and the role of gamma zein in kernel endosperm modification. Thus it is important to understand the role of additive and non-additive variance components or the role of GCA and SCA effects in the breeding of kernel modification so that more QPM varieties can be produced easily.

The other frustrating experience included the differential decline in the protein quality with some genetic backgrounds despite the presence of the *opaque-2* gene (Vasal, 2002). Earlier on, the experience with kernel endosperm modifiers had shown that endosperm modifiers were difficult to find and accumulate in dent maize generally (Vasal et al., 1980). The low levels of enthusiasm and success in breeding QPM varieties is probably due to this reason. Most breeding programmes in sub-Saharan Africa use dent germplasm. However most of the unimproved landraces maintained by the communities tend to be of the flint type. These flint types could present advantages in attempts to combine the QPM trait with other agronomic and quality traits that are already preferred by the farmers.

Thus despite several studies on the inheritance and role of kernel endosperm modifiers in the expression of the QPM trait, Vasal (2002) lamented the need for more research to be conducted on the genetics of endosperm modifiers in QPM. This was before the discovery of the third genetic system of amino acid modifiers (Vivek et al., 2008). The fact that certain genotypes possessing both the opaque-2 gene and a good level of endosperm modifiers could test poor for protein quality (tryptophan levels) could be one of the reasons why there was confounding in the expression of the QPM phenotype and hence Vasal (2002) encouraged more research to be done on the genetics of kernel endosperm modifiers. The idea of this project was conceived without the knowledge of the existence of the third genetic system – the amino acid modifiers genetic system reported in Vivek et al. (2008). Therefore, the research thrust was directed towards generating more information in the form of general combining abilities, specific combining abilities and reciprocal-cross effects from the current set of elite QPM germplasm. Otherwise it would have been prudent to generate more information on the role of the amino acid modifier system.

Another challenge for most poor breeding programmes in sub-Saharan Africa is the requirement for laboratory tests to validate the presence of the *opaque-2* gene in progenies. During the development of QPM materials in conversion programmes, it is important for progenies to be regularly tested for the presence of the *opaque-2* gene as indicated by Vivek et al. (2008). The testing requires establishment of a laboratory capable of conducting the tryptophan tests or if not then at least the capacity to prepare and send samples to other laboratories that can run the tests. Most breeding programmes in sub-Saharan Africa do not have the capacity to conduct these quality tests and also the budgets to pay other parties for conducting the tests. This can be costly for some poor breeding programmes and, hence, it remains as a major constraint.

1.9 Genotype by environment interactions

The maize growing environments in sub-Saharan Africa are characterised by both temporal and seasonal fluctuations of weather patterns. This leads to the significance of genotype x environment interactions in addition to the genotype main effects and the environment main effects in the development and evaluation of crop varieties for the sub-Saharan Africa environments. Genotype by environment interaction is defined as differential genotypic expression across environments (Fox et al., 1997) or a situation where there are inconsistent differences between genotypes across environments (Hohls et al., 1995). Three common types of genotype by environment (GE) interaction are recognised and these are cultivar x location interaction, cultivar x year interaction and cultivar x location x year interaction effects (Crossa, 1990; Fox et al., 1997). Genotype x environment interaction is influenced by the components of cultivar, locations and year differently such that in some cases the location factor is more important than both year and cultivar factor in which case the environment can be partitioned so as to allow specific genotypes to be deployed to sub-environments of specific adaptation (Fox et al., 1997). When the cultivar factor has the greatest influence, then genotype x environment can be addressed by choosing relatively stable cultivars across the target environments. Tthe season factor is difficult to deal with. Therefore, arguably the evaluation of the breeding potential in the form of combining abilities and reciprocal-cross effects of new QPM hybrids would require their evaluation for agronomic performance in several environments across both locations and seasons. The consistent expression of kernel endosperm quality traits across environments is also another important aspect in QPM variety development and deployment. Abiotic stresses are known to influence the expression of some of the quality aspects of QPM such as kernel endosperm modification. Official reports argue that this is only in some genetic backgrounds (Krivanek et al., 2007). It would be interesting to investigate the breeding potential of the

currently available elite germaplasm in the context of adaptation and stability across the local environments.

1.9.1 Genotype x environment evaluation techniques

The set of available techniques for the analysis of genotype and environment interactions include analysis of variance, linear regression analysis, nonparametric tests which include rank analyses, and multivariate techniques which include principal component analysis, additive main effects and multiplicative interaction (AMMI), Genotype Genotype x environment (GGE) biplot, and cluster analysis. The analysis of variance (ANOVA) technique is able to measure and detect genotype main effects, environment main effects and genotype x environment interactions. However the ANOVA technique cannot determine the pattern of GxE interactions so as to shed more light on areas of specific adaptation of genotypes (Zobel et al., 1988).

The linear regression method of Finlay and Wilkinson (1963) was the most commonly used technique in the analysis of adaptation of genotypes. It has the advantage of being able to simplify complex interactions to an orderly linear response but it is mostly useful when the genotypes exhibit a linear response to the environments in the study (Crossa, 1990). Furthermore linear regression analysis is poor at handling genotype or environmental outliers or incomplete data sets.

Nonparametric tests for GE evaluation were reviewed by Lin et al. (1986) and their advantage is that unlike linear regression the statistics generated by nonparametric tests are not influenced by the set of cultivars under evaluation. Furthermore the assumptions about the distribution of data, homogeneity of variances, and linearity are not necessary conditions (Huehn, 1990).

In the family of multivariate techniques, principal component analysis is able to analyse the GE but is not able to provide information on the genotype and environment main effects and so it would need to be complemented by another technique that can analyse the main effects (Zobel et al., 1988). Cluster analysis can be used to reveal patterns but the difficulty is in the choice of method as a poor choice of method would force unwarranted structure on a data set producing misleading results (Westcott, 1986). Furthermore the computational requirements for cluster analysis tend to be complex.

The two other methods, AMMI and GGE are more or less similar in that both rely on the singular value decomposition of data. This means that all the biological variables that can lead to GE

influences are expressed in a single axis which is simple to analyse and explain. The AMMI analysis combines both the advantages of the ANOVA and the principal component analysis into one method (Zobel et al., 1988). The GGE biplot is based on plotting the first two interaction principal component axes (IPCAs) against each other with IPCA2 as the ordinate and IPCA1 the abscissa. The GGE biplot does not provide information on the environment main effects but it has been successfully used to classify QPM inbred parents into heterotic groupings by Bhatnagar et al. (2004). Currently there is debate between AMMI proponents (Gauch Jr, 2006; Gauch Jr et al., 2008) and GGE proponents (Yan et al., 2007) on the issue of superiority between the two techniques. The method chosen for this study was the AMMI technique mostly because it combines both the advantages of the principal component analysis and the ANOVA technique.

1.9.2 Genotype x environment studies in QPM germpalsm

The analysis of QPM germplasm for response to GE using the AMMI model would reveal the patterns of stability and specific adaptation to local environments of the QPM hybrids constituted from the locally available elite QPM germplasm. One of the few reported GE studies on QPM on the African continent is that of Hohls et al. (1995). This study managed to identify adapted inbred lines and crosses using an adaptation of the less used Hohls et al. (1994) genetic models into joint regression analysis combined with the Gail and Simon (1985) test. The germplasm involved in the study of Hohls et al. (1995) was well adapted to South African environments but not well adapted to the subtropics and tropical environments where most of the malnutrition occurs. Furthermore due to intellectual property rights the germplasm is relatively less accessible.

The ANOVA, linear regression and AMMI techniques were used by Pixley and Bjarnason (2002) in the analysis of both grain yield and kernel endosperm modification in QPM germplasm whilst Bhatnagar et al. (2004) used the GGE biplot. Pixley and Bjarnason (2002) found that open pollinated cultivars (OPCs) were relatively more stable for grain yield followed by double cross hybrids, three way hybrids and single cross hybrids but the OPCs were less stable for kernel endosperm modification. This has implications for QPM breeders serving the smallholder farmers in Africa. It can be difficult to get QPM open pollinated cultivars accepted by the smallholder farmers because of unstable modification which can often result in undesirable kernel appearances.

The kernel quality traits studied by Pixley and Bjarnason (2002) namely protein content, tryptophan content, tryptophan concentration of protein (Quality Index) and endosperm modification were found to be generally stable in both hybrids and OPCs across the target environments using stability measures of Eberhart and Russell (1966). This stability if it can be attained under local conditions, would give hope to the solving of malnutrition problems in poor communities of Africa where maize is the staple crop.

Across the world there is scarcity of literature on genotype x environment interaction and adaptation of QPM materials, particularly under subtropical conditions. The analysis of genotype by environment would reveal the patterns of adaptation of elite QPM inbred lines for various traits. This would facilitate the maximum exploitation of the agronomic potential of the QPM genotypes when the genotypes are deployed to farmers in their environments of specific adaptation.

1.10 Pollen contamination of QPM by normal endosperm maize

Successes and achievements in both the subject of farmer participation, and the subject of genetics and breeding of QPM alone, are not sufficient to spur an increase in the level of adoption of QPM technology. This is because the other obstacle is the myth that the nutritional advantage of QPM crops ceases to exist due to foreign pollen contamination when the QPM crops coexist with normal endosperm maize crops. This is related to the skepticism on performance of QPM varieties mentioned by Vivek et al. (2008). Therefore, there is need to study the potential of coexistence of QPM and normal endosperm maize crops under conditions not designed to absolutely prevent cross pollination or intermating. Lauderdale (2000) cites fear of contamination of QPM crops by normal endosperm maize varieties as a major obstacle to QPM adoption. Vasal (2002) implored that there is need to develop studies and strategies for the prevention of contamination of QPM crops by normal endosperm maize pollen. In addition he advocated for the development of simple procedures to distinguish QPM grain from normal endosperm maize grain. Also the absence of a premium on QPM grain discourages maize growers from venturing into QPM production especially when the general belief is that QPM cultivars are not as competitive as normal endosperm maize cultivars for grain yield. These concerns are evidence of how the problem of normal maize endosperm pollen contamination is a deterrent to the adoption of QPM varieties and as long as the precise contamination levels that occur in QPM fields during coexistence with normal endosperm maize pollen remain unknown, QPM proponents would not be confident to encourage the adoption of QPM varieties under conditions of coexistence with normal endosperm maize varieties.

1.10.1 Studies on foreign pollen contamination of QPM

Literature on contamination of QPM crops by normal endosperm maize crops is limited and most of the recommended practices for producing QPM under coexistence with normal endosperm maize are based on either seed production practices reported in Havazvidi (1990) and Beck (2004) or results from the isolation studies of Sears and Stanley-Horn (2000), Burris (2001), Brookes et al. (2004), Halsey et al. (2005) on the coexistence of transgenic crops and normal endosperm maize. The only reported study on pollen contamination is that of Twumasi-Afriye et al. (1996).

Confronted by the belief that the nutritional advantage of QPM is lost in farmers' production plots mainly because of their small areas that expose the bulk of the crop to normal endosperm maize pollen contamination, Twumasi-Afriye et al. (1996) conducted experiments in Ghana to estimate the level of contamination in a white endosperm QPM crop. The highest level of contamination in the QPM crop obtained from two cropping-seasons data was 11% (Vivek et al., 2008). The QPM crop was conventionally grown and completely surrounded by a yellow normal endosperm maize crop of the same maturity. The normal endosperm maize crop and the QPM crop were allowed to cross freely at flowering. Details of separation distances between the adjoining rows of the two different crops were not given but it was observed that severe contamination occurred within the nearest 12m from the normal endosperm maize crop. Rat-feeding experiments were conducted to compare the nutritional quality and no significant differences were detected between the bulked grain from the most contaminated plot and the pure QPM grain.

Although Twumasi-Afriye et al. (1996) study managed to estimate the average level of contamination and also observe that severe contamination was within the first 12m from the normal endosperm maize crop, the study was not able to present the detailed pattern and distribution of contamination in each QPM field in a single realisation. Thus it is important to be able to visualise the pattern and relative distribution of foreign pollen contamination so that recommendations for farmer practices can be made with more confidence and conviction.

1.10.2 Spatial analysis and geostatistical techniques

The description of the spatial variation of a variable or phenomenon occurring in a random manner can be achieved through two groups of interpolation methods which are deterministic interpolation methods, and geostatistical methods (Johnston et al., 2001). The deterministic interpolation methods are inverse distance weighting (IDW), global polynomial interpolation,

local polynomial interpolation and radial basis functions whilst some of the classical geostatistical techniques are from the kriging family and these are simple kriging, ordinary kriging, universal kriging, indicator kriging, multiple indicator kriging, disjunctive kriging and lognormal kriging (ESRI®, 2001; Isaaks and Srivastava, 1989; Webster and Oliver, 2001). Kriging is a procedure where data collected from minimum scattered sampling points is used to estimate values at unsampled points resulting in optimal interpolation of the spatial distribution of the measured variable. This makes kriging appropriate in the study of foreign pollen contamination in fields of QPM where it is difficult to measure the level of contamination on every single ear.

Kriging makes use of the linear least squares estimation procedure (Tonkin and Larson, 2002). The advantages of kriging are that it is a robust method that reliably provides the best linear unbiased estimator, minimum estimation variances and optimal interpolation (Dubrule, 1983; Dubrule, 1984; Laslett et al., 1987; Oliver and Webster, 1990; Royle et al., 1981). Unlike other interpolation techniques kriging is able to provide both prediction maps and prediction error maps. This is important as it provides a measure of the degree of reliability of the results obtained.

Kriging has been used in mining (Emery, 2005; Richmond, 2003), hydrogeolology (Ahmadi and Sedghamiz, 2007; Gundogdu and Guney, 2007; Tonkin and Larson, 2002; Zimmerman et al., 1998), environmental science (Bayraktar and Turalioglu, 2005; Mutanga and Rugege, 2006; Oliver and Webster, 1990), natural resources (Emery, 2005; Olea, 1974), remote sensing, forestry science (Zas et al., 2007), black box modeling in computer experiments (Sacks et al., 1989), agriculture (mostly crop yield estimates) (Oliver and Webster, 1990) and soil properties (Laslett et al., 1987; McBratney and Webster, 1986). In all these applications the condition is that the variable of interest should be regionalised or spread out in space as described by Matheron (1965) quoted by Laslett et al. (1987). The distribution of pollen contamination satisfies these requirements although the plants that are contaminated are not continuous as other attributes like air pollution, groundwater, soil pH or mineral deposits. Hence, the kriging technique currently provides the best method to estimate pollen contamination in QPM fields.

There are no known reports on the application of kriging in the study and analysis of spatial variation of cross pollination by yellow normal endosperm maize pollen in maize crops. This study is the first time that kriging is being considered for decision making in seed sciences,

breeding, and genetics in relation to the dissemination of QPM varieties. The generation of information on statistics and graphs representative of the typical spatial variation of foreign pollen contamination in a QPM crop growing in a typical maize producing area in Zimbabwe and elsewhere would help extension agents, agronomists, seed scientists, and breeders, in advising farmers about nutritional advantages that can be gained through QPM varieties dissemination, adoption and utilisation.

1.11 Nutritional value of contaminated QPM

The nutritional advantage of QPM over normal maize is due to the presence of the simply inherited recessive *opaque-2* gene that confers the production of double the quantities of both lysine and tryptophan over normal endosperm maize (Mertz et al., 1964). The nutritional value of contaminated QPM is uncertain due to xenia effects of normal maize pollen gene on the single recessive *opaque-2* gene leading to the loss of nutritional superiority of normal endosperm maize pollen contaminated kernels. Xenia is the immediate effects of a foreign parent on nonmaternal tissue of the kernel (Kiesselbach, 1960). Literature on nutritional value of contaminated QPM is limited and the subject has not been researched much except for the work of Twumasi-Afriye et al. (1996) in Ghana that reported nutritional superiority of QPM up to a maximum of 20% inclusion of normal endosperm maize grain in a mixture of QPM and normal endosperm maize grain. The need to promote QPM in other countries and regions certainly demands that more studies of the nutritional value of contaminated QPM be conducted under different settings so that the stability of the QPM trait is thoroughly investigated for the benefit of correctly advising farmers and other stakeholders in the QPM value chain. Furthermore xenia were found to be influenced by the environment (Weiland, 1992) and therefore the influence under environments in Zimbabwe could be found to be different under environments in Ghana. Therefore, it is recommended that this gap be addressed through investigations into the nutritional value of contaminated QPM under local growing conditions. Certainly this would boost the confidence of the scientists and agronomists in advising farmers.

1.12 Summary

The importance of diagnosing the genetic architecture of the available breeding materials was highlighted. The gene action involved in most traits of economic importance in QPM varieties is of a quantitative nature except for the expression of the *opaque-2* gene which is recessive to the normal endosperm maize wild type gene. Both additive and non additive genetic variances were previously reported as important in the control of grain yield in QPM. Kernel endosperm quality traits were found to be mostly under the influence of additive genetic effects. Study of genetic

effects requires the utilisation of mating designs of which the diallel design is one of them. There are various forms of the diallel but since kernel endosperm traits are of equal interest compared to agronomic traits in QPM germplasm, diallel designs that measure reciprocal-cross differences were considered appropriate because of the need to precisely measure the genetic effects without confounding other forms of genetic effects. Most of the studies reviewed were based on other environments which are not in Southern Africa. Relatively less effort has been expended in research on QPM relative to normal endosperm maize varieties and as a result adapted QPM varieties are not prevalent in most sub-Saharan Africa regions. Therefore, there is not much evidence on the superiority or competitiveness of QPM relative to normal endosperm maize under local conditions. However, currently there is elite QPM germplasm available to the public in the sub-Saharan Africa region. It would be prudent to determine the agronomic potential of the QPM germplasm in the form of combining abilities using the diallel analysis of Griffing Method 3. Taking into consideration the dynamic nature of weather conditions in maize growing environments of sub-Saharan Africa that causes genotype by environment interactions the growing of multienvironment trials is arguably one of the approaches to pursue since environmental influences were previously experienced in the performance of QPM varieties in some genetic backgrounds. The AMMI technique was proposed as the method of choice for studying the adaptation of the QPM germplasm under the set of environments in this study. There is limitation of literature on the research of both the levels of contamination that can occur in QPM under coexistence with normal maize varieties, and the nutritional value of the mixture of contaminated QPM grain and pure QPM harvested from the coexisting plots. Both sets of information are critical in determining whether to recommend the production of QPM varieties under conditions that do not guarantee absolute isolation. Addressing the gaps identified in this review is expected to generate information and genotypes that would enhance the adoption and production of QPM by the nutritionally disadvantaged communities.

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2 Farmers' preferences and perceptions of maize varieties and their implications on development and dissemination of quality protein maize (QPM) varieties in Zimbabwe

Abstract

Quality protein maize (QPM) technology is relatively new in Zimbabwe and farmer awareness of QPM was low. Participation of smallholder farmers in the development and setting up of QPM breeding objectives and dissemination strategies was solicited through participatory rural appraisal (PRA) techniques. A total of 76 farmers involved in the Mother Baby Trial (MBT) focus groups in four villages from three districts (Zvimba, Wedza and Murehwa) of Zimbabwe participated. The PRA techniques used included work-sharing, village or resource mapping, venn diagramming, semistructured interviewing, matrix scoring and ranking and pairwise ranking. The results suggest that protein malnutrition was prevalent in the districts. Maize was the most important food crop and the three types of seed planted, namely landrace ("Hickory King"), open pollinated varieties (OPV) and hybrid varieties were normal endosperm maize. Hybrids were dominant and were produced mainly for sale, while "Hickory King", although not supported by the seed system, continued to be produced for home consumption because of its superior taste, white colour, large kernel size, high kernel density, kernel hardness, and resistance to weevils. Its lateness and susceptibility to foliar diseases were the main disadvantages of the variety. The ideal maize variety should be one with high yield potential, early maturity, drought tolerant, foliar disease resistant and pest tolerant (stem borers). For any QPM variety to be acceptable, farmers expected it to combine the agronomic attributes of hybrids and the quality characteristics of "Hickory King". To effectively promote the adoption and dissemination of QPM, farmers thought the Agricultural Research and Extension (AREX) arm of government would be the ideal conduit.

2.1 Introduction

As in many countries in southern Africa, maize is a staple food crop in Zimbabwe (Rusike, 1998). Nearly all the varieties cultivated are normal endosperm maize and, hence, deficient in two essential amino acids, lysine and tryptophan. With the development of quality protein maize (QPM) varieties, there is hope for the provision of an affordable source of balanced protein to millions of inhabitants of the maize growing regions (Graham et al., 1990; Vasal, 2002). The dissemination and adoption of QPM is still lagging behind normal endosperm maize especially in regions such as sub-Saharan Africa where it is needed most. In this region, total maize area is estimated at 22 million hectares (FAOSTAT, 2004), and only 1% (or 200 000 hectares) is

under QPM (Krivanek et al., 2007) yet the agronomic practices of both normal endosperm maize and QPM are similar (Vasal, 2001).

Anthropometric measures of morbidity, wasting away, stunting and underweight in children aged zero to five years classified Zimbabwe as one of the countries with a high risk of malnutrition in sub-Saharan Africa yet QPM varieties utilisation is still in the “embryonic” stage. Protein-energy malnutrition is one of the factors contributing to the undesirable anthropometric measures and in Zimbabwe 16.6% of the children were found to be underweight, 29.4% stunted, 6.4% wasted and only 27.6% of the children were breastfed up to 24 months (UNICEF, 2006). On the other hand QPM is known to possess about 80% of the biological value of cow milk (Bressani, 1992). This, therefore, means that there is a nutritional gap in the infant age group that can be filled up with QPM complemented by other interventions that can be implemented to improve nutrition security.

In Zimbabwe currently, there are no commercial QPM cultivars available for the farmers. The first step in assisting farmers in accessing QPM grain is to develop the cultivars. Least cost cultivar development requires setting up of breeding goals, objectives and strategies according to those for normal endosperm maize since the two types of maize have similar agronomy (Vasal, 2001). This requires the use of secondary data to establish a general direction of the breeding objectives for the environment in Zimbabwe.

However, unlike other technologies, adoption of agricultural productivity improvement technologies is overly constrained by social and cultural contexts when dealing with smallholder farming communities. The need for the involvement and participation of farmers in the development of new crop varieties for smallholder farmers was highlighted by DeVries and Toenniessen (2001). The importance of cowpea grain quality characteristics in targeting research was confirmed by Langyintuo et al. (2004) in a study of consumer preferences in West Africa. According to DeVries and Toenniessen (2001), farmers should be involved in all aspects of variety development that include priority setting, early generation breeding, variety testing and selection so that breeders obtain regular input from farmers that enables them to structure their selection indices accurately. Based on empirical results, Langyintuo et al. (2004) recommended that cowpea breeding programmes for the Ghanaian market should emphasise black eye colour but those for the Cameroonian markets should avoid black-eyed grains. In Malawi, because of poor interactions with the farmers, breeders of the national maize programme produced

improved high yielding dent varieties of poor grain characteristics such as milling qualities and consequently not adopted by farmers who prefer flint grains for home consumption (Smale and Heisey, 1994). Also Derera et al. (2006) reported the continual use of landraces by the farmers in the eastern belt of Zimbabwe despite the availability of new and high yielding hybrid varieties.

The use of secondary data alone in setting QPM breeding objectives and dissemination strategies has been criticised by DeVries and Toenniessen (2001) but could still be adopted under budgetary limitations. According to Rubey et al. (1997), three approaches can be pursued to incorporate farmers' preferences in the early stages of research planning. These include "political approach," the "presumptive approach," and the survey approach. They asserted that both the political approach where the interests of the most powerful users is catered for, and the presumptive approach where researchers implicitly and explicitly make assumptions about what the users need were found to be associated with high costs in designing maize breeding programmes. The potentially high costs stem from the fact that the produced variety could easily be unacceptable to the users (Smale and Heisey, 1994) and also some essential genetic material could be erroneously excluded from the active genetic pool because of incorrect notions about what the users want. Due to these limitations, Rubey et al. (1997), suggested an alternative approach for incorporating maize users' preferences into breeding strategies by using survey data on consumer preferences.

Earlier on Chambers (1994a) advocated for the use of the PRA technique, arguing that the technique empowers the people to generate, analyse, share and own the generated information contrary to the views of Gladwin et al. (2002) that PRA techniques were necessary but not sufficient because they overlook the heterogenous nature of farmer behaviour and there is no procedure to validate the universality of conclusions reached or constraints identified. An example of where QPM was introduced successfully in Africa is in Ghana where the promotional activities involved the national policy makers (State President), health ministry, research and extension departments, Sasakawa Global 2000, and radio and television broadcasts (Twumasi-Afriye et al., 1996). In addition the researchers in Ghana disapproved several myths about QPM and linked up very well with both the seed producers and industrial users of QPM grain. It is not indicated whether they conducted PRAs but their approach involved creating a good rapport with all the stakeholders involved in the production and utilisation of "Obatampa" (QPM). "Obatampa' literally means the good nursing mother.

Although Gladwin et al. (2002) recommended combining participatory approaches with scientific rigour and testing, several reports indicate that participation was sufficient to guarantee favourable outcomes of projects (Cleaver, 2001; Godfrey and Obika, 2004; Narayan, 1993; Pretty and Voudouhue, 1996; Prokopy, 2005). Can the participatory rural appraisal technique be used successfully to set QPM breeding goals and objectives in Zimbabwe? The postulation was that farmers have preferences and percepetions on maize varieties and these have implications for QPM varieties breeding and dissemination. The objective of the study was to solicit the participation of smallholder farmers in the development and setting of QPM breeding goals, objectives and dissemination strategies. The approach served to preempt any potential problems that might arise in the adoption of QPM cultivars by smallholder farmers in Zimbabwe.

2.2 Methodology

The convenience sampling approach (Saunders et al., 1997) was used to select Murehwa, Wedza, and Zvimba districts out of the 52 districts in Zimbabwe based on their proximity to Harare, the base station for CIMMYT Zimbabwe. The three districts are located in agroecological zones (Natural Region II) where the rainfall is above 700mm per annum and, hence, are suitable for maize production. In these districts, some "embryonic" work on QPM had also been conducted in the previous cropping seasons through the Mother and Baby Trial[2] (MBT) activities organised by the department of Agricultural Research and Extension (AREX).

In each district, a purposive sampling approach (Saunders et al., 1997) was used in identifying the groups of farmers for the PRA exercises. Groups of farmers that participated in the 2006/7 MBT were targeted as the nucleus but those that had not participated in the MBT were also included. The earlier group of farmers was expected to have some knowledge about QPM because a QPM variety was included in the MBT experiments. For the convenience of the farmers and ease of contrasting the phenotype of a typical QPM hybrid variety to normal maize plants, extra plots of ZS261, a QPM hybrid variety were planted in all Baby Trial sets that initially did not have a QPM variety.

[2] **Mother Baby Trial design**: A design where the mother trial contains all the entries in a trial and the baby trials are made up of the different entries organised according to the incomplete blocks that make up the mother trial. The mother trial is managed by researchers and each of the baby trials is managed by a different host farmer. The performance of the entries in the mother trial is systematically cross checked against the performance in the baby trial. The smaller baby trial is easily understood and managed by the farmer.

The PRA exercises were conducted during May to June 2007 when most smallholder farmers were expected to be less busy. The districts, the villages, and the numbers of participants are listed in Table 2.1.

Table 2.1 Selected districts, villages and number of farmer participants for PRA activities

District	Village	Number of farmers	
		Female	Male
Zvimba	Njiri	8	8
Wedza	Payarira	18	7
Murehwa	Chiweshe	6	4
Murehwa	Chidawaya	15	6

2.2.1 Data collection

The opening of PRA meetings in all the four villages followed a standard procedure starting with formal discussions with AREX staff on the objectives of the PRA and introduction of the PRA team to the farmers by the AREX staff followed by the subject of QPM by the facilitator. Highlights of the introduction were awareness of the existence of QPM, the nutritional advantages of QPM, and the need for isolation from normal maize crops in order to maintain the nutritional superiority of QPM over normal maize. Farmers were then asked to highlight any unusual aspect that they noticed about ZS261, the QPM variety that was included in the trials.

The following techniques by Chambers (1994a), Cornwall et al. (1993) and Pretty et al. (1995) and cited by Campbell (2002) were used to collect the data:

a) Worksharing: harvesting of MBT trials together with the farmers so as to create a rapport between the researchers and the farmers
b) Village or social or resource mapping was used by farmers to introduce their environments and surroundings to the PRA facilitators and in the process created a good rapport between the two groups. The establishment of a good rapport was paramount for the conduct of the later activities.
c) Venn diagrams were employed in the identification of important institutions in the maize production and consumption systems.
d) The identification and ranking of problems and constraints related to maize production and utilization was done using matrix ranking and scoring (Figure 2.1). Farmers' perceptions about the relative importance of the problems and constraints were

established for each village. Where applicable, pair wise ranking techniques were employed. The role and significance of different varieties grown by the farmers and their attributes were investigated using either matrix ranking and scoring, or pair wise ranking techniques.

e) Semi-structured interviews were conducted to investigate the nature and extent of problems and for probing to get more information on various subjects of discussion.

f) For PRA activities or exercise where the farmers' groups were too large the groups were split according to gender for easier management of discussions within a village.

g) Farmers' preferred modes / means of disseminating QPM technology information were investigated through the pairwise ranking technique.

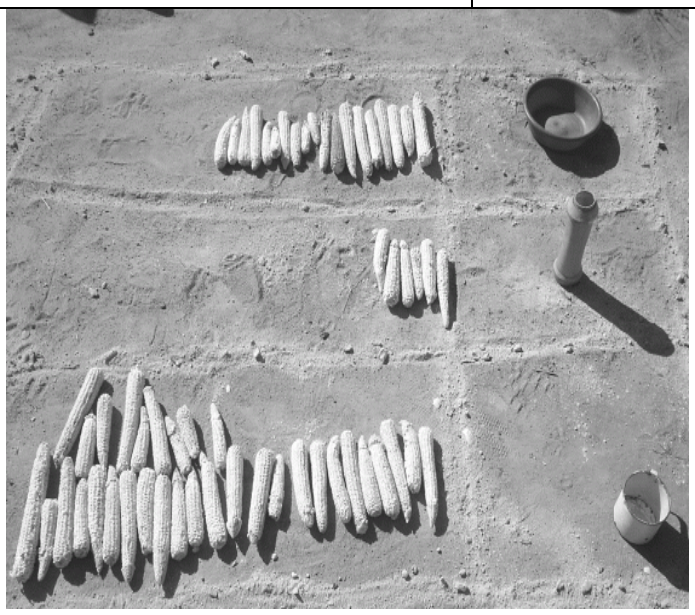
Seed Type	Scores using maize cobs cores	Key or Object
Improved Open Pollinated Variety (OPV) – ZM521, ZM521		
Local Variety – Landrace ('Hickory King")		
Hybrid		

Figure 2.1 An example of how the scores in Table 2.4 were obtained. The picture is showing Women farmers' matrix scores for preference of maize seed types in Payarira village (Small number of maize cobs cores indicate less preference and large number higher preference)

2.3 Results

The discussions during the opening of the PRA meetings indicated that there was low awareness of QPM in all the villages. Interviews with Nutrition / Health workers in the Njiri and Payarira villages confirmed the prevalence of protein malnourishment which could potentially

lead to *kwashiorkor*[3]. The farmers in all the four villages were enthusiastic about the possibility of growing QPM and all were committed to provide the necessary isolation requirements to prevent contamination from normal maize

2.3.1 Relative ranks of crops

The crops grown in each of the villages and their relative rankings are presented in Table 2.2. Although farmers recognised more or less the same crops, their perceptions of the importance of most crops judged by their relative rankings differed from village to village. Maize (*Zea mays* L) was the most important in all villages followed by groundnuts (*Arachis hypogea* L.) except in

Table 2.2: Relative rankings of crops grown in each of the four villages

Crop	Payarira	Njiri	Chidawaya	Chiweshe
Bambara groundnut	3	10	5	9
Bean	8	6	7	8
Cowpea	4	5	6	7
Cucumber	9	*	*	*
Groundnut	2	2	2	3
Maize	1	1	1	1
Okra	9	7	*	*
Finger millet	9	3	*	2
Sorghum	9	9	8	6
Soya bean	7	11	4	*
Sunflower	9	8	*	5
Sweet potato	4	4	3	4
Tobbacco	9	*	*	*

* Crop not listed in village; 1 = most important; 9 = least important.

Chiweshe where groundnut was third to finger millet (*Eleusine coracana* L.), a crop that was the third most important in Njiri, but ranked very low in Payarira, and was not listed in Chidawaya. Sweet potato (*Ipomea batatas* L.) ranked third in Njiri but fourth in the other three villages. Other crops not listed in some villages were cucumbers (*Curcubit spp*), tobacco (*Nicotiana tabacum*),

[3] **Kwashiorkor** is protein calorie malnutrition which can lead to infant morbidity and mortality. It disables the immune system such that the child is susceptible to a host of infectious diseases. The term is from Ivory Coast where it means the deposed child (weaned off) Source:MedicineNet.com. 1998. Webster's New World Medical Dictionary [Online]. Available by MedicineNet.com *http://www.medterms.com/script/main/art.asp?articlekey=4124* (posted 12 October 2008).

sunflower (*Helianthus annus),* soyabeans (*Glycine max* L.) *and* okra (*Hibiscus esculentus* L.). Bambara groundnut (*Vigna subterranea*), a crop that is both rich and balanced in protein was ranked lowly in all the villages except in Payarira village where it was ranked as the third most important. Crops such as cowpeas (*Vigna unguiculata*), and sorghum (*Sorghum bicolor* L.) were not ranked highly among the farmers.

2.3.2 Maize varieties grown by the farmers

Hybrid SC513, improved OPV ZM521 and the landrace "Hickory King" appeared popular among farmers in all the villages. Note that all the varieties listed in Table 2.3 were not QPM. With the assistance of the PRA facilitator, farmers grouped the varieties into Hybrid, OPV landrace, and improved OPV.

Table 2.3: Varieties of maize grown in each of the four villages

Seed type	Variety	Payarira	Njiri	Chidawaya	Chiweshe
Hybrid	AC 31	√		√	√
Hybrid	AC71	√		√	
OPV, Landrace	Hickory King	√	√	√	√
Hybrid	PAN 413		√		√
Hybrid	PAN 6479	√			
Hybrid	PAN 67		√		
Hybrid	PHB 30G19	√			
Hybrid	PHB 30G97				√
Hybrid	PHB 3253	√			
Hybrid	R 201	√			
Hybrid	SC 403	√			√
Hybrid	SC 411	√	√		
Hybrid	SC 513	√	√	√	√
Hybrid	SC 517	√			√
Hybrid	SC 628	√	√		
Hybrid	SC 633	√		√	
Hybrid	SC631			√	
Hybrid	SC635		√	√	
Hybrid	SC636		√		
Improved OPV	ZM 521	√	√	√	√
Improved OPV	ZM421		√	√	

Key: √ = variety grown by villagers; OPV = Open Pollinated Variety

2.3.3 Matrix scoring for the different seed types

The scores and ranks of type of seed maize varieties grown by the farmers are presented in Table 2.4. In all the groups, the hybrid variety was the most preferred type of seed except for the male farmers in Chidawaya who preferred OPVs. The reasons for their preference for hybrid

varieties included high yield potential, good tolerance to drought, good levels of resistance to diseases and insect pests, availability and easy access of seed in adequate quantities, and well adaptability to their environments. The group that preferred the OPVs argued that the varieties were drought tolerant. OPVs and "Hickory King" were ranked second in four and three occasions, respectively. The reasons for ranking OPVs second included their earliness to maturity thereby providing early harvests to poor farmers and the possibility of recycling the seed to save money while "Hickory King" was perceived to have desirable taste, higher flour retention when dehulled before milling, extremely white flour for making white "sadza", denser and bigger kernels, better resistance to kernel weevils than improved seeds, stable yield levels year after year, and recyclable seed.

Table 2.4 Translated results of maize cobs cores scores in each of the villages

Village	Gender	Type of seed	Score	Rank 1	Rank 2	Rank 3
Njiri	**F**	**Hybrid**	12	Hybrid		
		OPV	5		OPV	
		"Hickory King"	3			"Hickory King"
	M	**Hybrid**	10	Hybrid		
		OPV	4			OPV
		"Hickory King"	6		"Hickory King"	
Payarira	**F**	**Hybrid**	33	Hybrid		
		OPV	19		OPV	
		"Hickory King"	6			"Hickory King"
	M	**Hybrid**	15	Hybrid		
		OPV	1			OPV
		"Hickory King"	4		"Hickory King"	
Chiweshe	**F and M**	**Hybrid**	16	Hybrid		
		OPV	8		OPV	
		"Hickory King"	3			"Hickory King"
Chidawaya	**F**	**Hybrid**	10	Hybrid		
		OPV	8		OPV	
		"Hickory King"	5			"Hickory King"
	M	**Hybrid**	14			Hybrid
		OPV	33	OPV		
		"Hickory King"	21		"Hickory King"	

N.B. The total number of maize cobs cores used for each village was decided by the farmers. F = Female; M = Male.

Farmers were less confident about the agronomic performance of "Hickory King" but rather more confident about the desirable quality attributes of its kernels. "Hickory King" was ranked third in groups where farmers thought the landrace variety was relatively late maturing or its maturity rarely fitted well in their growing seasons and seed was not readily available in the

agro-dealer shops. Most farmers who grew “Hickory King” successfully planted it in the months of September and October in the vegetable gardens where they could both irrigate the crop and protect it from the animals. An unsubstantiated claim from the farmers who ranked “Hickory King” at third position was that the Grain Marketing Board – the sole official buyer of maize grain in Zimbabwe, was not accepting “Hickory King” grain, and so farmers that grew lots of it could not have markets for it.

It was intriguing to find out that although “Hickory King” was discontinued by the seed authorities and companies more than 20 years ago, it was still being “secretly[4]” produced in all the four villages mainly for home consumption whilst the hybrid varieties were being grown for sale to the Grain Marketing Board. Farmers openly confessed that “Hickory King” was their most preferred variety but lamented that it was late maturing and the seed quantities were hardly adequate.

To maintain good quality seed of “Hickory King” since it is not produced by seed companies, farmers indicated that they planted it in the relatively small gardens using both temporal and spatial isolation strategies. When planted in the fields, “Hickory King” could easily be contaminated by yellow maize but fortunately the flowering period of “Hickory King” and most other varieties did not overlap because of the different times of planting. At harvest the farmers would select well filled eight rowed ears for use as seed for the following cropping season. In storage, some of the farmers treated their “Hickory King” seed with “Shumba-Cooper” a grain storage pesticide containing fenitrothion *(O,O-Dimethyl O-(3-methyl-4-nitrophenyl) phosphorothioate)* and deltamethrin (*(S)--cyano-3-pehoxybenzyl(1R)-cis-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane carboxylate)* as active ingredients. Some farmers mixed the shelled seed with fine chaff from finger millet (*Eleusine coracana* L), whilst others simply hung the cobs above fireplaces until the next planting season[5].

Further investigation on farmers’ claim of resistance to weevils revealed that weevil-damaged seed lots of “Hickory King” germinated better than weevil-damaged hybrid seed lots because

[4] Secretly because the official position has been 100% maize area in Zimbabwe is planted to hybrids Rusike, J. 1998. Zimbabwe, p. 303 – 319 *In* M. L. Morris, ed. Maize Seed Industries in Developing Countries. Lynne Rienner Publishers in association with CIMMYT.

[5] The practice was investigated and found to be beneficial to indigenous maize seeds by Modi, A. 2002. Indigenous storage method enhances seed vigour of traditional maize. South African Journal of Science 98:138-139. and Modi, A. 2004. Short term preservation of maize landrace seed and taro propagules using indigenous storage methods. South African Journal of Botany 70:15-23.

the larger size of the “Hickory King” kernels made it possible for the germ to escape the weevil damage. For this reason, “Hickory King” was treasured like gold in Payarira village: Those who had the seed did not want to share it with others because they claimed that it was their secret weapon to guarantee their being considered the best farmers in their area. In Chidawaya village the farmers liked the “Hickory King” variety to the extent that it is affectionately known as “Mabhagu” meaning big.

Two main reasons why farmers would not plant their entire maize area to “Hickory King” despite their likeness for the variety were its late maturity and it not being accepted by the Grain Marketing Board. In Chidawaya village, the farmers estimated that when “Hickory King” flour is produced through the refined milling process the farmers were able to retain 75% of the original volume of grain whereas with the hybrid varieties and open pollinated varieties only 50% of the original volume of grain was retained as flour. Thus the farmers perceived that the other maize types lose more endosperm material during refined milling than the “Hickory King” variety. In all the villages the farmers indicated that they did not like eating culinary products from hybrid varieties but those from “Hickory King” grain. The grain millers also charged relatively more to mill per unit of “Hickory King” because of its hard kernel endosperm.

2.3.4 Desirable traits in maize production

The list and the rankings of traits considered important in maize production and, hence, could be used in developing QPM varieties are presented in Tables 2.5, 2.6 and 2.7 for Njiri, Payarira, and Chidawaya villages respectively. Both Njiri village men and women farmers concurred in their rankings for earliness, but the men ranked drought tolerance second whilst the women ranked it third (Table 2.5). Yield potential was ranked second by the women whilst the men ranked it at third position. Seed price, disease resistance, and pest resistance were ranked fourth, fifth and sixth, respectively, by both groups. The farmers explained that earliness was the most important attribute because in their area earliness guarantees a high chance of getting at least a good harvest. They pointed out that even if a variety was drought tolerant but not early maturing it would not satisfy the need of ensuring that they did not run out of grain between two consecutive harvests. The women farmers wanted varieties that were in the extra early maturity category – earlier than the FAO 500 classification.

In Payarira village the farmers listed six attributes, namely, high yield potential, adaptation, medium maturity, high resistance to diseases, drought tolerance, and weevil resistance. By adaptation, the farmers meant the ability to grow well in their locality. A pairwise ranking

exercise was conducted as presented in Table 2.6. Yield potential was ranked as the most important attribute followed by medium maturity. Resistance to diseases was ranked third whilst adaptation fourth. Drought tolerance was rated lowly at position 5 with weevil resistance 6.

Farmers in Chidawaya village rated resistance to stem borer as the most important trait followed by both tolerance to drought and resistance to foliar diseases (Table 2.7). These were then followed by both yield potential and earliness in maturity. Weevil resistance was ranked sixth ahead of taste (organoleptic taste). On probing on the issue of stem borers being ranked as the most important trait it was found out that stem borers had caused a severe loss of stand in the Chidawaya area during the previous (2006/7) cropping season.

Table 2.5 Pairwise ranking of criteria for choosing maize seed varieties in Njiri Village

		Women							
Men	**Criteria**	**Yield potential (Y)**	**Earliness to maturity (EM)**	**Price (PR)**	**Disease resistance (D)**	**Drought tolerance (DT)**	**Pest resistance (P)**	**Total**	**Rank**
	Yield potential (Y)		EM	Y	Y	Y	Y	4	**2**
	Earliness to maturity (EM)	EM		EM	EM	EM	EM	5	**1**
	Price (PR)	Y	EM		PR	DT	PR	2	**4**
	Disease resistance (D)	Y	EM	PR		DT	D	1	**5**
	Drought tolerance (DT)	DT	EM	DT	DT		DT	3	**3**
	Pest resistance (P)	Y	EM	PR	D	DT		0	**6**
	Total	3	5	2	1	4	0		
	Rank	**3**	**1**	**4**	**5**	**2**	**6**		

Table 2.6 Pairwise ranking of important maize attributes by Payarira village farmers

Criteria	Yield potential (Y)	Adaptation (A)	Medium maturity (MM)	Resistance to diseases (RD)	Drought tolerance (DT)	Weevil resistance (WR)
Yield potential (Y)						
Adaptation (A)	Y					
Medium maturity (MM)	Y	MM				
Resistance to diseases (RD)	Y	RD	MM			
Drought tolerance (DT)	Y	A	MM	RD		
Weevil resistance (WR)	Y	A	MM	RD	DT	
Total	**5**	**2**	**4**	**3**	**1**	**0**
Rank	**1**	**4**	**2**	**3**	**5**	**6**

Table 2.7 Pairwise ranking of important maize attributes by Chidawaya village farmers

Criteria	Yield potential (Y)	Weevil resistance (R)	Drought tolerance (DT)	Taste (T)	Foliar diseases resistance (F)	Earliness to maturity (EM)	Stem borer resistance (SR)
Yield potential (Y)							
Weevil resistance (R)	Y						
Drought tolerance (DT)	DT	DT					
Taste (T)	Y	R	DT				
Foliar diseases resistance (F)	F	F	DT	F			
Earliness to maturity (EM)	Y	EM	EM	EM	F		
Stem borer resistance (SR)	SR	SR	SR	SR	SR	SR	
Total	**3**	**1**	**4**	**0**	**4**	**3**	**6**
Rank	**4**	**6**	**2**	**7**	**2**	**4**	**1**

The matrix scoring and ranking of the attributes for Chiweshe farmers is presented in Table 2.8. The top five attributes in Chiweshe village were yield potential, earliness to maturity, drought tolerance, disease resistance, and kernel size. These traits were considered as important integral attributes in the development of QPM varieties for the area.

Table 2.8 Relative rankings of important maize attributes by Chiweshe village farmers

Trait	Score using maize cobs cores	Rank
Earliness to maturity	39	2
Weevil Resistance	4	8
Yield potential	49	1
Drought tolerance	21	3
Low soil fertility tolerance	7	7
Good husk cover	4	8
Foliar disease tolerance	18	4
Lodging resistance	3	10
Resistance to termites	2	11
Big kernel size	13	5
Apomix+	9	6

+ Ability to set seed without sexual mating so that farmers avoid buying seed every season

2.3.5 Farmers' perception of the three different types of seed.

The perceptions of farmers in Payarira, Chiweshe and Chidawaya villages about the attributes in the three types of seed are presented in Table 2.9. The list of traits used in the analysis in each village was constructed by the particular village's corresponding group of farmers. In Payarira village there were six traits, and in Chiweshe village there were five traits whilst in Chidawaya village there were seven traits. Only three traits, yield potential, disease tolerance and drought tolerance were common across the three villages. Weevil resistance was common in Payarira village and Chidawaya village whilst earliness was common in Chiweshe village and Chidawaya village. Adapatation and medium maturity occurred in Payarira village whilst kernel size was listed as an important trait in Chiweshe, and taste and stem borer resistance were listed as important traits in Chidawaya village. Although kernel size and taste were identified as important in variety choice by only one village it emerged from discussions across all the four villages that "Hickory King" was perceived to have the most desirable taste, kernel size and kernel hardiness.

In Payarira village, Hickory King scored 5 for all the traits except for drought tolerance where it had a score of 4. They perceived hybrid varieties to be equal to OPV for disease tolerance but better than open pollinated varieties in all the other traits except drought tolerance and weevil resistance.

In Chiweshe village, open pollinated varieties were perceived to be inferior to both hybrids and “Hickory King” for all the traits except for earliness which was better than that for “Hickory King”. “Hickory King” was rated the best for both yield potential and kernel size. Farmers considered hybrid varieties as the best in terms of earliness to maturity and drought tolerance.

In Chidawaya village open pollinated varieties were scored 5 for all the attributes except for stem borer resistance, which was rated 4. Hybrid varieties were rated 5 for both yield potential and earliness but were rated the worst for weevil resistance, stem borer resistance and taste. Farmers considered “Hickory King” the best variety for stem borer resistance, taste, disease tolerance and weevil resistance but it had the lowest scores for yield potential, drought tolerance and earliness to maturity. Farmers in all the four villages indicated the attributes wanted in a QPM variety as extremely white, large, flat, hard, weevil resistant, relatively denser kernels with taste similar to that of “Hickory King” and with a relatively high yield potential.

Table 2.9 Farmers' perception about different categories of maize varieties

Village	Trait	Hybrid	OPV	"Hickory King"
Payarira Village	Yield potential	4	2	5
	Adaptation	4	1	5
	Medium maturity	5	2	5
	Disease tolerance	4	4	5
	Drought tolerance	3	5	4
	Weevil resistance	2	4	5
Chiweshe Village	Yield potential	4	3	5
	Earliness	5	3	1
	Drought tolerance	5	2	3
	Disease tolerance	3	2	4
	Kernel size	4	3	5
Chidawaya Village	Yield potential	5	5	3
	Weevil resistance	3	5	5
	Drought tolerance	4	5	2
	Taste	3	5	5
	Disease tolerance	4	5	5
	Earliness	5	5	3
	Stem borer resistance	3	4	5

Key: 1= poor and 5 = very good. N.B. There was no opportunity to conduct the ranking exercise in Njiri Village (Zvimba).

2.3.6 Important organisations in the production of QPM varieties

Figures 2.2 and 2.3, respectively, show the Njiri and Payarira village farmers' lists of organisations that are important in the production of maize and the promotion, adoption and production of QPM varieties. Using the venn diagramming technique, farmers demonstrated the relative importance of these organisations: The bigger the circle, the more important the organisation was perceived.

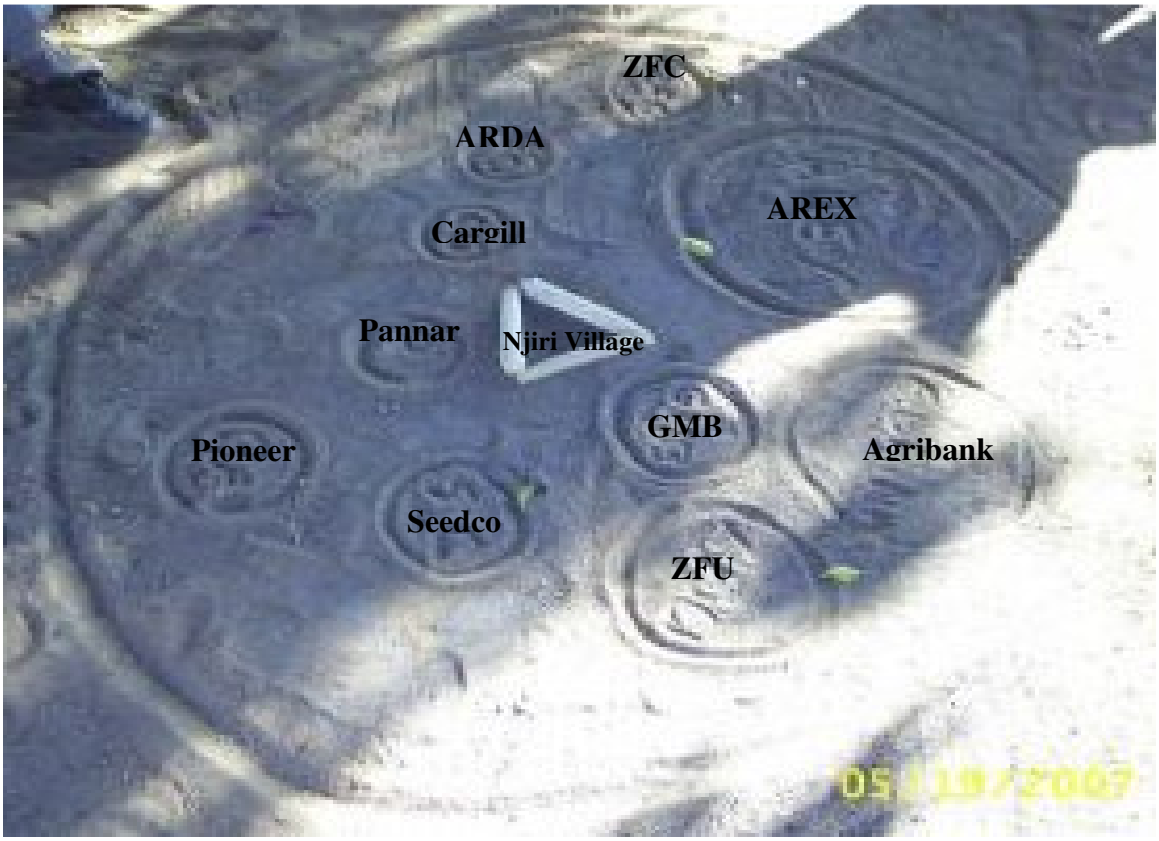


Figure 2.2 Venn diagram showing the relative importance of different organizations that the Njiri Village farmers perceived will be important in the promotion and adoption of QPM. (The triangle represented the village. The bigger the circle, the more important the organisation was perceived)

In the Njiri village farmers' opinion, AREX was the most important organization to them in the production of maize followed by Agribank[6], and then Zimbabwe Farmers Union (ZFU). The Grain Marketing Board, which provided them with inputs through the government input supply programmes, was ranked fourth followed by the Zimbabwe Fertiliser Company (ZFC) and Seedco, Pioneer, Agricultural and Rural Development Authority (ARDA), Cargill and Pannar, in that order. The fact that Agribank was ranked second showed the role that credit plays in these farmers' maize production systems. The farmers valued access to and availability of credit more than anything else except support from AREX. The farmers perceived SeedCo Ltd to be the most important seed company in their community and could be used as conduit for the promotion of QPM. In Njiri village, ZFU membership made farmers eligible for benefits that came through other

[6] Agribank is the state controlled financial institution that mostly finances farming activities in Zimbabwe.

organisations and hence it was considered the next important organisation after Agribank.

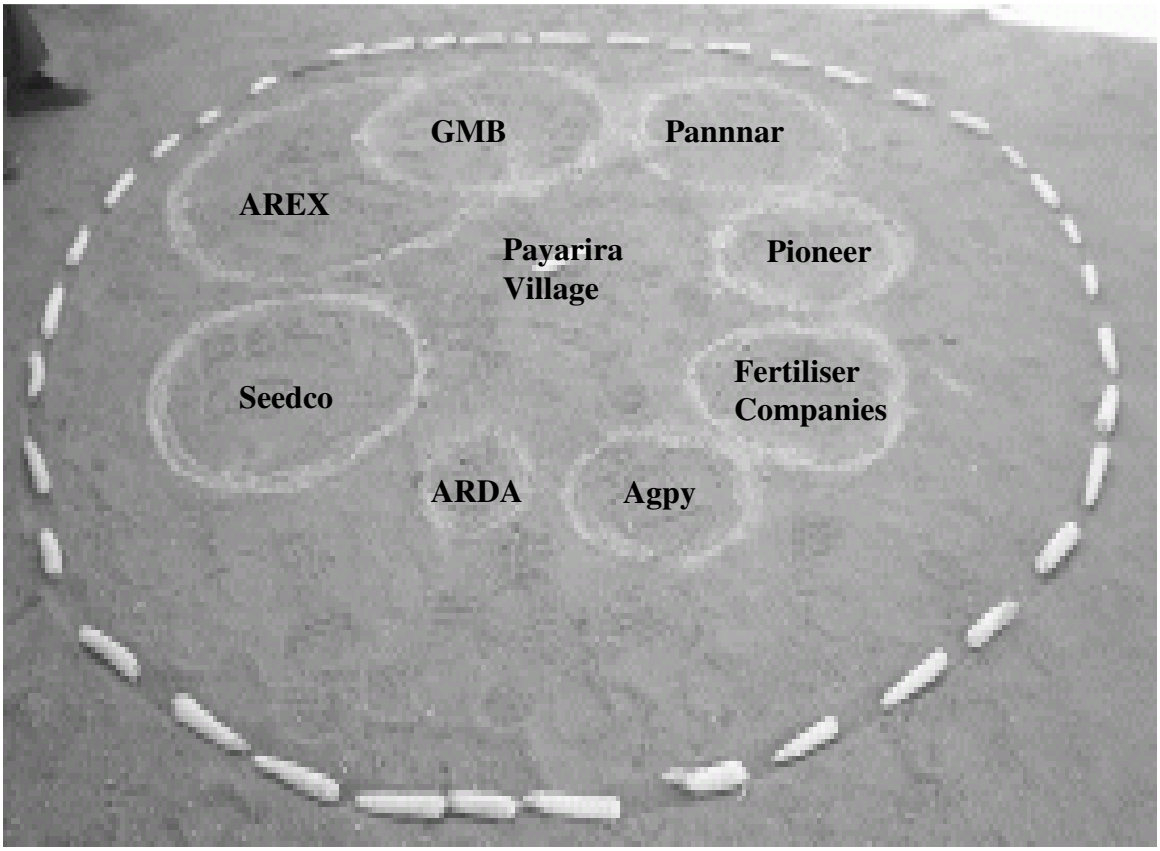


Figure 2.3 Venn diagram showing the relative importance of different organizations that the Payarira village farmers percieved to be important in the promotion and adoption of QPM. (The bigger the circle, the more important the organisation was perceived.)

In Payarira village (Figure 2.3) AREX was said to be the most important organization followed by GMB. According to the farmers, AREX interacted with GMB as shown by the intersection of the two circles. After GMB the farmers said Seed Co Ltd was the most important seed company followed by Pioneer, Pannar, Agpy and ARDA. The fertilizer companies were perceived to be playing a role bigger than that of Pioneer but smaller than that of Seed Co Ltd.

2.3.7 QPM information dissemination

The farmers in the four villages listed and ranked the ways and means of accessing new information in their area, and thus indicated information dissemination techniques or methods that could be useful in QPM promotion and adoption (Tables 2.10 -2.13). The Njiri village farmers in Zvimba district (Table 2.10) preferred getting information on QPM varieties from their local AREX representative followed by the radio and Cadec (a church funded non-governmental organisation). The farmers did not like the farmers' magazine as they strongly argued that it was difficult for them to access it and also they did not enjoy the task of reading the farmers' magazine.

Table 2.10 Pairwise ranking of preferred modes of QPM information dissemination by Njiri village farmers

	AREX	Radio	Televison set	Newspaper	Farmers Magazine	Church (Cadec)
AREX						
Radio	AREX					
Television set	AREX	Radio				
Newspaper	AREX	Radio	Television set			
Farmers Magazine	AREX	Radio	Television set	Newspaper		
Church (Cadec)	AREX	Radio	Church	Church	Church	
Total	5	4	2	1	0	3
Rank	**1**	**2**	**4**	**5**	**6**	**3**

The Payarira village farmers' most preferred way of accessing QPM information was through their local AREX officer (Table 2.11). This was followed by the radio and television. Newspapers were the least preferred sources of information claiming that they had no access to the daily newspaper or time to read it.

Table 2.11 Pairwise ranking of preferred modes of QPM information dissemination by Payarira village farmers

	AREX	Radio	Television set	Farmers Magazine	Newspaper
AREX					
Radio	AREX				
Televison set	AREX	Radio			
Farmers Magazine	AREX	Radio	Television		
Newspaper	AREX	Radio	Television	Farmers Magazine	
Total	4	3	2	1	0
Rank	**1**	**2**	**3**	**4**	**5**

The farmers in Chiweshe village preferred getting information on QPM technology through the local AREX representative followed by radio and television (Table 2.12). The farmers' magazine was the least preferred mode of information dissemination.

Table 2.12 Pairwise ranking of preferred modes of QPM information dissemination by Chiweshe farmers

	AREX	Radio	Television set	Farmers Magazine
AREX				
Radio	AREX			
Television set	AREX	Radio		
Farmers Magazine	AREX	Radio	Television	
Total	3	2	1	0
Rank	**1**	**2**	**3**	**4**

The Chidawaya village farmers' choice was the local AREX representative followed by the non-governmental organisation COMMUTECH (Table 2.13). The village councillor was ranked third and the radio fourth. The farmers' magazine was ranked on the fifth position whilst the television set medium was the least preferred at position six. The second choice for Chidawaya village, Commutech, was different from all the other villages' second choice of radio.

Table 2.13 Pairwise ranking of preferred modes of QPM dissemination by Chidawaya village farmers

	Commutech	AREX	Radio	Councillor	Television set	Farmers Magazine
Commutech						
AREX	AREX					
Radio	Commutech	AREX				
Councillor	Commutech	AREX	Councilor			
Television set	Commutech	AREX	Radio	Councillor		
Farmers Magazine	Commutech	AREX	Radio	Councillor	Farmers Magazine	
Total	4	5	2	3	0	1
Rank	**2**	**1**	**4**	**3**	**6**	**5**

2.3.8 Demystifying the term QPM

According to the Njiri village farmers' understanding about the subject of QPM and its potential benefits their local term for QPM was "Godzamhuri" which means family-nourisher. In Payarira village the farmers unanimously agreed on the term "Mupedzakwashi" which means kwashiorkor terminator. In both Chiweshe and Chidawaya villages of Murehwa, the farmers could not come up with a local term for QPM.

2.4 Discussion

In Njiri and Payarira village the authorities responsible for community health and nutrition noted the existence of the problem of malnutrition affecting the poor and the orphaned families in both villages, which was confirmed by the farmers. The immediate short term solution would be to implement feeding programmes. However, feeding programmes are often unsustainable because they can be adversely affected by factors influencing accessibility of the area such as poor infrastructure (Andersen, 2003) and political instability. Delivery of the nutrition enhancing technology through improving the nutritional value of the staple crop such as QPM is the best sustainable option available since maize is the most important staple food crop in the area.

Farmers grow hybrids, improved open pollinated varieties and a local variety (landrace). "Hickory King", a local variety, was important contradicting official reports that maize area in Zimbabwe is 100% hybrid (Doswell et al., 1996). Hybrid varieties, which replaced OPVs about thirty years ago, definitely play an important role in the cropping systems in

the three districts mainly because of their availability, better adaptation, high yield potential and tolerance to diseases and insect pests. Nevertheless, there were still pockets of “Hickory King” because of its high kernel density, intense white flour colour, desirable taste of food preparations, high level of flour recovery during pounding or refined milling, perceived tolerance to drought and foliar diseases, perceived resistance to weevils, and perceived higher yield levels. Some farmers could not plant their whole annual maize crop to “Hickory King” because of lack of adequate seed. Farmers in all the villages confessed that growing of improved open pollinated varieties is a practice that they adopted because of shortage of hybrid maize seed during the period of 2002/3 to 2006/7.

Maize is a controlled grain in Zimbabwe and only the parastatal, Grain Marketing Board (GMB), is authorised to buy it. This has implications for QPM that is not consumed on the farm. If urban and other consumers are to benefit from the QPM, separate marketing and storage arrangements have to be made for QPM varieties. A single handling system for both QPM and normal maize would contaminate the QPM. The other question to be addressed is should there be a premium on QPM seed and grain? The presence of a premium is likely to discourage consumers from consuming QPM. On the other hand, lack of a premium on QPM seed might discourage private seed companies from devoting a lot of resources to QPM breeding. The contentious questions, therefore, are: If there is a premium to be paid on QPM, who pays it since the consumers are not willing to do so? But if there is no premium, who produces the seed since most seed companies are not willing to do so? In this case deploying new QPM varieties as synthetics could be better since farmers can try to maintain their seed for several seasons before getting fresh seed.

Across all the villages, hybrid varieties were preferred by the farmers despite their perceived weaknesses except the male farmers in Chidawaya village who preferred OPV because the OPV seeds could be easily recycled. In developing QPM cultivars, therefore local breeders should consider OPVs as well. These have the advantage of competing well with “Hickory King”. However in areas where there is a lot of normal maize growing in the vicinity of open pollinated QPM varieties, the farmers would need to be discouraged from recycling seed if it is possible but if there is no option then they can be advised to harvest ears for seed from the centre of their plots.

The top six attributes farmers perceived as desirable for maize cultivars and by extension for QPM cultivars are yield potential, earliness, drought tolerance, resistance to weevils, resistance to stem borers, and resistance to foliar diseases. Although ranked differently from village to village, grain yield and earliness were among the top three. Farmers in Payarira and Chiweshe villages ranked them similarly but slightly different from those in Chidawaya village. For disease and drought tolerance, farmers' perceptions differed from village to village as indicated by the ratings. The demand for both high yielding and early maturity in the same cultivar has implications for both normal and QPM breeding in general because the two traits are negatively associated and, hence, it is difficult to maintain them in the same genotype. However, it is important to acknowledge that for the farmers to voluntarily grow and consume the QPM grain, these six attributes need to be combined with the desirable attributes of "Hickory King" which are large kernel size, high kernel density, intense white colour, kernel hardiness, and the desirable taste. According to Twumasi-Afriye et al. (1996) this approach worked in Ghana when the QPM trait was bred into soft, white and dent backgrounds which were already popular and preferred by both farmers and housewives. Before then all the QPM varieties released in Ghana were not accepted by the farmers because they lacked the preferred background phenotype.

Each of the four villages acknowledged the growing and production of "Hickory King" and it should be borne in mind that although the colour, taste, kernel shape and size, number of rows could still be typical of the original "Hickory King" variety, the other agronomic attributes such as yield, disease and insect tolerance, maturity category could have been affected through genetic drift emanating from the "Founder Effect" if measures were not taken to broaden the genetic base of "Hickory King" parents for the next generation (Falconer and Mackay, 1996). Thus, some of the agronomic attributes of "Hickory King" are likely to be different from village to village depending on parents selected by the farmers and foreign pollen contamination levels in each generation.

Farmers across all the four villages considered AREX represented by the local AREX officer as the most preferred organisation for QPM information dissemination followed by radio. In general the farmers had less preference for radio, television set, newspapers, and the farmers' magazine. The potential role of nongovernmental organisations in

promoting new technology was established in two of the villages. The potential influence of national politics in the power relations in the community was observed on the insistence that the village councillor could be used as a medium of communication in Chidawaya village. Several organisations including AREX, GMB, fertiliser companies, and seed companies were listed as important in the growing and promoting of maize. Among the seed companies, Seed Co Ltd was prominent because of the performance, availability and awareness of its products by the farmers. Therefore, marketing a new QPM product through Seed Co Ltd would likely lead to a better chance of adoption.

Farmers mainly grew “Hickory King” for home consumption and not for sale, and ate “sadza” from other varieties when the supply of “Hickory King” grain was depleted. Hybrid varieties were freely sold. This suggested that a QPM variety that does not meet the desired characteristic of “Hickory King” could still be produced by the farmers and not consumed but sold to GMB. This can potentially defeat the objective of trying to improve nutrition at the smallholder farmer level. A similar experience was observed in Malawi where farmers would grow both hybrids and landrace OPVs and keep the landrace OPVs for home consumption because of their desirable flint characteristic but sell the dent hybrid varieties grain to the state authorities (Smale and Heisey, 1994)

Two local names that farmers suggested for QPM were “Godzamhuri” and “Mupedzakwashi”. “Godzamhuri” simply means family-nourisher whilst “Mupedzakwashi” means *kwashiorkor* terminator. Both names are potentially suitable in taking the QPM message to other smallholder farmers who have not heard about QPM before. There could be need to probe certain issues further through a survey, such as in villages where the rankings for certain topics of discussion were not the same across gender, and even on subjects of discussion where the farmers had a consensus it would be intellectually fulfilling to use the results as content for triangulation through a questionnaire based survey.

2.5 Conclusion

The awareness of QPM by the farmers was low and so PRA activities were related to normal endosperm maize but effectively contributed towards the setting of goals for QPM breeding. Farmers listed the most preferred traits in QPM varieties as high yield potential, earliness, drought tolerance, disease resistance, and pest resistance but there were slight differences in rankings. The “Hickory King” landrace variety was preferred for

its taste, large kernels, kernel hardness, white flour, and weevil resistance. The inclusion of these attributes in a QPM variety could potentially lead to quick adoption. Whilst the presumptive approach to QPM breeding goal setting was going to capture most of the important agronomic traits, it would have missed out on the quality aspects that make farmers prefer “Hickory King” to the normal varieties. Agricultural Research and Extension was identified as the most important organization working with maize across all the four villages. The most preferred means of disseminating QPM information was through the local AREX representative. Use of the PRA technique in both QPM breeding goal setting and appraisal of dissemination strategies was an improvement over use of the presumptive technique. The presumptive technique could result in farmers growing the QPM varieties for sale and not for consumption. Therefore, when breeding new QPM varieties for the rural communities it is important to combine the taste, kernel size, kernel hardiness, kernel density, and intense white colour of “Hickory King” with drought tolerance, high yield potential, diseases resistance and earliness. These findings underscore the importance of smallholder farmers’ participation in QPM breeding goal setting and why it is important to incorporate their preferences in the breeding of QPM varieties.

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3 Combining ability and reciprocal cross effects analysis of elite CIMMYT QPM inbred lines in subtropical environments

Abstract

The generation of information on general combining ability (GCA), specific combining ability (SCA) and reciprocal (REC) effects is expected to facilitate the effective and efficient utilization of inbred lines in maize breeding programmes. A diallel analysis of nine inbred lines (36 F_1 hybrids and reciprocals) from CIMMYT was evaluated across seven environments during 2006/7 and 2007/8 seasons in Zimbabwe. Data was analysed for grain yield, anthesis days, kernel endosperm modification, protein content, tryptophan content and Quality Index (QI). General combining ability estimates for QI, tryptophan content, protein content, kernel endosperm modification and anthesis days were all highly significant ($p < 0.001$). Specific combining ability effects estimates were significant for grain yield ($p < 0.001$), anthesis days ($p < 0.001$) and QI ($p < 0.05$). Reciprocal cross effects were significant for QI ($p < 0.001$), tryptophan content ($p < 0.05$) and anthesis days ($p < 0.001$). There was preponderance of GCA effects in the genetic control of tryptophan content, protein content, kernel endosperm modification and anthesis days, and preponderance of SCA effects for grain yield. Specific combining ability effects though significant for QI, tryptophan content and anthesis days were not the most important form of genetic effects. Reciprocal cross effects though significant for QI, tryptophan content and anthesis days were the least important form of genetic effects in all the traits. The cross with the highest and most desirable SCA for grain yield was CZL03016 X CML144. Inbred line CZL03016 had the most desirable GCA effect for kernel endosperm modification but the least desirable score for QI. The most desirable GCA effects for QI, tryptophan content and protein content were found in inbred line CML264Q. In all the crosses where maternal effects were significant (QI and tryptophan content) except for anthesis days, inbred CML264Q was involved. There was highly significant hybrid X environment interactions for all traits (grain yield, anthesis days, kernel endosperm modification, protein content, and tryptophan content) except QI which suggested that the QI trait can be confidently selected for using one environment.

3.1 Introduction

Maize is cultivated on more than 22 million hectares in sub-Saharan Africa (SSA) (FAOSTAT, 2004). The relatively high productivity and easy agronomy of maize makes it one of the most important crops in sub-Saharan Africa (Doswell et al., 1996). Unfortunately maize is deficient in two essential amino acids, lysine and tryptophan (Mertz et al., 1964). Consequently, weaned infants, the sick, and adults who can not afford other sources of protein rich foods but maize are predisposed to malnutrition. In worst cases malnutrition manifests as kwashiorkor[7]. Despite that the potential role of QPM in alleviating human nutrition related problems in SSA, which was reported by Vasal (2002) and Graham (1989) QPM adoption levels remain low due to several factors. These include lack of locally adapted cultivars, lack of adequate information on the breeding potential of available QPM inbred lines that retards the breeding processes. The utilisation and evaluation of QPM germplasm generally demands more effort and resources than normal endosperm maize germplasm due to the need to put more emphasis and resources on kernel quality traits in QPM than in normal endosperm maize. However, both types of germplasm require the same level of effort and attention on what are broadly referred to as agronomic or field adaptation traits. In this study the focus will be on grain yield, anthesis days, and quality traits which include kernel endosperm modification, protein content, tryptophan content and Quality Index (QI).

Due to the relatively high costs and difficulties linked to QPM breeding, in SSA, the development of locally adapted QPM cultivars hinges on the exploitation of publicly available QPM donor germplasm in the form of inbred lines, open pollinated varieties, and synthetics. Several SSA maize breeding programmes spend considerable effort towards hybrid cultivar development and as such find QPM inbred lines are more attractive than the other types of germplasm. The utilisation of exotic elite CIMMYT QPM germplasm both directly and in conversion programmes is being conducted by most breeding programmes in SSA (CIMMYT, 2004; CIMMYT, 2005). The effective and efficient exploitation of the exotic sources of QPM germplasm merits the constant and regular evaluation of the breeding potential of introduced exotic materials under local conditions. This includes assessment of GCA and SCA effects (Sprague and Tatum, 1942). Previous evaluations of CIMMYT QPM germpalsm were of materials that were

[7] **Kwashiorkor** is protein calorie malnutrition which can lead to infant morbidity and mortality. It disables the immune system such that the child is susceptible to a host of infectious diseases. The term is from Ivory Coast where it means the deposed child (weaned off) Source: MedicineNet.com. 1998. Webster's New World Medical Dictionary [Online]. Available by MedicineNet.com *http://www.medterms.com/script/main/art.asp?articlekey=4124* (Verified 12 October 2008).

developed without regard for heterotic groupings (Pixley and Bjarnason, 1993). Currently there is high regard for heterotic groupings in CIMMYT breeding programmes and it is desirable to evaluate the genetic potential of locally available inbred lines under subtropical environments.

The general findings in the earlier studies of Vasal et al. (1993b), Vasal et al. (1993a), and Pixley and Bjarnason (1993) was that GCA effects were significant and important for grain yield and the quality traits. However, the later studies of Fan et al. (2004) and Jompuk et al. (2007) found both GCA effects and SCA effects to be significant and more important for grain yield. In the study of Bhatnagar et al. (2004), only SCA effects were significant for grain yield. Hence it would be interesting to investigate the components of genetic variation that are exhibited by elite CIMMYT QPM inbred lines under subtropical environments.

For the quality traits, all the earlier studies cited above found GCA effects to be more important than SCA effects. However, quality traits are mostly kernel endosperm traits whose expression can be influenced by reciprocal differences in the crosses and yet in most of the previous studies there has been no effort to utilise experimental designs that cater for reciprocal cross differences. This could be due to the consideration of both the relatively high costs and cumbersome nature of employing designs that cater for reciprocal cross differences and also that most traits of economic importance in maize are free from reciprocal cross differences. When previously used diallel designs that cater for reciprocal cross differences revealed reciprocal cross differences in some of the cases (Vasal, 1975; Vasal et al., 1980). There were several studies that revealed reciprocal cross differences in maize for different traits (Hallauer and Martinson, 1975; Mann and Pollmer, 1981; Mann et al., 1981; Pollmer et al., 1979), but their instability and low magnitude makes it difficult to exploit them in breeding programmes (Mann et al., 1981; Pollmer et al., 1979).

Failure to detect reciprocal cross differences when they are present would lead to the inflation of estimates for GCA (Roach and Wulff, 1987a) and SCA effects (Lopez et al., 2003). Therefore, it is prudent to utilise a mating design that can detect the presence or absence of reciprocal differences among crosses of the available QPM inbred lines. The generated information will facilitate efficient direct utilisation and introgression of exotic elite QPM germplasm into local germplasm pools. Therefore, the objective of the study was to estimate GCA, SCA and reciprocal cross effects on quality traits and grain yield among the publicly available elite CIMMYT QPM inbred lines for the benefit of local breeding programmes. The hypothesis for the

study was that the inbred lines in the study were diverse for GCA, SCA, and reciprocal cross (REC) effects.

3.2 Methods and materials

3.2.1 Germplasm

A nine parent diallel experiment with reciprocals was created using the QPM inbred lines listed in Table 3.1. The F_1 crosses were made during the summer cropping seasons of 2005/6 at CIMMYT Harare, and during the winter of 2006 at Muzarabani, Zimbabwe. Some more F_1 seed was re-made during the summer cropping season of 2006/7, and the winter cropping season of 2007 at Harare and Muzarabani nurseries respectively. In addition to the F_1 crosses and reciprocal crosses from the nine parent diallel, three locally adapted varieties (SC721, SC633, and SC527Q) were included as check varieties. Cultivar SC721 and SC633 are normal endosperm maize varieties and SC527Q is a QPM variety.

Table 3.1 QPM inbred parents for the diallel of experimental hybrids

Entry	Inbred line	Pedigree	H G[s]	ADT[s]	ECGT[s]
1	**CZL03016**	[CML159/[CML159/[MSRXPOOL9]C1F2-205-1(OSU23i)-5-3-X-X-1-BB]F2-3sx]-8-1-2-B	A	MA	WF
2	**CML144**	P62C5F182-2-1-2-BB-3-1-##	B	LT	WF
3	**CML159**	P63C2F5-1-3-1-B-2-1-1-B-#	A	LT	WF
4	**CML175**	P68C0F77-2-3-7-B-2-3-1-B-1-BB	B	ST	WD
5	**CML176**	(P63-12-2-1/P67-5-1-1)-1-2-BB	B	LT	WDF
6	**CML181f**	UWO417-B-2-1-1-BB	B	ST	WF
7	**CML264Q**	(CML176*CML264)-BxCML264)-7-1xCML264]xCML264-F2(65)-B*4	B	LT	WF
8	**CML492**	P62C3F163-3-3-3-2-#-1-1-2-BB	B	LT	WF
9	**CZL01006**	GQL5	B	LT	W /nc

[s]Key HG = heterotic group; ADT = adaptation; MA = midaltitude; LT = lowland tropical; ST = subtropical; ECGT = endosperm colour and grain texture; WF = white flint, WD = white dent; WDF = white dent flint; nc = not classified grain texture; Group A = Tuxpeno, B73 types; Group B = Eto, Ecuador, and Mo17 types.

The CML coded lines were internationally accepted as elite CIMMYT maize inbred lines by then, and the CZL coded lines were developed at CIMMYT Zimbabwe and were still in the process of being considered for CML coding if found to possess good agronomic properties. The inbred

lines were developed from CIMMYT germplasm pools 62, 63, and 68 and UWO417. The source GQL5 is from Ghana. Most of the lines were of lowland tropical adaptation except CML181f, CML175 and CZL03016. All the inbred lines were of white endosperm colour and CML176, and CML175 were classified as of a dent grain texture whilst CZL01006 was not classified.

3.2.2 Environments

The field evaluation of 75 entries composed of 36 F_1 crosses, 36 reciprocals and three check varieties was done at CIMMYT Harare (CH) (1409 m a.s.l., 17º43' S, 31º01' E), Agricultural Research Trust (ART) farm (1527 m a.s.l.; 17º43' S, 31º05' E), Rattray Arnold Research Station (RARS) (1341 m a.s.l., 17º40' S, 31º13' E) and Kadoma Research Centre (KRC) (1149 m a.s.l., 18º19' S; 29º 17' E). The experiments were conducted at each site for two cropping seasons (2006/7 and 2007/8) except at KRC where the crop was poorly established during 2006/7 creating a total of seven environments (made up of season x site combinations).

3.2.3 Management

Standard cultural practices for growing maize were followed at all the sites. Different fertiliser rates were applied as follows: 166 kg N-56 kg P-28 kg K ha-1 at CH; 180 kg N-84 kg P-42 kg K ha-1 at ART farm; 145.30 kg N-56 kg P-28 kg K ha-1 at RARS; and 88.4 kg N-56 kg P-28 kg K ha-1 at KRC. The weeds were effectively controlled by herbicides and hand weeding at all the sites. Rainfall totals for the relatively drier 2006/7 cropping season were 582mm (CH), 673mm (RARS), 529mm (ART farm) and 490mm (KRC). The 2007/8 cropping season was relatively wetter with rainfall totals of 978mm (CH), 837.5mm (ART farm), 902.5mm (RARS) and 433mm (KRC). The KRC site received a higher total and poor temporal distribution in 2006/7 but better temporal distribution and a lower total in 2007/8.

3.2.4 Experimental design

The experiment was designed as a 15 x 5 alpha (0, 1) lattice design with two replicates for each environment. The experimental unit was two 4-m rows spaced at 0.75m and an effective spacing of 0.25m in the row at all the sites. The crop was thinned to one plant per station at three weeks growth stage to give a plant population density of approximately 53 000 plants ha^{-1}.

Agronomic trait data were recorded for grain yield (GWT) for the whole plot, grain moisture (MOI), anthesis date (AD) measured as the number of days to 50% pollen shedding after planting, silking date (SD) measured as number of days after planting when 50% of the plants had emerged silks. A sample of 100 kernels from two self pollinated ears per plot was used to

determine kernel endosperm modification (KMod) ratings (1-5) according to Vivek et al. (2008) and the ratings were interpreted as follows:

1 = not opaque and endosperm wholly translucent

2 = 25% opaque

3 = 50% opaque

4 = 75% opaque

5 = 100% opaque

Samples of 20 self pollinated kernels per plot for the three 2006/7 environments (CH, ART farm, and RARS) were analysed in Mexico at the CIMMYT Soil and Plant Analysis Laboratory (SPAL) for the determination of nitrogen content (%), protein content (%), and tryptophan content (%). Nitrogen and protein content were determined using the Technicon Autoanalyser II protocol in Vivek et al. (2008). The protocol for tryptophan content determination was the Opienska-Blauth et al. (1963) calorimetric method modified by Hernandez and Bates (1969) and is described in Vivek et al. (2008).

There were statistically derived traits and these were grain yield (GY) adjusted to 12.5% H_20 and tons ha^{-1}. The Quality Index (QI), which is a measure of QPM nutritional value, was derived from the ratio of tryptophan to total protein and multiplied by 100 according to Vivek et al. (2008).

3.2.5 Statistical analyses

Analysis of variance was conducted for grain yield, anthesis date, kernel endosperm modification, protein content (Prot), tryptophan content (Try), and QI using lattice adjusted means for each environment and then combined across environments with significant data. The genotypes were considered to be fixed and both the replications within environments and the environments were random.

Griffing (1956) method 3 (with F_1 and reciprocals and excluding parents) model 1 (fixed effects model) was used to analyse the data for GCA, SCA and REC effects for the different traits across the environments. All data were analysed over seven environments except the QI and tryptophan data which were determined for three environments only due to high laboratory costs. The GCA, SCA and REC effects were calculated based on averages across 7 or 3 sites.

Diallel-SAS05, a comprehensive program for Griffing's and Gardner-Eberhart analyses developed by Zhang et al. (2005) was used in performing the diallel analysis in SAS computer package (SAS, 2004).

According to Zhang and Kang (1997) the multilocation model for Griffing's diallel method 3 model 1 can be presented as shown below;

"$Y_{ijklc} = \mu + \alpha_l + b_{kl} + v_{ij} + (\alpha v)_{ijl} + e_{ijklc}$

Where Y_{ijklc} = *observed trait value for each experimental unit (*i *and* j *parents;* k*, replication;* l*, location;* c*, sample);* μ = *population mean;* α_l = *environment effect;* b_{kl} = *block or replication within environment effect;* v_{ij} = *F_1 hybrid effect =* $g_i + g_j + s_{ij} + r_{ij}$

[where g_i *is the general combining ability (GCA) effect of the* i*th parent,* g_j = *GCA effect of the* j*th parent,* s_{ij} = *specific combining ability of the* ij*th F_1 hybrid,* r_{ij} = *reciprocal effect for the* ij*th or* ji*th F_1 hybrid =* $m_i + m_j + n_{ji}$ *(where* m_i = *maternal effect of parental line i,* m_j = *maternal effect of parental line* m_j, n_{ij} = *nonmaternal effects of the* ij*th or* ji*th F_1 hybrid];*

$(\alpha v)_{ijl}$ = *interaction between F_1 hybrids and environments =* $(\alpha g)_{il} + (\alpha g)_{jl} + (\alpha s)_{ijl} + (\alpha r)_{ijl}$

[where $(\alpha g)_{il}$ = *interaction between GCA effect for the* i*th parent and environments,*
$(\alpha g)_j l$ = *interaction between GCA effects for the* j*th parent and the environments,*
$(\alpha s)_{ijl}$ = *interaction between SCA effect for the* ij*th F_1 hybrid and environments, and*
$(\alpha r)_{ijl}$ = *interaction between reciprocal effect for the* ij*th or* ji*th F_1 hybrid]*

Environments $= (\alpha m)_{il} + (\alpha m)_{jl} + (\alpha n)_{ijl}$

(where $(\alpha m)_{il}$ = *interaction between environments and maternal effects of parental line* i*,*
$(\alpha m)_{jl}$ = *interaction between environments and maternal effects of parental line* j*,*
(αn)ijl = interaction between environments and nonmaternal effects of the ij*th or the* ji*th F_1 hybrid; and* e_{ijklc} = *residual effect. Restriction for GCA effects:* $\sum g_i = 0$*, and restriction for SCA effects:* $S_{ij} = S_{ji}$*, and restriction for reciprocal effects* $r_{ij} = -r_{ji}$ (Zhang and Kang, 1997)"

The interaction terms were used to test for the significance of the corresponding main effects in all the cases (Zhang and Kang, 1997) because the Griffing 3 model was fixed. The environments and replications within environments were considered to be random and tested against the residual error term. Relative importance of genetic effects was computed using the proportion of F_1 hybrids (entry) sum of squares due to each of the three genetic effects (GCA, SCA, and REC). Similarly relative importance of maternal and nonmaternal effects was computed as a proportion of reciprocal effects sum of squares.

3.3 Results

3.3.1 Hybrid variation for grain yield and quality traits

The analyses of variances for the diallel analyses are presented in Tables 3.2 and 3.3. The mean values for grain yield, anthesis days, kernel endosperm modification, protein content, tryptophan content, and QI were 6.05 t ha^{-1} , 73.82 days, 2.64 (1-5 rating), 9.77%, 0.078%, and 0.804, respectively (Tables 3.2 and 3.3). The hybrids were significantly different for all traits in Tables 3.2 and 3.3 ($p<0.01$). The SCA effects were highly significant for grain yield and days to anthesis, and QI but were non significant for the rest of the traits. The GCA effects were highly significant for anthesis days and kernel modification (Table 3.2), and also highly significant for protein content, tryptophan content and QI (Table 3.3). For grain yield all the interaction terms were significant whilst for anthesis days REC x E and N x E were not significant, and for kernel modification M x E was not significant. For protein content all interaction terms were highly significant except for reciprocal cross differences and their subdivisions of maternal and nonmaternal effects. General combining ability by environment was the only significant interaction term for tryptophan content. QI had no significant interaction terms. There were significant reciprocal differences for both tryptophan content and QI and these were of the nonmaternal type for tryptophan and both maternal and nonmaternal types for QI. Inbred line mean values for the significant genetic effects in Tables 3.2 and 3.3 are presented in the following sections.

Table 3.2 Diallel analysis of QPM hybrids for grain yield, anthesis days, kernel modification using Griffing's Method 3 fixed model for seven environments

Trait		Grain Yield t ha^{-1}			Anthesis Days			Modification (1-5)		
Sources	**DF**	**MS**	**F**	**Prob**	**MS**	**F**	**Prob**	**MS**	**F**	**Prob**
Envnts (E)	6	253.06	208.53	<0.001	4103.29	1764.19	<0.001	7.43	44.76	<0.001
Reps/E.	7	13.01	10.72	<0.001	7.93	3.41	<0.010	0.35	2.11	<0.050
Hybrids	71	7.61	2.98	<0.001	51.43	14.03	<0.001	1.20	4.41	<0.001
GCA	8	12.95	1.87	n.s.	353.54	59.25	<0.001	8.13	17.07	<0.001
SCA	27	13.69	5.70	<0.001	20.27	4.80	<0.001	0.42	1.52	n.s.
REC	36	1.85	1.09	n.s.	7.66	2.80	<0.001	0.24	1.08	n.s.
M	8	2.23	1.09	n.s.	13.99	4.13	<0.001	0.23	1.49	n.s.
N	28	1.75	1.10	n.s.	5.85	2.29	<0.001	0.24	1.00	n.s.
Hybrids X E	426	2.55	2.10	<0.001	3.67	1.58	<.0001	0.27	1.63	<0.001
GCA X E	48	6.91	5.69	<0.001	5.97	2.57	<0.001	0.48	2.87	<0.001
SCA X E	162	2.40	1.98	<0.001	4.22	1.81	<0.001	0.28	1.67	<0.001
REC X E	216	1.69	1.40	<0.010	2.74	1.18	n.s.	0.22	1.33	<0.010
M X E	48	2.05	1.69	<0.010	3.39	1.46	<0.050	0.15	0.92	n.s.
N X E	168	1.59	1.31	<0.050	2.55	1.10	n.s.	0.24	1.45	<0.010
Error	497	1.21			2.33			0.17		
CV %		18.22			2.07			15.41		
Hybrids Mean		6.05t ha^{-1}			73.82 days			2.64		
% GCA (SS)		19.18			77.46			76.52		
% SCA (SS)		68.46			14.99			13.42		
% REC (SS)		12.35			7.55			10.06		

Key : Envnts = Environments, Hybrids = F_1 Single crosses, GCA = general combining ability, SCA = specific combining ability, REC = reciprocal effects, M = maternal effects, N = nonmaternal effects., CV = coefficient of variation.

Table 3.3 Diallel analysis of QPM for protein content, tryptophan content, Quality Index using Griffing's Method 3 over three environments during 2006/7

Trait		Protein %			Tryptophan %			Quality Index		
Sources	**DF**	**MS**	**F**	**Prob**	**MS**	**F**	**Prob**	**MS**	**F**	**Prob**
Envnts (E)	2	103.06	181.130	<0.001	0.00677	70.960	<0.001	0.18788	20.950	<0.001
Reps/E.	3	3.58	6.300	<0.001	0.00168	17.630	<0.001	0.10832	12.080	<0.001
Hybrids	71	2.89	3.530	<0.001	0.00081	8.440	<0.001	0.06350	8.130	<0.001
GCA	8	18.74	16.699	<0.001	0.00622	32.023	<0.001	0.44573	35.773	<0.001
SCA	27	1.34	1.286	n.s.	0.00009	0.943	n.s.	0.01392	1.698	<0.050
REC	36	0.54	0.920	n.s.	0.00015	1.890	<0.050	0.01574	2.425	<0.001
M	8	0.43	0.524	n.s.	0.00024	2.429	n.s.	0.02322	6.829	<0.001
N	28	0.57	1.100	n.s.	0.00012	1.681	<0.050	0.01360	1.845	<0.050
Hybrids X E	142	0.82	1.440	<0.010	0.00010	1.000	n.s.	0.00781	0.870	n.s.
GCA X E	16	1.12	1.973	<0.050	0.00019	2.037	<0.050	0.01246	1.389	n.s.
SCA X E	54	1.04	1.827	<0.010	0.00009	0.952	n.s.	0.00820	0.915	n,s.
REC X E	72	0.59	1.030	n.s.	0.00008	0.812	n.s.	0.00649	0.724	n.s.
M X E	16	0.82	1.445	n.s.	0.00010	1.021	n.s.	0.00340	0.379	n.s.
N X E	56	0.52	0.911	n.s.	0.00007	0.752	n.s.	0.00737	0.822	n.s.
Error	213	0.57			0.00010			0.00897		
CV %		7.72			12.457			11.772		
Hybrids Mean		9.77 %			0.078 %			0.804		
% GCA (SS)		72.99			86.784			79.098		
% SCA (SS)		17.57			4.032			8.336		
% REC (SS)		9.44			9.184			12.566		
% M					35.914			32.791		
% NM					64.086			67.209		

Key: Envnts = Environments, Hybrids = F_1 Single crosses, GCA = general combining ability, SCA = specific combining ability, REC = reciprocal effects, M = maternal effects, N = nonmaternal effects., CV = coefficient of variation.

3.3.2 General combining ability effects

The inbred line GCA effects for anthesis days, kernel endosperm modification, protein content, tryptophan content and Quality Index are presented in Figures 3.1 and 3.2. The inbred line with the best GCA values for both anthesis days ($p < 0.001$) and kernel endosperm modification ($p < 0.001$) was CZL03016. The second best line for anthesis days was CZL01006 ($p < 0.001$) followed by CML176 and CML175 both of which were not significantly different from zero. The worst inbred line for anthesis days was CML144 ($p < 0.001$) followed by CML181f ($p < 0.001$), CML492, and CML264Q both of which were significantly different from zero at $p < 0.05$. The

second best line for anthesis days, CZL01006 ($p < 0.001$), was also the second best line for kernel endosperm modification at $p < 0.001$. Inbred lines CML144, CML181f and CML492 were not significantly different from zero for kernel endosperm modification. The worst line for kernel endosperm modification was CML264Q ($p < 0.001$) followed by CML175 ($p < 0.001$) and CML176 ($p < 0.01$). The worst line for kernel endosperm modification GCA values, CML264Q ($p < 0.001$), was contrastingly the best line for protein content GCA values with $p < 0.001$ level of significance (Figure 3.1 and Figure 3.2). The second best line for protein content GCA was CML144 but was not significantly different from zero. There were only two inbred lines with positive protein content GCA values, CML264Q and CML144. The best line for protein content, CML264Q, was also the best inbred line for both tryptophan ($p < 0.001$) and QI ($p < 0.001$) GCA values (Figures 3.1, 3.2 and 3.3).

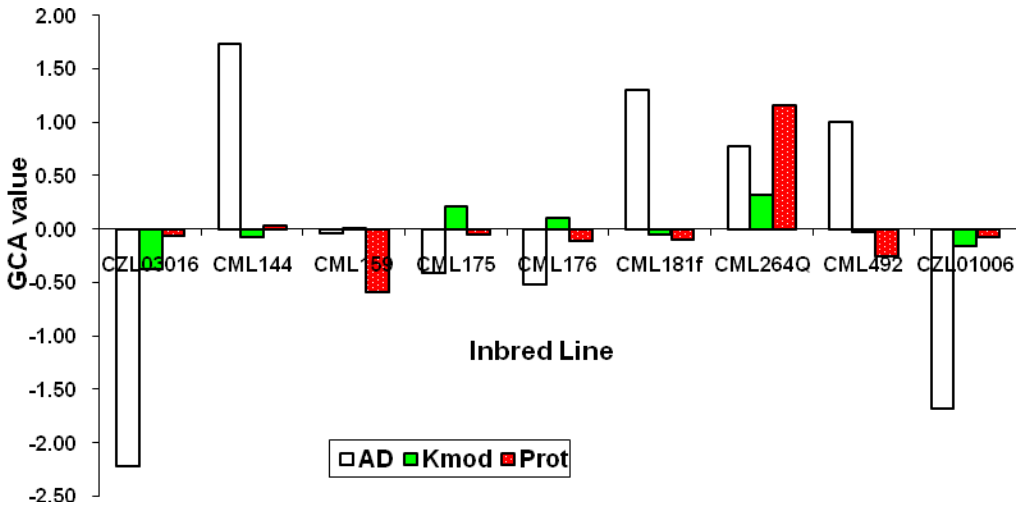

Figure 3.1 GCA values for anthesis days, kernel endosperm modification and kernel protein content of nine inbred lines.

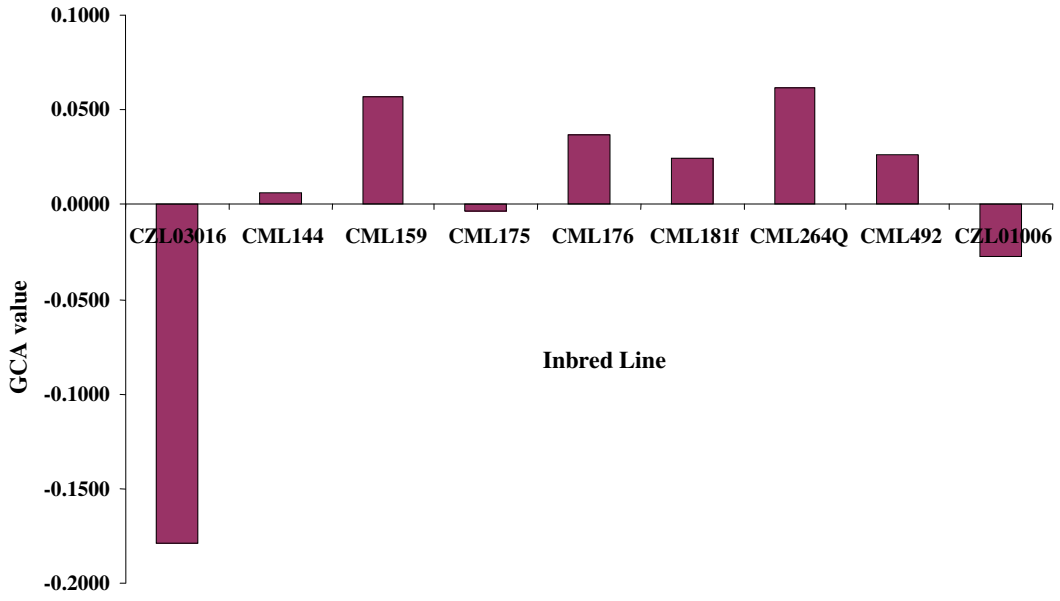

Figure 3.2 GCA values for Quality Index of nine inbred lines over three environments

Inbred line CZL03016, was the worst line for both tryptophan content ($p < 0.001$) and QI ($p < 0.001$) GCA values in Figures 3.3 and 3.2 respectively. The next worst line for both tryptophan content ($p < 0.05$) and QI ($p < 0.01$) GCA values was CZL01006. Inbred line CML175 was a negative but nonsignificant GCA value for QI. Inbred lines CML264Q, CML159 and CML176 had positive QI GCA values that were significantly different from zero at $p < 0.001$ whilst inbred lines CML492 and CML181f were positive and significant ($p < 0.05$). The QI GCA value for inbred line CML144 was positive but not significant.

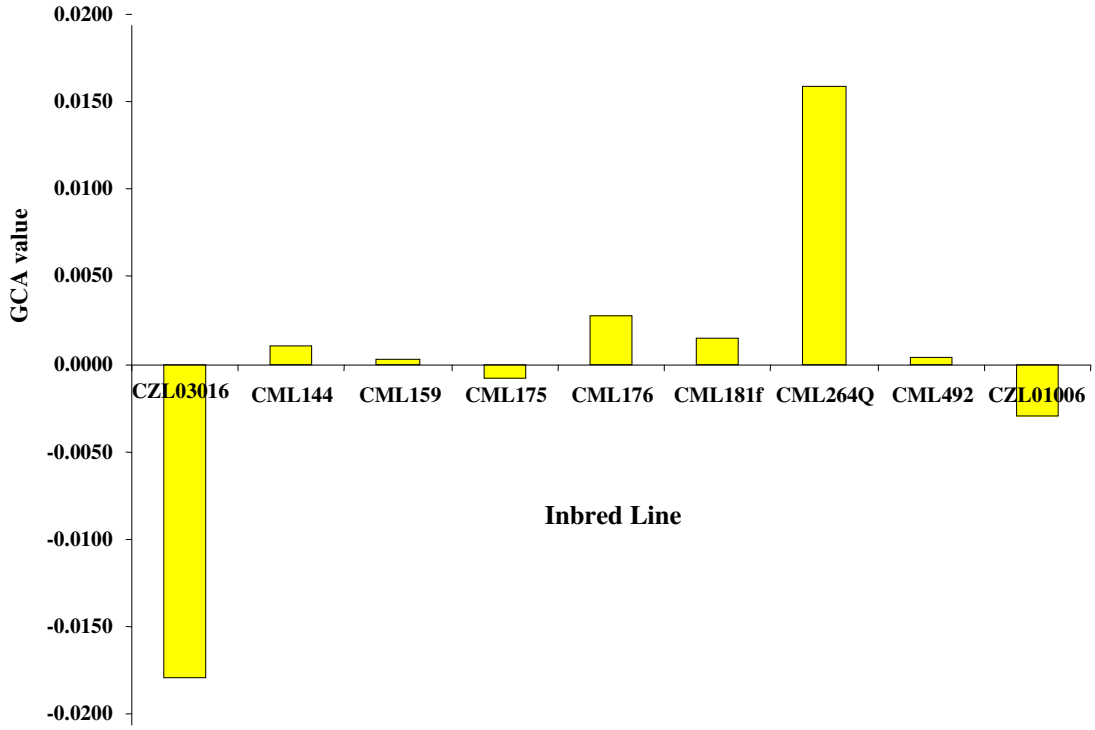

Figure 3.3 GCA values for kernel tryptophan content of inbred lines over three environments

Inbred line CZL03016, was the worst line for tryptophan content ($p < 0.001$) GCA values in Figure 3.3. The next worst line for both tryptophan content ($p < 0.05$) GCA values was CZL01006. Inbred line CML176 had significant ($p < 0.05$) positive GCA for tryptophan content followed by CML175 which was both negative and not significant. Inbred lines CML144, CML159, CML181f and CML492 had positive but nonsignificant GCA values for tryptophan content. The best line for tryptophan content was CML264Q with a significant ($p < 0.001$) positive value.

3.3.3 Specific combining ability and reciprocal cross effects

3.3.3.1 Grain yield

In Table 3.4 the highest SCA value as for the cross between line 1 (CZL03016) and line 2 (CML144) and the lowest was between line 2 (CML144) and line 6 (CML181f). The crosses with significant ($p < 0.05$) positive SCA values were between line 1 (CZL03016) and line 2 (CML144), line 5 (CML176) and line 6 (CML181f), line 6 (CML181f) and line 8 (CML492), and the cross of

line 3 (CML159) and 9 (CZL01006), Three of the F_1 hybrid crosses had significant negative SCA values for grain yield and these were between line 1 (CZL03016) and line 3 (CML159), line 2 (CML144 and line 6 (CML181f) both crosses significant at $p < 0.001$, and line 5 (CML176) and 7 (CML264Q) which was significant at $p < 0.05$. Although GCA effects for grain yield (not shown) were not significant, CZL03016 had the highest GCA effects in addition to being part of the cross with the highest SCA effects.

Table 3.4 Specific combining ability of QPM hybrids for grain yield (t ha^{-1})

Inbred Line	1	2	3	4	5	6	7	8	9
1		0.78**	-1.19***	0.14	-0.24	0.37	0.14	0.09	-0.08
2			-0.29	0.34	0.57*	-2.41***	0.58*	0.22	0.21
3				0.18	0.18	-0.40	0.30	0.52*	0.70**
4					0.09	0.29	0.08	-0.61*	-0.52*
5						0.74**	-0.64*	-0.49	-0.21
6							0.12	0.71**	0.57*
7								-0.18	-0.40
8									-0.27
9									

Key: LSDs: V(sij) = 0.50077* and 0.6609** for testing significance of SCA effects from zero, V(sij-sik) = 0.75709* and 0.99932** and V(sij-skl) = 0.69113* and 0.9125** are both for comparing significance of differences between SCA effects. Where * is for significance at 5%, and ** is for significance at 1% and *** for significance at 0.1%.

3.3.3.2 Anthesis days

For days to anthesis the selection is for both lines and crosses with the smallest values. Therefore, the cross with the best SCA value was between CZL03016 and CML144. General combining ability values were included in Table 3.5 so as to highlight that the inbred line with the best GCA value, inbred line 1 (CZL03016) produced the best SCA value when crossed to the inbred line with the worst GCA value, inbred line 2 (CML144). The worst reciprocal cross effects were between line 5 (CML176) and line 2 (CML144) and the best were between line 5 (CML176) and line 8 (CML492). The reciprocal cross effects were both of the maternal and nonmaternal type (Table 3.6).

Table 3.5 Specific combining ability (above diagonal), general combining ability and reciprocal cross differences (below diagonal) for anthesis days

Inbred Line	1	2	3	4	5	6	7	8	9	**GCA**
1		-1.78*	1.73**	-0.63	0.71*	-0.64	-0.33	0.09	0.84*	**-2.21*** **
2	0.21		0.01	1.00**	0.06	1.24**	0.23	-0.74*	-0.03	**1.73*** **
3	0.32	-0.54		0.44	-0.22	0.15	-1.61**	0.02	-0.52	**-0.03**
4	-0.50	0.14	0.32		-0.62	-0.90**	0.23	0.40	0.08	**-0.41**
5	0.61	1.25**	0.29	0.36		0.02	0.65	-0.04	-0.57	**-0.51**
6	-0.64*	0.04	0.04	-0.39	-0.71		0.69	-0.96**	0.40	**1.31*** **
7	-0.57	-0.29	1.11**	-0.71*	-0.04	-0.82**		0.78*	-0.65	**0.78***
8	-0.64*	0.39	-0.32	-0.46	-1.00**	-0.82**	0.11		0.45	**1.01****
9	-0.21	0.21	-0.25	-0.25	-0.21	-0.07	0.07	-0.39		**-1.67*** **

Key: GCA, SCA and REC effects were significant for anthesis days. LSDs V(gi-gj) = 0.4962* and 0.6619** for testing significance of differences between GCA effects; V(r) = 0.6164* and 0.8127** for testing significance of reciprocal effects; V(rij-rkl) = 0.8717* and 1.1493** for testing significance of differences between reciprocal effects; V(sij) = 0.6639* and 0.8763** for testing significance of SCA effects, V(sij-sik) = 1.0037* and 1.3248** and V(sij-skl) = 0.9162* and 1.2093** are both for comparing significance of differences between SCA effects. Where * is for significance at 5%, and ** is for significance at 1% and *** is for significance at 0.1%.

The most desirable line for maternal effects was line 5 (CML176) and the least desirable was line 8 (CML492) (Table 3.6). The most desirable cross for nonmaternal effects was between line 6 (CML181f) and line 7 (CML264Q) and the least desirable cross was between line 2 (CML144) and line 5 (CML176) and this was followed closely by crosses between lines 1 (CZL03016) and 3 (CML159), and 5 (CML176) and 7(CML264Q).

Table 3.6 Maternal (extreme right hand column) and non maternal effects (upper half of diagonal) for anthesis days

Inbred Line	1	2	3	4	5	6	7	8	9	Maternal effects
1		0.48	0.63*	-0.50	0.27	-0.49	-0.25	-0.22	0.07	-0.16
2			-0.49	-0.13	0.64*	-0.08	-0.23	0.54*	0.23	0.11
3				0.01	-0.37	-0.12	1.12**	-0.21	-0.28	0.15
4					0.02	-0.24	-0.39	-0.04	0.03	-0.16
5						-0.22	0.63*	-0.24	0.40	-0.50**
6							-0.65*	-0.56*	0.06	0.00
7								0.20	0.03	0.17
8									-0.53	0.26*
9										0.12

Key: LSDs V(m) = 0.2198* and 0.2932** for testing significance of maternal effects from zero; V(mi-mj) = 0.3296* and 0.4397** for testing significance of differences between maternal effect values; V(nij) = 0.5257* and 0.6938** for testing significance of nonmaternal effects from zero, V(nij-nik) = 0.7948* and 1.0489** and V(nij-nkl) = 0.7435* and 0.9812** are both for comparing significance of differences between nonmaternal effects. Where * is for significance at 5% and ** is for significance at 1% and *** is for significance at 0.1%.

3.3.3.3 Tryptophan content

The maternal effect values and the reciprocal cross effects data for tryptophan content are presented in Table 3.7. The highest reciprocal cross effects for tryptophan content were in the cross between inbred 7 (CML264Q) and inbred 1 (CZL03016) whilst the lowest reciprocal cross effects were in the cross between inbred 6 (CML181 f) and inbred 5 (CML176). The highest value for maternal effects (presented in the last column) was with inbred line 1 (CZL03016) and the lowest maternal effects occurred in inbred line 3 (CML159).

Table 3.7 Reciprocal cross (below diagonal) and maternal effects for tryptophan content

Inbred	1	2	3	4	5	6	7	8	9	Maternal
1										**0.0035****
2	0.0031									**-0.0012**
3	0.0021	0.0008								**-0.0016**
4	0.0022	-0.0053	-0.0021							**0.0003**
5	0.0069**	-0.0025	0.0003	-0.0026						**-0.0005**
6	0.0050	-0.0029	0.0004	0.0010	-0.0058*					**0.0007**
7	0.0119***	0.0033	-0.0056*	-0.0026	0.0028	-0.0004				**-0.0005**
8	-0.0009	-0.0007	-0.0043	0.0004	0.0023	0.0028	0.0038			**-0.0005**
9	0.0011	-0.0003	-0.0004	0.0012	-0.0020	0.0014	0.0009	-0.0013		**-0.0001**

Key: LSDs: V(m) = 0.001897* and 0.002614** for significance of maternal effects from zero; V(mi-mj) = 0.002846* and 0.003921** for testing significance of differences between M effects; V(r) = 0.005063* and 0.00672** for testing significance of reciprocal cross effects; V(rij-rkl) = 0.00716* and 0.009503** for testing significance of differences between reciprocal cross effects; Where * is for significance at 5% and ** is for significance at 1% and *** is for significance at 0.1%.

3.3.3.4 Quality Index

The estimated values for SCA effects and reciprocal cross effects for QI data are presented in Table 3.8, whilst the values for both maternal effects and nonmaternal effects are presented in Table 3.9. The highest SCA effects of 0.0766 were exhibited by the cross of inbred line 7 (CML264Q) and inbred line 1 (CZL03016) (Table 3.8). The reverse cross of CML264Q to CZL03016 exhibited the highest reciprocal cross effects (Table 3.8) and the highest maternal effects were found with inbred line CZL03016 (Table 9). The lowest reciprocal cross effects of -0.0625 occurred in the cross of inbred line 3 (CML159) and inbred line 8 (CML492) (Table 3.8).

Table 3.8 Specific combining ability (above diagonal) and reciprocal cross effects (below diagonal) for Quality Index

Line	1	2	3	4	5	6	7	8	9
1		-0.0504*	-0.0246	-0.0100	0.0480	0.0075	0.0766**	-0.0312	-0.0159
2	0.0175		0.0055	0.0443	-0.0110	-0.0390	0.0250	0.0048	0.0209
3	0.0225	0.0108		-0.0232	0.0356	0.0210	0.0100	0.0073	-0.0316
4	0.0150	-0.0225	-0.0558		-0.0172	0.0215	-0.0545*	-0.0089	0.0480*
5	0.0650*	-0.0375	0.0217	-0.0317		-0.0047	-0.0115	-0.0334	-0.0057
6	0.0592*	-0.0208	0.0083	0.0083	-0.0458		-0.0237	0.0294	-0.0120
7	0.1383***	0.0233	-0.0075	-0.0325	0.0208	-0.0083		0.0068	-0.0288
8	-0.0083	0.0175	-0.0625*	-0.0058	0.0150	0.0392	0.0083		0.0251
9	0.0017	-0.0300	-0.0150	-0.0058	0.0025	0.0108	0.0075	0.0192	

Key: LSDs V(r) = 0.0464* and 0.0615** for testing significance of reciprocal effects; V(rij-rkl) = 0.0656* and 0.0870** for testing significance of differences between reciprocal cross effects; V(sij) = 0.0454* and 0.0605** for testing significance of SCA effects from zero, V(sij-sik) = 0.0686* and 0.0914** and V(sij-skl) = 0.0627* and 0.0834** are both for comparing significance of differences between SCA effects. Where * is for significance at 5% and ** is for significance at 1% and *** is for significance at 0.1%.

3.3.4 Maternal and nonmaternal effects

The highest maternal effects for QI were found in inbred line 1 (CZL03016) and the lowest were in inbred line 3 (CML159) (Table 3.9). The highest nonmaternal effects for QI were in the cross of inbred line 1 (CZL03016) and inbred line 7 (CML264Q) whilst the lowest values were in two crosses; inbred line 4 (CML175) crossed to inbred 7 (CML264Q), and inbred line 3 (CML159) crossed to inbred 8 (CML492).

Table 3.9 Maternal (extreme right hand column) and nonmaternal effects (upper half diagonal) for Quality Index

Inbred	1	2	3	4	5	6	7	8	9	Maternal
1		-0.026	-0.028	-0.020	0.028	0.028	0.091**	-0.041	-0.032	**0.035****
2			0.003	-0.014	-0.032	-0.009	0.019	0.028	-0.020	**-0.009**
3				-0.040	0.035	0.028	-0.005	-0.045*	0.002	**-0.016***
4					-0.034	0.012	-0.045*	-0.004	-0.004	**0.000**
5						-0.039	0.010	0.020	0.006	**-0.003**
6							-0.025	0.037	0.008	**0.004**
7								0.023	0.022	**-0.013***
8									0.018	**0.002**
9										**0.001**

Key: LSDs V(m) = 0.0112* and 0.0154** for testing significance of maternal effects from zero; V(mi-mj) = 0.0168* and 0.0232** for testing significance of differences between maternal effect values; V(nij) = 0.0438* and 0.0583** for testing significance of nonmaternal effects from zero, V(nij-nik) = 0.0662* and 0.0881** and V(nij-nkl) = 0.0619* and 0.0824** are both for comparing significance of differences between nonmaternal effects. Where * is for significance at 5% and ** is for significance at 1% and *** is for significance at 0.1%.

3.3.5 Relative importance of GCA, SCA, and REC cross effects sums of squares

Relative importance of GCA, SCA and reciprocal cross effects for each of the traits is presented in Figure 3.4. General combining ability effects were significant for QI, tryptophan content, protein content, kernel endosperm modification and anthesis days and were also the major component of sums of squares of the total genetic effects in these traits (Figure 3.4) with more than 72% of total sum of squares. Specific combining ability was significant for grain yield, anthesis days, and QI but was the major component of sum of squares of genetic effects only for grain yield with 68.5% of the total sum of squares (Figure 3.4). Reciprocal cross effects were significant for anthesis days, tryptophan content, and QI but were not the major genetic component in all the three traits based on total sums of squares for GCA, SCA and REC effects.

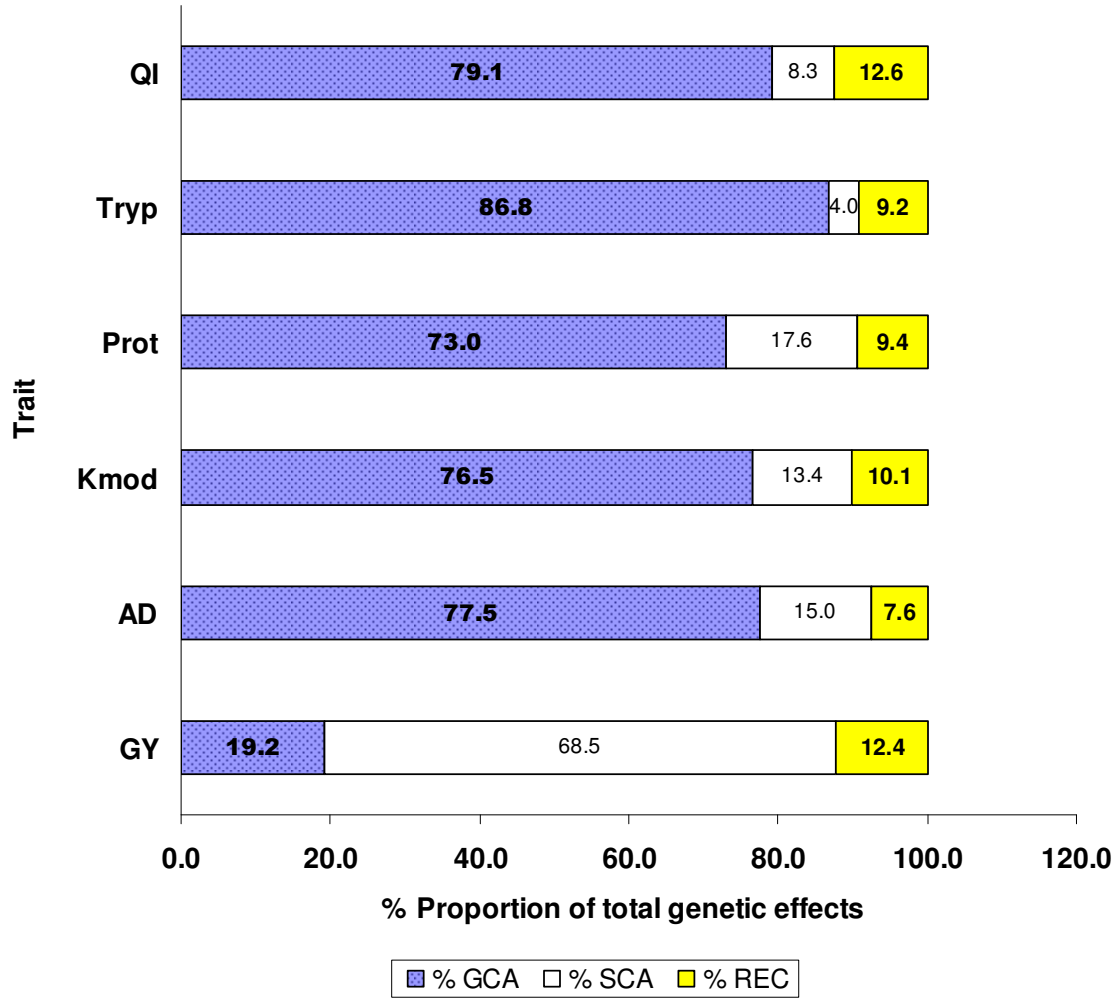

Figure 3.4 Relative importance of general combining ability (GCA), specific combining ability (SCA) and reciprocal (REC) cross effects sums of squares for grain yield (GY), anthesis date (AD), kernel endosperm modification (Kmod), protein content (Prot), tryptophan content (Tryp), and Quality Index (QI)

3.3.6 Relative importance of maternal and nonmaternal effects

The relative importance of maternal and nonmaternal effects based on sum of squares in each of these traits is presented in Figure 3.5. At least forty percent of reciprocal cross effects sum of squares for anthesis days was due to significant ($p < 0.001$) maternal effects whilst the remainder was due to significant ($p < 0.001$) nonmaternal effects which were a result of the interaction of the nuclear and extranuclear factors in crosses. Nonmaternal effects sum of squares constituted the major proportion (64.1%) of reciprocal cross effects sum of squares for tryptophan content and they were significant, whilst the maternal effects (35.9%) were nonsignificant. An almost similar picture is displayed by reciprocal cross effects sum of squares

for QI where both components of reciprocal cross effects were highly significant and 67.2% were nonmaternal effects and 32.8% were maternal effects.

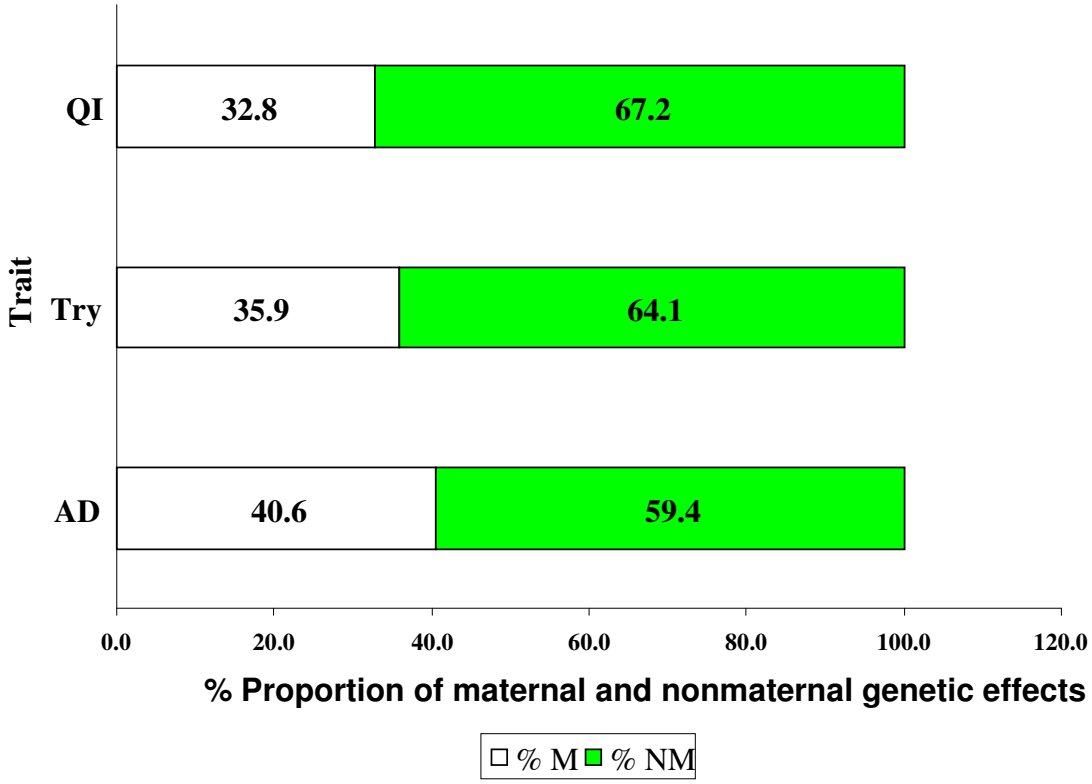

Figure 3.5 Partitioning of significant reciprocal cross effects (REC) into maternal (M) and nonmaternal (NM) sums of squares for anthesis days (AD), tryptophan (Try) content and Quality Index (QI).

3.4 Discussion

3.4.1 General and specific combining ability

The results in Tables 3.2 and 3.3 indicated that either some or all the nine parents were genetically different for the traits studied. This indicated the presence of considerable genetic variation which could be exploited in a QPM breeding programme. General combining ability was nonsignificant whilst SCA was highly significant for yield. This meant that nonadditive gene action was more important for grain yield than additive gene action. These results are in contrast to earlier studies on CIMMYT QPM populations. In earlier studies on CIMMYT QPM populations (Vasal et al., 1993a; Vasal et al., 1993b) and CIMMYT QPM inbred lines (Pixley and Bjarnason, 1993) GCA effects were found to be significant and more important than SCA effects

for grain yield. This difference could be due to the fact that both CIMMYT QPM populations and inbred lines were previously not oriented towards the development of QPM hybrid varieties and hence not polarised along heterotic groupings. The set of materials used in this study were developed with due regard to heterotic groupings.

In a recent study on QPM inbred lines including inbred lines CML176, CML181 and CML184 from CIMMYT, Bhatnagar et al. (2004) found SCA effects to be significant and more important than GCA effects in the genetic control of grain yield. Also in a recent study but on normal endosperm maize, Long et al. (2004) found both GCA and SCA effects to be significant for grain yield but SCA effects were more important than GCA effects. The set of inbred lines used in this study can be exploited for grain yield through targeting SCA effects. Quality protein maize breeders using the set of inbred lines in this study can achieve high grain yield levels by growing the F_1 crosses of lines with the best SCA for grain yield and these were CZL03016 and CML144. The high level of SCA effects for grain yield between the two inbred lines also confirmed that CZL03016 and CML144 belong to opposite heterotic groups as indicated in the background information in Table 3.1.

Specific combining ability effects were also found to be significant in both anthesis days and QI but were not the major component of sums of squares for these two traits. The major component of genetic effects sums of squares were GCA effects and these were highly significant in both anthesis days and QI. Thus for both anthesis days and QI both additive and non additive genetic effects were significant but additive effects were more important than non additive effects as indicated by the proportion of additive sums of squares in Figure 3.4. Vasal (1993a) and Bhatnagar et al. (2004) both found SCA effects to be nonsignificant but highly significant GCA effects for silking days. The significance of SCA effects for QI suggest that it could be logical to develop heterotic groupings for QI to facilitate development of QPM hybrids so that this trait can be exploited through F_1 crosses like grain yield for the benefit of the farmers. Currently heterotic groupings are based on grain yield.

All the kernel endosperm quality traits including kernel endosperm modification, protein content, tryptophan content and QI had significant GCA effects, and GCA effects were the major component for sums of squares in each of these traits (Figure 3.4). This implied that additive gene effects were involved in the expression of the trait and were more important than both SCA effects and reciprocal cross effects. Bhatnagar et al. (2004) studied pericarp removal, endosperm hardness, 1 000 -kernel weight, and test weight, and found GCA effects to be more

important than SCA effects. Similarly, Long et al. (2004)'s study in normal endosperm maize found that kernel quality traits (iron and zinc content) were mostly controlled by the significant GCA effects as opposed to the nonsignificant SCA effects.

In the studies of Vasal (1993a and 1993b) and Pixley and Bjarnason (1993) GCA effects were found to be the only significant component for the studied kernel endosperm quality traits. Vasal (1993a and 1993b) studied kernel endosperm hardness whilst Pixley and Bjarnason (1993) studied protein concentration in grain, tryptophan concentration in grain and tryptophan concentration in protein which is similar to what is currently referred to as QI. Earlier research findings on kernel endosperm modifying factors also reported that additive variance (which is derived from general combining ability) was more important than non-additive variance (which is derived from specific combining ability) (Bjarnason et al., 1976; Hohls et al., 1996; Vasal, 1975; Vasal et al., 1980). Sriwatanapongse (1974) also found that kernel endosperm vitreosity which is related to kernel endosperm modification level was controlled by additive genetic variance. Therefore, for the set of inbred lines in the present study desirable levels of the kernel endosperm modification trait can be best imparted using inbred lines with the best GCA values (CZL03016 and CZL01006) for kernel endosperm modification, whilst protein content, tryptophan content, and QI can all be imparted through the use of inbred line CML264Q.

In this study inbred line CML264Q had the best GCA effects values for protein content, tryptophan content, and QI but the worst GCA effects value for kernel endosperm modification. Thus quality protein maize breeders can improve the kernel nutrition quality attributes of their crosses, or breeding crosses by using CML264Q for protein content, tryptophan content and QI but not for grain yield.

The best line for kernel endosperm modification, CZL03016, was worst for both tryptophan content and QI GCA values, whilst for protein content the line with the worst GCA value was CML159. The inbred line (CZL03016) with the best level of kernel endosperm modification produced the best cross for grain yield but was poor in all the other kernel quality traits except in producing the cross with highest SCA for QI with inbred line CML264Q. Quality Index is the overall deciding attribute for protein quality in QPM and since CZL03016 showed the highest SCA effects for both grain yield (with CML144), and QI (with CML264Q), QPM breeders who want to obtain high grain yield level without losing on the QI can try to do so by test crossing CZL03016 to recombinant inbred lines from the cross of CML264Q and CML144. Also since there is a possibility of exploiting SCA effects in QI for some of the crosses, QPM breeders

could explore this avenue before discarding QPM inbred lines that are not very good for nutritional traits but with good levels of modification.

Relative maturity is an important trait and in this study CZL03016 was found to have the best GCA for anthesis date and it also produced the cross with the best SCA for grain yield when crossed to CML144. This is an advantage to breeders who are seeking early maturing and high yielding germplasm in that both earliness to flowering, and high grain yield occurred in the same cross. Although CML144 had the worst GCA effects for days to anthesis its cross with CZL03016 had the best SCA effect for anthesis days.

It was found that when a QPM breeder wants to use this set of inbred lines the two key lines will be CZL03016 (for kernel endosperm modification, grain yield, and days to anthesis), and CML264Q (for protein content, tryptophan content and QI). All the other lines with good GCA scores for quality traits and good levels of SCA for yield with CZL03016 can be grouped with CML264Q. On the other hand all the lines with good GCA levels for protein content, tryptophan content, and QI but with poor SCA with CZL03016 can be grouped on the same side with CZL03016. The combination of CZL03016 with CML264Q had the third best SCA value for grain yield, but nonsignificant, and the best and significant SCA for QI and thus the hybrid combination of the two inbred lines is already the best for quality traits and what is needed is to improve it for grain yield.

3.4.2 Reciprocal cross differences

Differences between the F_1 crosses and their reciprocal crosses were significantly expressed for anthesis days, tryptophan content and QI. Thus the days to anthesis, tryptophan content and QI values were dependent on the direction of the cross. In order to ensure that the desirable levels of expression for these three traits are maintained in future, the crosses have to be done with parents designated according to the direction of the cross with the desirable outcome. Reciprocal cross effects were not the major component of sums of squares in all the traits studied and at most they were only 12.6% in QI. Although not very important in most agronomic traits of maize, Vasal (1975) and Vasal et al. (1980) reported significant reciprocal cross effects in QPM for kernel endosperm traits in the form of maternal effects.

In this study both QI and anthesis days had both maternal and nonmaternal effects significant whilst for tryptophan content only the nonmaternal type of reciprocal cross effects was significant. The maternal component of reciprocal cross effects represents the amount of reciprocal cross variation that can be influenced by the maternal parent whereas the

nonmaternal component represents differences due to the interaction of cytoplasmic and nuclear genes and are generally unexplainable (Lopez et al., 2003). In this study, relative importance of nonmaternal effects was higher than maternal effects in anthesis days, tryptophan content and QI meaning that unexplainable reciprocal cross differences were relatively more important than those that can be influenced by the effect of the maternal genotype on the progeny. Thus although CML264Q was found to have significant reciprocal cross effects for tryptophan content and QI the desirable expression of these traits might not always be achieved by using CML264Q as a female parent since the bulk of the reciprocal cross differences is controlled by unexplainable nonmaternal effects. Similarly for CML176 and anthesis days, the bulk of the reciprocal cross differences variation was unexplainable nonmaternal effects.

This validates the importance of using diallel designs that cater for reciprocal differences when dealing with kernel endosperm traits. The partitioning of the entries (hybrids) sum of squares into general GCA, SCA effects and reciprocal cross effects coupled with the current emphasis on heterotic groupings in CIMMYT breeding programmes could also be the reason why SCA effects were found to be significant for QI. This could be so because some of the types of maternal effects when present would inflate either the random error or the SCA sum of squares which in both cases could make the F-test for SCA less sensitive there by revealing insignificant SCA effects for QI (Roach and Wulff, 1987b).

3.4.3 Genotype by environment interactions

The significance of the hybrids by environment interaction terms for grain yield, anthesis days, kernel modification, and protein content implied that the performance of the genotypes was different for grain yield, anthesis days, kernel modification and protein content in the environments used in this study. This highlighted the need to use several environments in the estimation of genetic effects. Genetic component estimates based on data from single environments were found to be unreliable (Christie and Shattuck, 1992; Zhang and Kang, 1997). The hybrid entries source of variation and the corresponding genetic components for the QI trait had nonsignificant interactions with the environment. This suggested that the relative rankings of the genotypes involved in the study were consistent from one environment to another for QI such that only one environment can be reliably used for the study of QI

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3.5 Conclusions

The estimates for GCA, and SCA effects in crosses of QPM inbred lines revealed that GCA was significant and important for QI, tryptophan content, protein content, kernel endosperm modification and anthesis days whilst SCA was important and significant in grain yield, and less important but significant for both QI and anthesis days. Reciprocal cross effects were significant for QI, tryptophan content and anthesis days but were of lesser importance in all the three traits as indicated by the relative contribution of reciprocal cross effects to the hybrid entry sums of squares. Thus maternal effects need to be considered when evaluating anthesis days, tryptophan content and QI.

The best inbred combination for grain yield was the cross of CZL03016 to CML144 whilst the best inbred line for GCA values of all the quality traits (QI, tryptophan content and protein content) except kernel endosperm modification was CML264Q. The most desirable inbred line for kernel endosperm modification GCA was CZL03016. The significance of hybrid x environment interactions for all the different traits, except QI substantiated the need to use several environments in determining the genetic potential of QI Index in this set of inbred lines invokes the idea of trying to exploit the non additive genetic variation for QI through heterosis just like grain yield. This could be an advantage because inbred parents with mediocre levels of tryptophan content like CZL03016 can be expected to produce a hybrid genotype with high SCA values of the QI trait like the cross of CZL03016 to CML264Q.

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4 Evaluation of quality protein maize (QPM) F_1 hybrid genotypes for grain yield and kernel endosperm modification in subtropical environments

Abstract

The adaptation of 36 F_1 hybrids and their reciprocal crosses for kernel endosperm modification and grain yield was evaluated across seven environments using both analysis of variance (ANOVA) and additive and multiplicative interaction (AMMI) models. The ANOVA results revealed that the best four experimental (QPM) hybrids were not significantly different from the best normal endosperm maize (check) variety but the group mean of the experimental hybrids (QPM) was inferior to the group mean of check varieties or normal endosperm maize for grain yield ($p<0.05$). The highest mean grain yield (8.49 t ha^{-1}) was that of a normal endosperm check variety (SC721) whilst the most desirable score for kernel endosperm modification (1.86) was from the experimental QPM hybrid CZL01006/CZL03016. Mean kernel endosperm modification score of normal endosperm maize genotypes was significantly better but the mean kernel endosperm modification score for QPM genotypes was within acceptable ranges. Therefore, although there were some significant group mean comparisons, individual genotype comparisons indicated that both experimental and QPM hybrids were as competitive as both check hybrids and normal endosperm maize hybrids for both traits. AMMI analyses revealed that AMMI2 and AMMI1 models were suitable for grain yield and kernel endosperm modification, respectively. The AMMI2 biplot for grain yield revealed a relatively high degree of crossover interactions for most of the genotypes, and the Spearman Rank correlation coefficient for grain yield was -0.61 ($p<0.0001$) indicating medium strength correlations for genotype ranks across environments. Quality protein maize genotype CML176/CML181f was identified as the most stable experimental QPM hybrid with a mean yield of 6.51t ha^{-1} across all environments. Normal endosperm maize commercial hybrid, SC721, was the highest yielding genotype across all environments using AMMI but the performance was not stable. There were QPM hybrids ((CML181f/CML176), (CZL03016/CML144), (CML264Q/CML144) and SC527Q) comparable to normal endosperm maize hybrids (SC721, SC633) in terms of mean grain yield and, hence, were well adapted to the subtropical environments.

4.1 Introduction

The development of quality protein maize (QPM) varieties rich in both tryptophan and lysine brought hope to the improvement of nutritional security for millions of maize consumers in sub-Saharan Africa whose diets lack other sources of protein rich foods. The agronomy of QPM is the same as that of normal endosperm maize but the biological value of QPM is about 80%

whereas that of normal endosperm maize is 40 to 57% (Bressani, 1992). In Zimbabwe anthropometric measures linked to protein-energy malnutrition indicated that 16.6% of the children were underweight, 29.4% stunted, 6.4% wasted and only 27.6% of the children are breastfed up to 24 months (UNICEF, 2006). The development and promotion of QPM cultivars can contribute towards the alleviation of protein malnutrition, especially in regions of the world where maize is the staple crop. It is important that QPM production be encouraged as one of the intervention strategies that can improve the nutritional security of maize consuming nations in a sustainable manner. Maize growing environments in sub-Saharan Africa are characterised by both frequent and extreme climatic fluctuations which threaten the productivity and profitability of maize farming systems. Inevitably the expectation of the farmer is for the newly released QPM cultivars to perform well consistently in the target environments which are mostly diverse in terms of both soil and climatic factors.

Generally it is not easy for cultivars to perform in a consistent manner since extreme fluctuation of climatic factors (mostly rainfall) can potentially lead to undesirable high maize genotype by environment interactions. In addition to stable and consistent performance the newly released QPM varieties would also be expected to perform better than the existing cultivars. In a recent study by Bhatnagar et al. (2004) normal endosperm maize varieties were found to perform better than QPM cultivars but not significant ($p > 0.05$). In an earlier study by Pixley and Bjarnason (1993), on average, the best QPM hybrid in each of the trials studied yielded 14% more than the best normal endosperm check variety. The environments in these two studies were not the same and most of them were not representative of the subtropical environments. Therefore, it would be interesting to investigate both the performance and adaptation of experimental hybrids produced from the current set of CIMMYT elite QPM inbred lines relative to normal endosperm maize cultivars under subtropical environments.

Genotype by environment interaction is differential genotypic expression across environments (Fox et al., 1997). Genotypes with extremely high seasonal genotype x environment fluctuations across seasons or across several locations in the same growing season are undesirable since they adversely influence both the breeding process, and the productivity and profitability of maize farming systems if released to the farmers. Previous studies on QPM have highlighted concerns for stability of grain yield and kernel endosperm quality traits (kernel endosperm modification, protein content, tryptophan content, and Quality Index) with some genetic backgrounds (Bjarnason and Vasal, 1992; Vasal, 2000; Vasal et al., 1984). This is because the

opaque-2 gene that confers high levels of tryptophan and lysine is associated with low grain yield levels and undesirable kernel characteristics (Mertz, 1994; Villegas, 1994). These drawbacks were addressed through concerted breeding efforts that promoted the use of kernel endosperm modifiers (Vasal, 2002; Vasal et al., 1980; Vasal et al., 1984) however, expression of kernel endosperm modifiers was found to be both influenced by background genotype and environment. Therefore, the study of kernel endosperm modification and grain yield performance in target environments of elite contemporary CIMMYT QPM germplasm will assist in the identification of QPM genotypes with relatively high and stable performance.

In order to cater for unexpected poor agronomic performances due to genotype x environment interactions, breeders evaluate the performance of test materials across several locations in several growing seasons. This facilitates the identification of both high yielding and relatively stable genotypes, and their areas of specific adaptation. Two techniques currently used for identifying high yielding genotypes with stable performance and their corresponding areas of specific adaptation are additive main effects and multiplicative interaction (AMMI) analysis (Gauch Jr and Zobel, 1997; Zobel et al., 1988) and genotype, and genotype by environment (GGE) Biplot (Yan and Tinker, 2005; Yan et al., 2000). The two techniques are increasingly being used separately and independently by breeders compared to the traditional methods of a separate ANOVA (Snedecor and Cochran, 1980) followed by a linear regression (Eberhart and Russell, 1966; Finlay and Wilkinson, 1963), and principal component analysis (Hill and Goodchild, 1981). The main advantage of both AMMI and GGE biplot is that, in addition to incorporating the ANOVA technique, they can also analyse complex patterns of genotype x environment interaction whereas linear regression is appropriate when there is a linear response of the genotypes to the environments and yet this is not always the case. In 72% of the environments in a study by Crossa et al. (1990), AMMI selected a different highest yielding genotype than did treatment means. The basic difference between AMMI analysis and GGE Biplot is that AMMI analysis produces outputs with genotype main effects (G), environments (E) main effects and partitions the genotype x environment (GxE) interaction where as GGE Biplot produces all the other components but with no environment (E) main effects, and, hence, the name GGE biplot analysis. Currently there is an unresolved argument on which of the two methods is superior or better (Gauch Jr, 2006; Gauch Jr et al., 2008; Yan et al., 2007).

Both AMMI and GGE biplot require a two way data set displayed in rows and columns and in consideration of the available computing resources, AMMI was chosen for this study. In a

previous study of both hybrid and open pollinated QPM cultivars, Pixley and Bjarnason (2002) found that tryptophan concentration in protein (currently referred to as Quality Index) was the most stable trait, followed by protein concentration in grain, then kernel endosperm modification and grain yield in the order of decreasing stability. However, in all the genotypes studied by Pixley and Bjarnason (2002), protein concentration and kernel endosperm modification were found to be within the acceptable ranges for QPM. Most breeding programmes in sub-Saharan Africa are making use of the contemporary elite CIMMYT QPM germpalsm in their efforts to develop locally adapted QPM cultivars (CIMMYT, 2004; CIMMYT, 2005). Therefore, it is imperative to generate more information on the agronomic potential of the CIMMYT inbred lines for the benefit of sub-Saharan Africa QPM breeding programmes. The identification of high yielding and stable genotypes will both facilitate the subsequent breeding and release of new QPM cultivars by the sub-Saharan African breeding programmes, thereby, improving the nutritional security of the inhabitants of sub-Saharan Africa.

The first objective of the study was to compare the performance of the experimental QPM genotypes against selected check cultivars, and also compare the performance of QPM hybrids against normal endosperm maize hybrids for both grain yield levels and kernel endosperm modification scores. The second objective was to investigate the adaptation of QPM genotypes for grain yield and kernel endosperm modification in sub-Saharan Africa target environments using the AMMI technique. The hypotheses were that the set of experimental F_1 hybrids and their reciprocal crosses was adapted to the subtropics as the check varieties and also the agronomic performance of the QPM hybrids was comparable to that of normal endosperm maize hybrids.

4.2 Methods and materials

4.2.1 Germplasm

The F_1 hybrids and their reciprocal crosses were constituted using a full diallel of the nine QPM inbred parents listed in Table 4.1. Seed of the F_1 crosses and their reciprocals was made during both summer cropping seasons and winter nurseries of the period 2005/6 to 2007 using the CIMMYT Harare (summer nurseries) and CIMMYT Muzarabani (winter nurseries) field facilities. In addition to the F_1 crosses and reciprocals, three Seed Co varieties (SC721, SC633, and SC527Q) were used as check entries. Cultivar SC721 is a late maturing white endosperm single cross normal endosperm maize variety grown in the southern and central Africa region. The hybrid cultivar SC633 is a medium maturing normal endosperm maize single cross variety,

whilst SC527Q is an early maturing white endosperm single cross pre – commercial QPM hybrid variety.

The CML coded lines in Table 4.1 are internationally accepted as elite CIMMYT inbred lines and the CZL coded lines were developed at CIMMYT Harare and were still in the process of being considered for CML coding if proved to be of superior agronomic attributes. The inbred lines were developed from CIMMYT germplasm pools 62, 63, and 68 and UWO417. The source GQL5 is from Ghana. The majority of the lines were classified as members of the B heterotic group and only CZL03016 and CML159 belong to the A heterotic grouping. Most of the lines were of lowland tropical adaptation except CML181f, CML175 and CZL3016 which are subtropical or midaltitude. All the inbred lines were of white endosperm colour and CML176, and CML175 were classified as of a dent grain texture whilst CZL01006 was not classified.

Table 4.1 QPM inbred parents for the diallel of experimental hybrids

Entry	Inbred line	Pedigree	HG[s]	ADT[s]	ECGT[s]
1	CZL03016	[CML159/[CML159/[MSRXPOOL9]C1F2-205-1(OSU23i)-5-3-X-X-1-BB]F2-3sx]-8-1-2-B	A	MA	WF
2	CML144	P62C5F182-2-1-2-BB-3-1-##	B	LT	WF
3	CML159	P63C2F5-1-3-1-B-2-1-1-B-#	A	LT	WF
4	CML175	P68C0F77-2-3-7-B-2-3-1-B-1-BB	B	ST	WD
5	CML176	(P63-12-2-1/P67-5-1-1)-1-2-BB	B	LT	WDF
6	CML181f	UWO417-B-2-1-1-BB	B	ST	WF
7	CML264Q	(CML176*CML264)-BxCML264)-7-1xCML264]xCML264-F2(65)-B*4	B	LT	WF
8	CML492	P62C3F163-3-3-3-2-#-1-1-2-BB	B	LT	WF
9	CZL01006	GQL5	B	LT	W /nc

[s]Key HG = heterotic group; ADT = adaptation; MA = midaltitude; LT = lowland tropical; ST = subtropical; ECGT = endosperm colour and grain texture; WF = white flint, WD = white dent; WDF = white dent flint; nc = not classified grain texture; Group A = Tuxpeno, B73 types; Group B = Eto, Ecuador, and Mo17 types.

4.2.2 Environments

The experiment was conducted at CIMMYT Harare (CH) (1409 m a.s.l., 17º43' S, 31º01' E), Agricultural Research Trust (ART) farm (1527 m a.s.l.; 17º43' S, 31º05' E), Rattray Arnold

Research Station (RARS) (1341 m a.s.l., 17º40' S, 31º13' E) and Kadoma Research Centre (KRC) (1149 m a.s.l., 18º19' S; 29º 17' E). The experiment was conducted at each site for two cropping seasons (2006/7 and 2007/8) creating a total of eight test environments but seven were successful.

The targeted plant population density was 53000 plants per hectare in each of the environments and was achieved by a spacing of 0.75m between the rows, and an effective spacing of 0.25m in the row. Different fertiliser rates were applied as follows: 166kg N-56kg P-28kg K ha^{-1} at CH; 180kg N-84kg P-42kg K ha^{-1} at ART farm; 145.30kg N-56kg P-28kg K ha^{-1} at RARS; and 88.4kg N-56kg P-28kg K ha^{-1} at KRC. Historically both ART farm and CIMMYT Harare are classified as high potential environments, with RARS being classified as a medium potential environment and KRC as a low potential environment.

Rainfall totals for the relatively drier 2006/7 summer cropping season were 582mm (CH), 673mm (RARS), 529mm (ART farm) and 490mm (KRC). The 2007/8 cropping season was relatively wetter with rainfall totals of 978mm (CH), 837.5mm (ART farm), 902.5mm (RARS) and 433mm (KRC). The KRC site received a higher total in 2006/7 but poor temporal distribution and better temporal distribution and a lower total in 2007/8. Standard cultural practices for growing maize were followed at all the sites and the fields were maintained clean through both hand weeding and use of herbicides.

4.2.3 Experimental design

The experiment was designed as an alpha (0, 1) lattice design with two replicates for each environment, with five plots per incomplete block (giving 15 incomplete blocks in each replication). The experimental unit was two 4-m rows spaced at 0.75m. Plants were thinned to one per station at three weeks after planting resulting in a plant population density of approximately 53 000 plants ha^{-1}.

Grain yield in kilograms per plot was adjusted to tons ha^{-1} at 12.5% H_20 using Fieldbook software (Banziger and Vivek, 2007). A sample of 100 kernels from two self pollinated ears per plot was used to determine kernel endosperm modification (Kmod) ratings (1-5) according to Vivek et al. (2008) and the ratings were interpreted as follows:

1 = not opaque and endosperm wholly translucent

2 = 25% opaque

3 = 50% opaque

4 = 75% opaque

5 = 100% opaque

The overall kernel endosperm modification score for a plot was weighted according to the kernel numbers that belonged to each of the five categories.

4.2.4 Statistical analyses

The interaction of four locations by two years was used to produce the different environments and, hence, there were seven environments from the three successful locations of 2006/7 cropping season, and four successful locations from the 2007/8 cropping season. Analysis of variance was conducted for both grain yield (GY) and kernel endosperm modification (Kmod) in SAS (2004) using the GLM procedure for each environment separately (not shown) and then combined across environments after establishment of homogeneity of variances. The genotypes were considered to be fixed and both the replications within environments and the environments were random. Two separate contrasts were made in two different analysis of variance tests, experimental hybrids versus check varieties, and QPM germplasm versus normal endosperm maize germplasm. However, the QPM versus normal endosperm maize germplasm was slightly unfair since there was no early maturing normal endosperm maize germplasm among the checks.

The grain yield data in tons ha^{-1} and kernel endosperm modification scores were analysed using the AMMI macro in Genstat 11 (VSN, 2008). The AMMI analysis assumes all data to be from a randomised block design and makes use of adjusted (predicted) means (Talbot et al., 2008). According to (Ebdon and Gauch Jr, 2002a) the model for the ANOVA can be written as follows:

"$Y_{ger} = \mu + \alpha_g + \beta_e + \theta_{ge} + \varepsilon_{ger}$, *and the AMMI model is as follows;*

$$Y_{ger} = \mu + \alpha_g + \beta_e + {}^{N}\sum_{n=1} \lambda_n \varsigma_{gn} \eta_{en} + \rho_{ge} + \varepsilon_{ger},$$

Where Y_{ger} *is the grain yield level or endosperm modification score for genotype g in environment e for replicate r,* μ *is the grand mean,* α_g *are genotype mean deviations (mean*

minus the grand mean), β_e are the environment mean deviations, N is the number of SVD (singular value decomposition) axes retained in the model, λ_n is the singular value for SVD axis n, ς_{gn} are the genotype singular vector values for SVD axis n, η_{en} are the environment singular vector values for SVD axis n, θ_{ge} are the interaction residuals, ρ_{ge} are the AMMI residuals, and ε_{ger} is the error term. The term $^{N}\sum_{n=1} \lambda_n \varsigma_{gn} \eta_{en} + \rho_{ge}$ is equivalent to the interaction term in the ANOVA model. More background information on the AMMI model can be obtained from Gauch Jr (1992).

According to Ebdon and Gauch Jr (2002a) the eigenvalue for a given SVD axis is the sum of squares (SS) retained by that axis and it is equal to the square of the singular value, λ^2. The sum of the eigenvalues $\sum\lambda^2$ for the N axes, plus the residual SS for a reduced model, is equal to the GE interaction SS. Thus the interaction SS is partitioned by SVD into interaction SS and associated degrees of freedom, which allow for the use of F-Tests to determine the significance of a given SVD axis" (Gauch Jr, 1992).

4.2.5 Model selection

Several methods have been suggested for the determination of the optimum number of interaction principal component axes (IPCAs) to use in the AMMI model (Dias and Krzanowski, 2003; Dias and Krzanowski, 2006; Gauch Jr, 1988) and all require considerable computation which could not be done in this study. The computation suggested on page 147 of Gauch Jr (1992) was used to select the best AMMI model from the AMMIF (AMMI Full) model. A full model is one with all the significant IPCAs. The computation is based on estimating the level of noise using statistics from the AMMI ANOVA. Noise is defined as the discrepancy between a yield estimate and its true mean (Gauch Jr, 1992).

The percent level of noise in the GE interaction component is estimated as follows:

(100 x (Interaction DF x EMS))/Interaction SS

where interaction DF is equal to interaction degrees of freedom, EMS is equal to the expected error mean square for the AMMI ANOVA, Interaction SS is equal to interaction sum of squares. The number of IPCAs in the final model would be that with a residual sum of squares value either equal or close to the corresponding sum of squares for the estimated level of noise. The findings in most of the previous studies on determining the optimum number of IPCAs except

that of Sivapalan et al. (2000) were that at most AMMI2 should be the highest model used in explaining the biological patterns.

4.2.6 Biplots

Biplots were done according to the model selected as the best fitting model for each trait. There are two commonly used biplots AMMI1 biplot where IPCA1 is plotted against genotype and environment means and AMMI2 biplot where IPCA2 scores are plotted against IPCA1 scores. IPCA scores against both genotype and site means were done for each trait separately using the first one or two significant IPCAs. Biplots beyond AMMI2 tend to be complex and not easily explainable with a two dimensional graph.

4.3 Results

The analysis of variance results comparing the experimental varieties against the check varieties are presented in Table 4.2. The grain yield means for the entries (checks + experimental hybrids) were highly significant ($p < 0.001$) and their interaction with the environments was also highly significant ($p < 0.001$). The grain yield means of the check cultivars were not significantly different, and their interaction with the environments was significant ($p = 0.05$). The contrast of the grain yield mean for the check varieties against the experimental varieties was significant ($p < 0.05$) and the interaction with the environments was highly significant ($p < 0.001$). The grain mean yield of the checks was 7.78 tons ha^{-1} and the mean of the experimental hybrids was 6.05 tons ha^{-1}.Thus the checks yielded higher than the experimental hybrids and the presence of the interaction of the environments with entries justifies further analysis of genotype x environment through AMMI. The entry numbers and pedigrees of genotypes are presented in Appendix 4.1 at the end of Chapter 4.

Table 4.2 Analysis of variance for grain yield: Experimental versus check cultivars

Source	DF	SS	MS	F Value	Pr >F
Environments (Envts)	**6**	**1625.70**	**270.95**	**221.22**	**0.00**
REP(Envts)	**7**	**93.31**	**13.33**	**10.88**	**0.00**
ENTRY	**74**	**671.72**	**9.08**	**3.40**	**0.00**
Experimental (Exp)	71	540.00	7.61	2.98	0.00
Checks (Chks)	2	11.04	5.52	2.52	0.12
Chks vs Exp	1	120.68	120.68	10.12	0.02
Envts*ENTRY	**444**	**1184.40**	**2.67**	**2.18**	**0.00**
Envts*Exp	426	1086.54	2.55	2.08	0.00
Envts*Chks	12	26.28	2.19	1.79	0.05
Envts*Chks vs Exp	6	71.57	11.93	9.74	0.00
Error	**518**	**634.44**	**1.22**		
Corrected Total	**1049**	**4209.57**			
Mean (t ha^{-1})					
Experimental		**6.05t ha^{-1}**			
Checks		**7.78t ha^{-1}**			

The results of the contrast of grain yield performance of QPM hybrids (experimental hybrids + SC527Q) against the normal endosperm maize check entries (SC721 and SC633) are presented in Table 4.3. The source of variation for the QPM germplasm was significantly different ($p < 0.001$) and normal endosperm varieties source of variation was not significant. The contrast of normal endosperm varieties to QPM germplasm was significant ($p < 0.05$) and the mean of the best performing normal endosperm maize entry (SC721) was at least 1t ha^{-1} higher, but using LSD mean comparisons was not significantly different from the means of the four top yielding QPM varieties (Appendix 4.2 at the end of Chapter 4). All the interactions with the environment were significantly different ($p < 0.001$) except the interaction of normal varieties and the environment. The average grain yield levels were 8.02t ha^{-1} and 6.06t ha $^{-1}$ for normal maize hybrids and QPM hybrids, respectively.

Table 4.3 Analysis of variance for grain yield: QPM versus normal endosperm maize cultivars

Source	DF	SS	MS	F value	PR > F
Environments (Envts)	**6**	**1625.70**	**270.95**	**221.22**	**0.00**
REP(Envts)	**7**	**93.31**	**13.33**	**10.88**	**0.00**
ENTRY	**74**	**671.72**	**9.08**	**3.40**	**0.00**
QPM	72	561.19	7.79	3.05	0.00
Normal	1	5.97	5.97	2.98	0.14
Normal vs QPM	1	104.55	104.55	9.03	0.02
Envts*ENTRY	**444**	**1184.40**	**2.67**	**2.18**	**0.00**
Envts*QPM	432	1102.90	2.55	2.08	0.00
Envts*Normal	6	12.02	2.00	1.64	0.14
Envts*Normal vs QPM	6	69.48	11.58	9.45	0.00
Error	**518**	**634.44**	**1.22**		
Corrected Total	1049	4209.57			
Mean (t ha^{-1})					
QPM		6.06**t ha^{-1}**			
Normal		8.02**t ha^{-1}**			

The results for analysis of variance for kernel endosperm modification with contrast of check varieties against experimental entries are presented in Table 4.4. Highly significant differences ($p < 0.001$) were observed for all the sources of variation except for the contrast of check varieties against experimental entries, the interaction of environments with checks and the interaction of the environments with the contrast of checks and experimental varieties. The mean scores for kernel endosperm modification for experimental and checks were 2.64 and 2.48, respectively.

Table 4.4 Analysis of variance for kernel endosperm modification: Experimental versus check cultivars

Source	DF	SS	MS	F Value	Pr > F
Environments (Envts)	**6**	**43.29**	**7.22**	**42.96**	**0.00**
REP(Envts)	**7**	**3.07**	**0.44**	**2.61**	**0.01**
ENTRY	**74**	**91.03**	**1.23**	**4.51**	**0.00**
Experimental (Exp)	71	84.96	1.20	4.41	0.00
Checks (Chks)	2	4.99	2.49	9.97	0.00
Chks vs Exp	1	1.08	1.08	2.53	0.16
Envts*ENTRY	**444**	**121.13**	**0.27**	**1.62**	**0.00**
Envts*Exp	426	115.58	0.27	1.62	0.00
Envts*Chks	12	3.00	0.25	1.49	0.12
Envts*Chks vs Exp	6	2.55	0.42	2.53	0.02
Error	**518**	**87.01**	**0.17**		
Corrected Total	1049	345.53			
Mean (1-5) rating					
Experimental		2.64			
Check		2.48			

The analysis of variance contrasting QPM and normal endosperm maize varieties for endosperm modification is in Table 4.5. In Table 4.5 kernel endosperm modification scores were not significantly different ($p > 0.05$) for both the normal endosperm varieties, and the interaction of normal endosperm varieties with the environments. All the other sources of variation were highly significant ($p < 0.001$) except the contrast of normal endosperm maize to QPM which was significant ($p < 0.05$). The mean ratings for kernel endosperm modification for normal endosperm maize hybrids and QPM hybrids were 2.24 and 2.65, respectively.

Table 4.5 Analysis of variance for kernel endosperm modification: QPM versus normal endosperm cultivars

Source	DF	SS	MS	F Value	Pr > F
Environments (Envts)	**6**	**43.29**	**7.22**	**42.96**	**0.00**
REP(Envts)	**7**	**3.07**	**0.44**	**2.61**	**0.01**
ENTRY	**74**	**91.03**	**1.23**	**4.51**	**0.00**
QPM	72	86.38	1.20	4.44	0.00
Normal	1	0.08	0.08	1.57	0.26
Normal vs QPM	1	4.57	4.57	6.76	0.04
Envts*ENTRY	**444**	**121.13**	**0.27**	**1.62**	**0.00**
Envts*QPM	432	116.77	0.27	1.61	0.00
Envts*Normal	6	0.31	0.05	0.30	0.93
Envts*Normal vs QPM	6	4.05	0.68	4.02	0.00
Error	**518**	**87.01**	**0.17**		
Corrected Total	1049	345.53			
Mean (1-5) rating					
QPM		2.65			
Normal maize		2.24			

4.3.1 AMMI ANOVA

4.3.1.1 Grain yield

The results of the AMMI1 analysis of variance for yield are presented in Table 4.6 with those for kernel endosperm modification in Table 4.7. For grain yield four interaction PCAs were highly significant ($p < 0.01$), and the fifth IPCA was not significant ($p > 0.05$). The treatments (genotypes + environments + interactions) explained 82.7% of the total grain yield sums of squares in Table 4.6. The genotypes explained 15.96% of the total sums of squares and 19.30% of the treatments sum of squares. The environments explained 38.6% of the total variation and 46.7% of the treatments sum of squares. The interactions were 28.1% of the total variation and 34.02% of the treatments variation. Thus the environments had more variation than the genotypes followed by the genotype x environment interactions and genotypes had the least variation among the components of the treatments source of variation.

For grain yield level (Table 4.6) the first IPCA explained 35.9% of the variation (sum of squares) using 17.8% of the interaction degrees of freedom. When the first two IPCAs were used the model explained 57.7% of the interaction using only 35.2% of the total interaction degrees of freedom. Addition of the third IPCA resulted in the model explaining 75.5% of the total interaction variation using only 52% of the total interaction degrees of freedom. The first four IPCAs explained 86.75% of the total GE interaction using only 68.5% of the total interaction degrees of freedom.

Table 4.6 AMMI analysis of grain yield levels with sequential sums of squares for the first five PCA axes.

Source	DF	SS	MS	F	F prob	% Total SS explained
Total	1049	4210	4.01	*	*	
Reps (Envts)	7	93	13.32	10.88	<0.01	2.2
Treatments	524	3482	6.65	5.42	<0.01	82.7
Genotypes	74	672	9.08	7.41	<0.01	15.96
Environments	6	1626	270.98	20.34	<0.01	38.6
Interactions	444	1185	2.67	2.18	<0.01	28.1
IPCA 1	79	427	5.41	4.41	<0.01	
IPCA 2	77	257	3.33	2.72	<0.01	
IPCA 3	75	211	2.81	2.29	<0.01	
IPCA 4	73	133	1.82	1.48	<0.01	
IPCA 5	71	95	1.33	1.09	0.302	
Residuals	69	63	0.91	0.74	0.93678	
Error	518	635	1.22	*	*	15.1

4.3.1.2 Kernel endosperm modification

In Table 4.7 the AMMI analysis captured 73.86% of the total variation as treatments variation for kernel endosperm modification in seven environments. The genotypes accounted for 26.23% of the total variation but this was 35.51% of the treatments sum of squares. The environments were responsible for 12.63% of the total variation and this was 17.09% of the treatments sum of squares. The interaction sum of squares was 35.00% of the total sum of squares but this was 47.39% of the treatments variation. The interactions (GXE) accounted for most of the variations in kernel endosperm modification and environments accounted for the least amount of variation.

Table 4.7 AMMI analysis of kernel endosperm modification levels with sequential sums of squares for the first five PCA axes.

Source	DF	SS	MS	F	F prob	% Total SS explained
Total	1049	344.71	0.3286	*	*	
Reps (Envnts)	7	3.08	0.4401	2.62	0.01151	0.89
Treatments	524	254.59	0.4858	2.89	0.00000	73.86
Genotypes	74	90.41	1.2217	7.27	0.00000	26.23
Environments	6	43.52	7.2529	16.48	0.00000	12.63
Interactions	444	120.66	0.2718	1.62	0.00000	35.00
IPCA 1	79	37.86	0.4792	2.85	0.00000	
IPCA 2	77	24.57	0.3191	1.9	0.00003	
IPCA 3	75	19.36	0.2581	1.54	0.00427	
IPCA 4	73	17.8	0.2439	1.45	0.01228	
IPCA 5	71	12.27	0.1728	1.03	0.41959	
Residuals	69	8.8	0.1275	0.76	0.92274	
Error	518	87.04	0.168	*	*	25.25

The first interaction PCA for kernel endosperm modification in Table 4.7 explained 31.4% of the interaction sum of squares against 17.8% degrees of freedom for the interaction. The combination of the first IPCA and the second IPCA explained 51.7% of the variation using 35.2% degrees of freedom. The first three IPCAs used 52% of the total interaction degrees of freedom whilst explaining 67.8% of the total interaction sum of squares. The four IPCAs explained 82.5% of the sum of squares variation against 68.5% of the total interaction degrees of freedom. In both analyses the residual was left with relatively more degrees of freedom and less sum of squares.

4.3.2 Levels of noise and pattern in AMMI ANOVA

The level of noise in both AMMI ANOVAs (Table 4.8 and 4.10) was relatively higher than what was found in other studies reviewed by Gauch (1992) and was of the same magnitude but still higher than 33.3% and 40.6% obtained by Ebdon and Gauch (2002b). The most suitable model for grain yield was selected as AMMI2 (Tables 4.9 and 4.11) because the noise sum of squares for grain yield, 541.68, lay between the residual sum of squares for AMMI2 and AMMI3 but was closer to AMMI2 and, hence, AMMI2 was selected.

Table 4.8 Levels of noise and pattern in the grain yield interaction sums of squares

Attribute	% Level	Sum of Squares
Noise	45.71	541.68
Pattern	54.29	643.32

Table 4.9 Residual sums of squares for grain yield in each AMMI model

AMMI model	AMMI1	AMMI2	AMMI3	AMMI4
Residual SS	757	635	290	157

Similarly the most suitable model for kernel endosperm modification was selected as AMMI1 (Tables 4.10 and 4.11) since the noise sum of square value of 74.592 was between AMMI1 and AMMI2 but closer to AMMI1.

Table 4.10 Levels of noise and pattern in the Kmod interaction sums of squares

Attribute	% Level	Sum of Squares
Noise	61.82	74.592
Pattern	38.18	46.068

where kmod = kernel endosperm modification

Table 4.11 Residual sums of squares for Kmod in each AMMI model

AMMI model	AMMI1	AMMI2	AMMI3	AMMI4
Residual SS	82.8	58.2	38.87	21.07

where kmod = kernel endosperm modification

4.3.3 Genotype selections according to yield

Genotype pedigrees are presented in Appendix 4.1 at the end of Chapter 4.The lowest yielding environment was KR08 with a mean yield level of 4.228t ha^{-1} whilst the highest was AR07 with a mean yield of 8.043t ha^{-1}. The mean grain yield levels of genotypes (data not shown) varied with environment ranging from 1.68t ha^{-1} (genotype 16 (CML144/CML181f) in environment KR08) to 11.72t ha^{-1} (genotype 3 (SC721) in environment CH07). The entry numbers of the top four yielding genotypes in each environment based on a model with two PCA axes are given in

Table 4.12. Genotype G3 (SC721) was ranked among the top 4 in six of the seven environments followed by genotype G48 (CML181f/CML176) which was among the top 4 in four of the seven environments. Genotypes G70 (CZL01006/CML159), G11 (CZL03016/CZL01006), G12 (CML144/CZL03016), G52 (CML264Q/CZL03016), and G53 (CML264Q/CML144) appeared once among the top four selections for grain yield.

Table 4.12 First four AMMI2 selections per environment for grain yield (t ha-1).

Environment	Code	Mean t ha^{-1}	PCA Score	Rank			
				1	2	3	4
ART 2008	**AR08**	7.591	2.282	G48	G37	G50	G52
CIMMYT 2008	**CH08**	5.917	1.286	G48	G3	G50	G12
RARS 2008	**RA08**	5.569	0.465	G3	G53	G48	G4
KRC 2008	**KR08**	4.288	0.377	G53	G3	G4	G48
RARS 2007	**RA07**	5.023	-0.849	G3	G4	G2	G1
ART 2007	**AR07**	8.043	-1.373	G3	G2	G4	G1
CIMMYT 2007	**CH07**	6.387	-2.188	G3	G2	G70	G11

Genotype pedigrees are presented in Appendix 1 at the end of Chapter 4

4.3.4 Genotype selections according to kernel endosperm modification

The environment means for kernel modification on a 1-5 rating ranged from 2.4 (the lowest and best) observed under AR07 and CH07 environments, to 3.0 (the highest but worst) observed under KR08 environment (Table 4.13). The genotype means were different under the different environments and the best rating was 1.3 for genotype 11 (CZL03016/CZL01006) in environment AR08, whilst the worst rating was 4.0 for genotype 54 (CML264Q/CML159) and obtained in environment RA08 (data not shown). The entry numbers of the first four genotypes (those with the least scores) are given in Table 4.13. Genotype G68 (CZL01006/CZL03016) was ranked among the top 4 in all the seven environments, whilst genotypes G20 (CML159/CZL03016) and G11 (CZL03016/CZL01006) both appeared among top 4 in six environments, genotype G10 (CZL03016/CML492) was among the top 4 three times, and genotypes G3 (SC721), and G12 (CML144/CZL03016) both appeared twice and genotypes G70 (CZL01006/CML159) and G50 (CML181f/CML492) once.

Table 4.13 First four AMMI1 selections per environment for modification (1-5) rating

Environment	Code	Mean score[a]	PCA Score	Rank			
				1	2	3	4
ART 2008	AR08	2.484	1.0764	G11	G20	G68	G12
KRC2008	KR08	2.967	0.8721	G11	G20	G68	G12
RARS 2008	RA08	2.809	0.2452	G68	G11	G20	G3
CIMMYT 2008	CH08	2.714	0.1698	G68	G11	G20	G3
RARS 2007	RA07	2.678	-0.4901	G68	G10	G11	G20
ART 2007	AR07	2.391	-0.5199	G68	G10	G11	G20
CIMMYT 2007	CH07	2.391	-1.3535	G10	G70	G50	G68

[a]Score 1-5, 1 = well modified and most desirable, 5 = poorly modified and least desirable. Genotype pedigrees are presented in Appendix 1

4.3.5 Biplots

Based on results of model selection, three biplots were plotted and these are presented in Figure 4.1 to 4.3. The first two were for the first IPCAs against both genotype and environment means of each of the two traits (grain yield and kernel endosperm modification), and the third was for the interaction of IPCA 1 and IPCA 2 for grain yield.

4.3.5.1 IPCA 1 against environment and genotype grain yield means

In Figure 4.1 the IPCA 1 classified the environments into two main groups. The first group was above the IPCA 1 = 0 score and the second group below. All the environments above the IPCA 1 = 0 score were from the 2007/2008 cropping season whilst those below were from the 2006/2007 cropping season. Environments AR08, AR07 and CH07 were classified above the mean grain yield for all the environments whilst environment CH08 was almost equal to the grand mean and RA08, RA07 and KR08 were below the grand mean grain yield level with KR08 being the lowest yielding environment.

A key to the entry numbers for the genotypes is given in Appendix 4.1 at the end of Chapter 4. Genotype 3 (SC721) was the highest yielding genotype under IPCA 1 scores followed by genotype 2 (SC633) and then genotypes 1 (SC527Q), 4 (CZL03016/CML144), 48 (CML181f/CML176), 53 (CML264Q/CML144) and 12 (CML144/CZL03016) (Figure 4.1). All the high yielding genotypes performed well in environments AR07 and CH07. The least yielding genotypes were entry 16 (CML144/CML181f), 45 (CML181f/CML144), and 5

(CZL03016/CML175) followed by 63 (CML492/CML175), 34 (CML175/CML492), 20 (CML159/CZL03016) and 21(CML159/CML144). Entries 16 (CML144/CML181f), 45 (CML181f/CML144), 5 (CZL03016/CML175), and 20 (CML159/CZL03016) were adapted to environments KR08 and RA08. Genotype 12 (CML144/CZL03016) and 40 (CML176/CML181f) yielded above the grand mean grain yield level and were more stable than genotypes above and below the IPCA 1 = 0 level. Entries 23 (CML159/CML176), 66 (CML492/CML264Q) and 64 (CML492/CML176) were also stable but they yielded below the grand mean yield (6.12 tons ha-1). Genotypes and environments with the same sign on the PCA axis interacted positively and those with different signs interacted negatively. The best yield performers in environment RA07 were entries 35 (CML175/CZL01006) and 71 (CZL01006/CML175). Genotypes 48 (CML181f/CML176), 53 (CML264Q/CML144) and 12 (CML144/CZL03016) performed well in environment AR08 and in addition to being a high grain yielder, genotype 12 (CML144/CZL03016) was relatively more stable since it was closer to the line IPCA 1 = 0. Most of the genotypes were found around the grand mean. Genotype 14 (CML144/CML175) had a stable performance (with a mean almost located at the origin of the graph) and the average yield was equal to the grand mean (6.12 tons ha^{-1}).

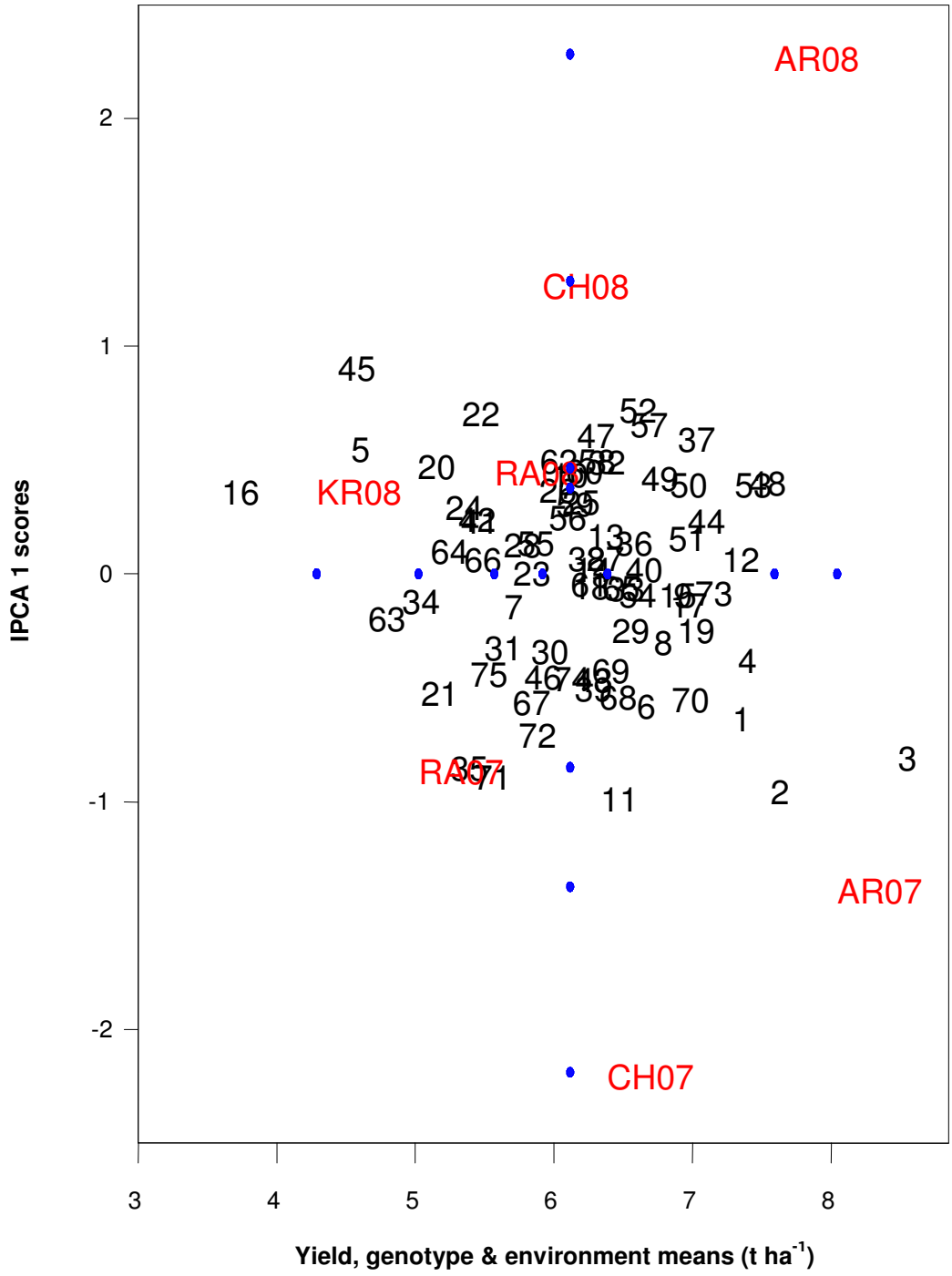

Figure 4.1 AMMI biplot of IPCA 1 scores against grain yield for seven environments. Environment scores represented by a four character symbol where AR = ART farm, CH = CIMMYT Harare, RA = Rattray Arnold Research Station, KR = Kadoma Research Centre, 07 represents 2007 cropping season and 08 represents 2008 cropping season.

4.3.5.2 Means for IPCA 1 against environment and genotype kernel endosperm modification

The biplot of IPCA 1 against both genotype and environment means for kernel endosperm modification is presented in Figure 4.2. The desirable genotypes for kernel endosperm modification were those with low scores. Therefore selection was done for genotypes that exhibited low scores. The classification of the environments for kernel endosperm modification in Figure 4.2 showed KR08 with the highest score and, hence, the worst environment, followed by RA08, CH08, RA07, AR08, and both AR07 and CH07 were the best environments with the least score. The genotypes with the best level of kernel endosperm modification were entries 68 (CZL01006/CZL03016), 11 (CZL03016/CZL01006), 20 (CML159/CZL03016), 10 (CZL03016/CML492), 3 (SC721), 36 (CML176/CZL03016), 44 (CML181f/CZL03016), 2 (SC633), 52 (CML264Q/CZL03016), and 12 (CML144/CZL03016).

The least desirable levels (high values) of kernel endosperm modification were found in genotypes 25 (CML159/CML264Q), 55 (CML264Q/CML175), 54 (CML264Q/CML159), 49 (CML181f/CML264Q), 8 (CZL03016/CML181f), 1 (SC527Q), 39 (CML176/CML175) and 33 (CML175/CML264Q). There was positive interaction between genotypes with the worst scores and environments KR08, RA08 and CH08. Genotypes 68 (CZL01016/CZL03016), 11 (CZL03016/CZL01006), 20 (CML159/CZL03016), 3 (SC721), 36 (CML176/CZL03016), 44 (CML181f/CZL03016), 2 (SC633), and 12 (CML144/CZL03016) interacted positively with environments CH07 and AR07. The genotypes found along or close to the IPCA1 =0 level, were relatively stable in performance. The best in terms of stability included genotypes 60 (CML492/CZL03016), 13 (CML144/CML159), 65 (CML492/CML181f) 16 (CML144/CML181f), 43 (CML176/CZL01006), and 34 (CML175/CML492).

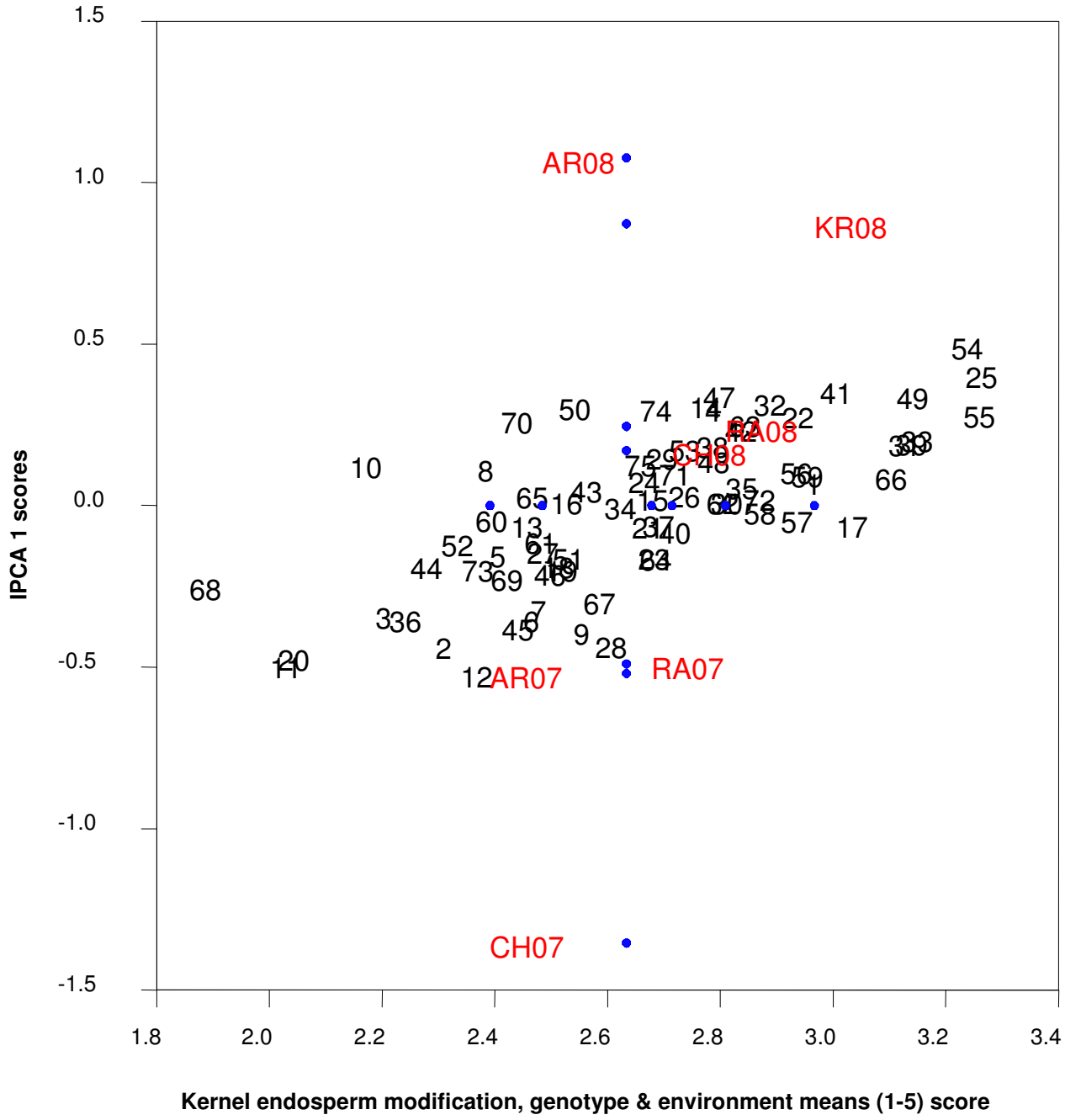

Figure 4.2 AMMI biplot of IPCA 1 scores against kernel endosperm modification for seven environments. Environment scores represented by a four character symbol where AR = ART farm, CH = CIMMYT Harare, RA = Rattray Arnold Research Station, KR = Kadoma Research Centre, 07 represents 2007 cropping season and 08 represents 2008 cropping season

4.3.5.3 Grain Yield: IPCA 2 against IPCA 1 Biplot

The plot of IPCA 2 against IPCA 1 for grain yield split the environments into four groups which can be interpreted as megaenvironments (Figure 4.3). ART farm 2007/8 (AR08) and CIMMYT Harare 2007/8 (CH08) were classified together in quadrant 1, whilst Rattray Arnold Research Station 2007/8 (RA08) and Kadoma Research Centre 2007/8 (KR08) were together in quadrant 2. In quadrant 3 there were two environments Rattray Arnold Research Station 2006/7 (RA07) and ART farm 2006/7 (AR07). Quadrant 4 had one environment CIMMYT Harare 2006/7 (CH07). All the seven environments interacted with the genotypes for grain yield level as indicated by the distances of their respective markers from the origin (centre). Environment CH07 was the most discriminating (longest distance from origin) whilst RA08 was the least discriminating because of the shortest length of its distance from the origin.

There was specific adaptation of genotypes since the set of genotypes was split into all the four quadrants (megaenvironments) with some genotype markers positioned further away from the origin. In quadrant 1, genotypes 45 (CML181f/CML144), 52 (CML264Q/CZL03016), 22 (CML159/CML175), 47 (CML181f/CML175), 20 (CML159/CZL03016) and 50 (CML181f/CML492) were specifically adapted to environments CH08 and AR08. The genotype entries 57 (CML264Q/CML181f), 58 (CML264Q/CML492), 38 (CML176/CML159), 55 (CML264Q/CML175) and 64 (CML492/CML176) were specifically adapted to environments RA08 and KR08. In environments AR07 and RA07 the adapted genotypes were 21 (CML159/CML144), 67 (CML492/CZL01006), 43 (CML176/CZL01006), 7 (CZL03016/CML176), and 71 (CZL01006/CML175) whilst genotypes 11 (CZL03016/CZL01006), 2 (SC633), 3 (SC721), 70 (CZL01006/CML159), and 9 (CZL03016/CML264Q) were adapted to environment CH07. The most stable genotype with no specific adaptation but performed the same in all environments was entry 40 (CML176/CML181f) which was found at the origin and a few other unlabelled entries that were located close to the origin of the biplot. Some entries were not labeled to avoid cluttering the graph. Genotypes located close together in the AMMI2 biplot will tend to have similar yield levels in all the environments whilst genotypes that are far apart will differ in their response pattern over the environments or even differ in mean yield (Kempton, 1984).

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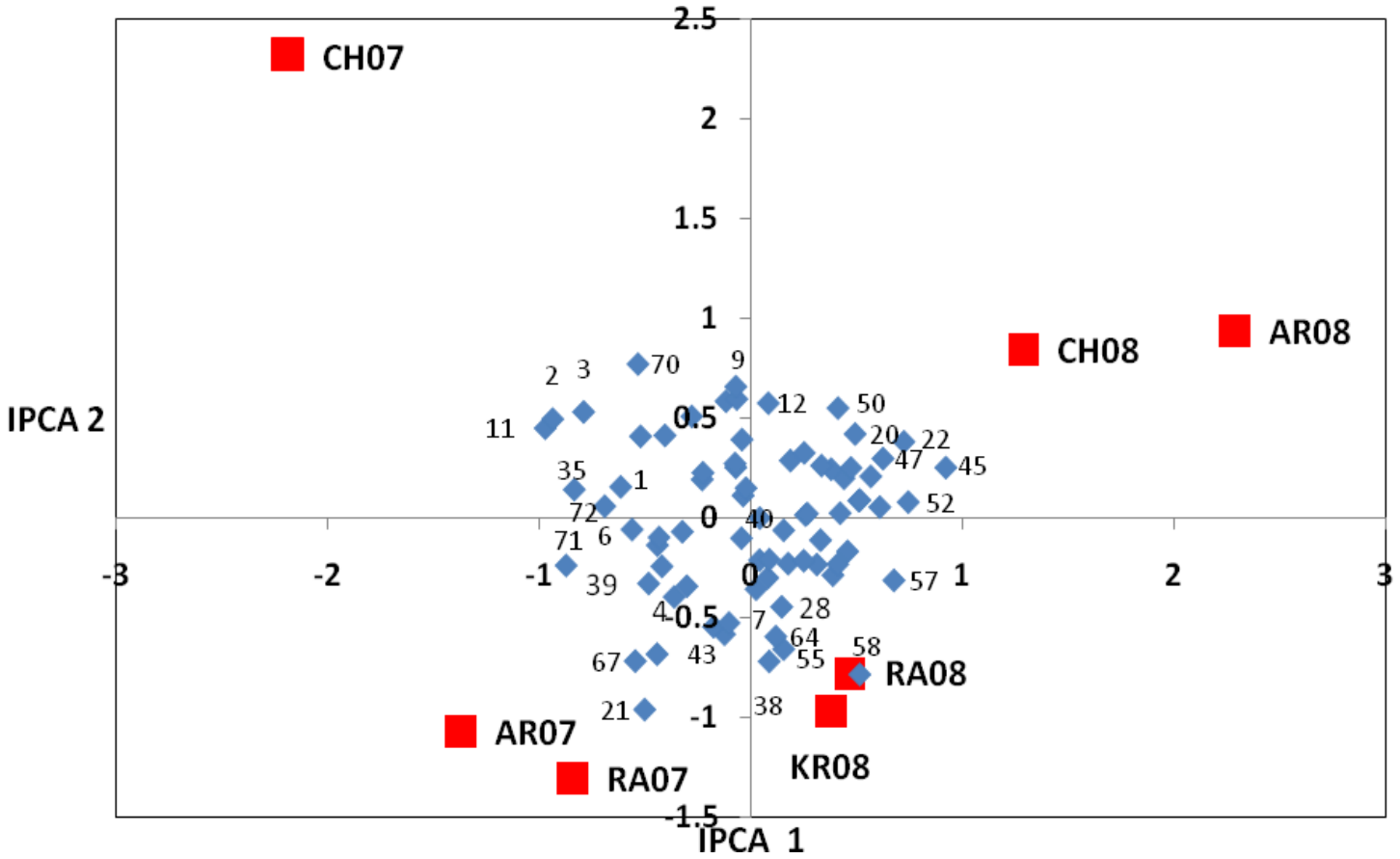

Figure 4.3 AMMI Biplot of IPCA 2 against IPCA 1 for grain yield The red squares indicate environments and the blue rhombuses represent genotypes and their corresponding entry numbers for those that could be labeled without cluttering the graph. Environment scores represented by a four character symbol where AR = ART farm, CH = CIMMYT Harare, RA = Rattray Arnold Research Station, KR = Kadoma Research Centre, 07 represents 2007 cropping season and 08 represents 2008 cropping season

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The pattern of distribution of genotypes among the four quadrants suggests a high degree of crossover type interactions. Spearman rank correlation coefficient was found to be -0.61 ($p < 0.0001$) which means high yielding genotypes were generally ranked the best (low) but the relationship is of medium strength and, hence, there was a considerable degree of interactions. This is evident in the ranks of the genotypes in Appendix 4.3 at the end of Chapter 4. Both Figure 4.1 and Figure 4.2 identified genotype 40 (CML176/CML181f) as stable for grain yield across all the environments since it was not specifically adapted to any environment. The ranking for genotype 40 (CML176/CML181f) was also stable in Appendix 4.3 at the end of Chapter 4.

4.4 Discussion

4.4.1 Comparison of experimental hybrids to check varieties for grain yield

The checks were significantly better than the experimental hybrids ($p<0.05$) but the checks were not significantly different among themselves. This probably indicated differences in germplasm adaptation since the QPM check which was not significantly different from the other checks was well adapted to the test environments having been bred in the subtropical environments. The experimental hybrids were mostly exotic germplam since six out of the nine inbred lines were of lowland tropical adaptation whereas the checks were constituted from wholly locally adapted inbred lines. The relative maturities of the experimental entries, based on anthesis days (data not shown in this study) were representative of the relative maturities of the check varieties. Since yield potential in maize is known to be positively correlated to relative maturity, one of the possible reasons for significant yield differences between experimental hybrids and the check entries could be relatively poor adaptation of the QPM hybrids to the test environments. This was likely since two thirds of the inbred lines used in the study were adapted to tropical lowlands and yet the growing environments in this study were subtropical (mid-altitude). However LSD comparison of individual means of the top yielding check entry (SC721) and the top three yielding experimental hybrids (Appendix 2 at the end of Chapter 4) revealed no significant differences.

4.4.2 Comparison of QPM hybrids to normal endosperm maize hybrids for grain yield

The mean of the normal endosperm maize hybrids was significantly higher than the mean for QPM hybrids ($p<0.05$) (Table 4.3). This is in contrast to the findings of Pixley and Bjarnason (1993) but similar to the findings of Bhatnagar et al. (2004) who despite not getting significant grain yield differences ($p >0.05$) suggested that QPM hybrids in their study were poorly adapted to the test environments. Individual genotype comparisons (Appendix 4.2) revealed that the best

yielding normal endosperm maize hybrid (though higher by 1.0 t ha^{-1}) was not significantly different from the top four yielding QPM hybrids.

The mean yield of QPM hybrids was inferior to that of normal endosperm maize hybrids partly because the normal endosperm maize hybrids group was made up of the best performing entries only whereas the QPM group was made up of both good performing entries (CML181f/CML176 and CZL03016/CML144), and poor performing entries such as entry 16 (CML144/CML181f) and the reciprocal entry 45 (CML181f/CML144). Furthermore it should be borne in mind that the QPM to normal endosperm maize hybrids comparison for grain yield potential was slightly unfair since the maturity (data not shown) of the normal endosperm maize entries was from medium to late whereas for the QPM hybrids the maturity ranged from early, through medium to late.

4.4.3 Comparison of experimental hybrids to checks for kernel endosperm modification

The contrast of the mean of the three check varieties to the experimental hybrids was not significant for kernel endosperm modification. Thus the experimental hybrids were as good as the check entries for kernel endosperm modification. The most desirable kernel endosperm modification score was from experimental hybrid entry 68 (CZL01006/CZL03016) (data not shown), and revealed in Figure 4.2. However the checks were significantly different among themselves whilst the experimental hybrids also were significantly different in their own group. Further exploration of the means of the check entries indicated that the mean for SC527Q was significantly different from the means of both SC721 ($p<0.001$) and SC633 ($p <0.05$). This indicated that the kernel endosperm modification in SC527Q, although good, was however, inferior to the kernel endosperm modification in the other check varieties. The results indicated that it was possible to get good and acceptable levels of kernel endosperm modification. In SC527Q, the modification was acceptable but not the same level as that in the best normal check entries in this study. The difference in kernel endosperm modification among the experimental hybrids indicated the presence of variability which can be exploited in the development of locally adapted hybrids.

4.4.4 Comparison of QPM hybrids to normal endosperm maize hybrids for kernel endosperm modification

The contrast of QPM germplasm to normal endosperm maize germplasm was significant ($p<0.05$) for kernel endosperm modification. The normal endosperm maize germplasm had a better mean score of 2.24 versus 2.65 for the QPM germplasm which included SC527Q. The

results indicated that in this set of genotypes the mean for kernel endosperm modification of QPM genotypes was inferior to that of normal endosperm maize genotypes. The normal endosperm maize entries source of variation was not significant ($p > 0.05$) for kernel endosperm modification but the QPM entries source of variation was significant ($p < 0.01$). Thus for SC527Q it was possible to improve the grain yield to the same level as that of normal endosperm maize germplasm, but for the kernel endosperm modification SC527Q was inferior to normal maize genotypes. However the mean score for kernel endosperm modification rating for QPM was still within the acceptable range of below 3 and hence the performance of the QPM germplasm was within acceptable range. Furthermore the most desirable genotype score for kernel endosperm modification (1.86) was from a QPM genotype, entry 68 (CZL01006/CZL03016) (data not shown) but was not significantly superior to the best normal endosperm maize score (2.19). Therefore, although not significantly better, there were QPM genotypes with absolute kernel endosperm modification scores better than those of normal endosperm varieties.

4.4.5 AMMI model fitting

In this study, a compromise was made between the AMMIF model (with four significant IPCAs) and AMMI0 model in selecting the best model for the data. This is because the AMMI model always contains both noise and pattern and as the number of IPCAs selected gets closer to the AMMIF model the level of noise increases. The objective should be to extract as much pattern as possible without tremendously increasing the level of noise. Therefore to leave out the undesirable noise a model lower than the AMMIF had to be selected. Based on Gauch (1992) guidelines AMMI2 was selected for grain yield and AMMI1 was suitable for kernel endosperm modification. This recommendation is in line with previous reports and findings where Gauch Jr and Zobel (1996) cited 31 reports in which AMM1 was used and only two for AMMI2. Also earlier on, Gauch (1992) showed that both AMMI1 and AMMI2 were a better compromise between the misfit of AMMI0 and the best variance (overfitting) of AMMIF. In the model fitting for grain yield, the expected level of noise was between model AMMI2 and AMMI3 but closer to AMMI2 (Tables 4.8 and 4.9) and, therefore, AMMI 2 was chosen, whereas for kernel endosperm modification the expected level of noise was between AMMI1 and AMMI2 but closer to AMMI1 (Tables 4.10 and 4.11) and, therefore, AMMI1 was chosen. In the study of Sivapalan et al. (2000) AMMI 4 was fitted as the best model but this was after reducing from the AMMIF which had eleven significant IPCAs and also Ebdon and Gauch (2002b) recommended AMMI2 and AMMI7 which were both reduced models.

Whilst in all the cases the best is to fit a reduced model and not the full AMMI model, according to Crossa et al. (1990), the degree of complexity of the best predictive AMMI model depends on the range of environmental conditions, the diversity of germplasm, and the type of crop. Sivapalan et al. (2000) suggested that the recommendation of AMMI4 in their study was an indication of highly contrasting environments and diverse genotypes. The environments in the present study were highly contrasting, with all the environments from the 2006/7 being drier than the environments from the 2007/8. In fact, according to climate records in Zimbabwe, the period of December 2007 to January 2008 was the wettest in 120 years and created a highly conducive environment for early crop growth whilst the 2006/7 cropping season was classified as a drought season (Zimbabwe Meteorology Office, personal communication). The set of inbred lines used in constituting the F_1 experimental hybrids was made up of six lowland tropical inbred lines and three subtropical (or midaltitude) lines and hence, diverse.

4.4.6 Distribution of treatment variation in the AMMI anova

The distribution of the treatment variation for grain yield in the AMMI study was; environment (46.7%), genotypes (19.30%) and genotypes x environment interaction (34.02%). The distribution of the treatment variation for kernel endosperm modification was; environment (17.09%), genotypes (35.51%), and genotypes x environment interaction (47.39%). The distribution of the treatments sums of squares among the components of the treatment source of variation for both grain yield and kernel endosperm modification deviated from the findings of Romagosa and Fox (1993), and the expectation of Gauch Jr and Zobel (1996). The expectation is that the proportion of the treatment components should be distributed on average in the ratio 70 : 20 : 10 for environment (E), genotypes x environment interaction (GE), and genotypes, respectively.

However, when the genotypes or environments are more diverse, GE can be expected to be as high as 60% (Gauch Jr and Zobel, 1996). The environments in this study were diverse in terms of rainfall distribution since the December 2007 to January 2008 period was recorded as the wettest season in 120 years in Zimbabwe whilst the 2006/7 cropping season was classified as a drought season (Zimbabwe Meteorology Office, personal communication). The recorded rainfall totals ranging from 433mm to 978mm (see methods and materials section) are evidence of the diversity of the environments.

The GE variation in the present study was 34.02% for grain yield and 47.39% for kernel endosperm modification which on average was one and half times more (grain yield) and at

least twice (kernel endosperm modification) the expected average level of 20%. The observation made for the G variation was about twice for grain yield (19.30%) and three and half times for kernel endosperm modification (35.51%) than the expected values of around 10%. The high G proportions in this study implied that the genotypes were different whilst on the other hand the high GE meant that for most of the genotypes it was difficult to widely recommend them in more than one of the four mega-environments identified in Figure 4.2. The high level of diversity in the genotypes for both grain yield and kernel endosperm modification was also revealed by the fact that the sum of squares for genotypes were greater than IPCA 1 sum of squares and yet in other studies cited by Gauch (1992) and Gauch Jr and Zobel (1996) IPCA 1 sum of squares were normally larger than the genotypes sum of squares.

The existence or occurrence of yield trials with proportions of components of treatment sum of squares that deviate from the general finding of 70:20:10 for environment: genotype x environment interaction: genotypes was not ruled out by Gauch Jr and Zobel (1996). Therefore, the finding in this study is one of those where GE is significant and the partitioning of components deviates from the mostly observed pattern of results possibly because of the highly contrasting environments (rainfall data in methods and materials section and the demarcation of environments in Figures 4.1 to 4.3) and the diversity of germplasm shown in Table 4.1 (lowland tropical versus subtropical or midaltitude germplasm).

4.4.7 Biplots patterns

4.4.7.1 Grain yield

The pattern observed for the environments in Figure 4.1 can be attributed to rainfall amounts since the 2006/7 environments (with low average rainfall) were grouped on the negative side of the IPCA 1 axis whilst all the 2008 environments were on the positive side of the IPCA 1 axis. The 2007/8 annual total rainfall figures were higher than the 2006/7 annual total rainfall figures. This resulted in highly contrasting environments. Although the environments were created by the interaction of four locations with two cropping seasons the high level of GE interaction revealed that it can be difficult to both identify and recommend widely adapted genotypes in these environments by testing for a few seasons as demonstrated by the interaction patterns for grain yield in Figure 4.3 where in one season CH08, AR08 are classified together, whilst RA08 and KR08 are in a separate group, and in another season AR07 and RA07 are in the same group whilst CH07 is a separate group. Although it can be difficult to recommend several varieties for grain yield across all the environments in this study, the advantage was that the

environments differed greatly thereby subjecting the hybrids to a wide range of agronomic performance influencing factors despite that ART farm (AR07 and AR08), and CIMMYT Harare (CH07 and CH08) are traditionally recognised as the same environment or agroecology.

4.4.7.2 Kernel endosperm modification

The IPCA 1 separated the environments into two main groups with the negative side of IPCA 1 containing 2006/7 environments and the positive side containing 2007/8 environments. This indicated high seasonal effects evident in differences in rainfall totals presented in the methods and materials section. The meteorological office in Zimbabwe classified the December 2007 to January 2008 period as the wettest in 120 years whilst the 2006/7 was a severe drought (Zimbabwe Meteorology Office, personal communication). Furthermore the biplot of IPCA 1 against both genotype and environmental mean for kernel endosperm modification (Figure 4.2) split the environments into each of the four segments of the graph. The KR08, RA08 and CH08 environments were in the same quadrant with the KR08 further away from both the origin and the other two environments. Thus although they could be classified as the same mega-environment there were differences within the group and, hence, the KR08 environment needed to be treated separately. Environment KR08 was the worst environment and responsible for large interactions and, hence, was good at discriminating the genotypes for kernel endosperm modification. The RA07 environment was in quadrant 2 with both AR07 and CH07 in quadrant 3. However, although the means for AR07 and CH07 were the same (Figure 4.2), the two environments differed in their interaction effects with CH07 being responsible for high interactions whilst AR07 interactions were of smaller magnitude. The AR08 environment could be treated as a separate environment and it had interactions of high magnitude.

4.4.8 Adaptation of genotypes

The interactions of IPCA 1 axis with IPCA 2 axis in Figure 4.3 made it difficult to visualise the best yielding genotype but in Figure 4.1 when IPCA 1 axis was used against both genotype and environmental means, the best yielding genotypes were identifiable. The distances of the positions of the best yielding genotypes in Figure 4.1 from the IPCA 1 =0 line indicated high levels of GE interactions which were confirmed in the IPCA 2 versus IPCA 1 biplot. Thus genotypes 3 (SC721), 2 (SC633) and 1 (SC527Q) were identified as the best yielding genotypes but not in all the environments since they all lacked stability (Figure 4.1 and 4.3).

The genotypes with high ratings for kernel endosperm modification are undesirable and selection was for genotypes with low scores. The best performing genotypes (with low scores)

entry 68 (CZL01006/CZL03016), 11 (CZL03016/CZL01006), 20 (CML159/CZL03016), 10 (CZL03016/CML492), 3 (SC721), 36 (CML176/CZL03016), 44 (CML181f/CZL03016), 2 (SC633), and 52 (CML264Q/CZL03016) were close to the IPCA 1 = 0 line and most of them do not seem to be specifically adapted to any environment but genotype 2 (SC633) was closer to AR07. Genotype entries 38 (CML176/CML159), 48 (CML181f/CML176) and 53 (CML264Q/CML144) were adapted to environment CH08 whereas genotypes 63 (CML492/CML159), 42 (CML176/CML492), 32 (CML175/CML181f) and 22 (CML159/CML175) were specifically adapted to environment RA08.

4.5 Conclusions

The experimental hybrids were inferior against the check cultivars for grain yield but not for kernel endosperm modification. The comparison of QPM varieties to normal endosperm varieties revealed that normal endosperm maize varieties were generally superior for both grain yield level and kernel endosperm modification. Although the mean for normal endosperm maize hybrids was significantly ($p < 0.05$) better than QPM hybrids for grain yield, the yield performance of the top yielding normal endosperm maize variety which was at least 1.0t ha^{-1} higher was not significantly different from the top four yielding QPM hybrids. Group (checks versus experimental hybrids and QMP versus normal endosperm maize hybrids) mean comparisons indicated significant differences pointing to the superiority of both checks and normal endosperm maize over experimental hybrids and QPM hybrids for grain yield, but individual genotype comparisons revealed that the checks were comparable to the best yielding experimental hybrids and the top three QPM hybrids were comparable to the normal endosperm maize hybrid.

The levels of noise in both traits (45.71% for grain yield and 61.82% for kernel endosperm modification) was relatively higher than for most previously reported experiments suggesting highly contrasting environments and diverse genotypes. The best models were AMMI2 for grain yield and AMMI1 for kernel endosperm modification. The seven environments were diverse in their influence on the diverse genotypes for both grain yield and kernel endosperm modification despite that they were created from four locations and two cropping seasons using the location x year factor. Genotypes with specific adaptation were identified for both traits and were recommended in the environments of specific adaptation. Genotype entry 40 (CML176/CML181f) was identified as the most stable and widely adapted genotype for grain yield in both AMMI1 and AMMI2 biplots and, therefore, can be recommended to be grown in all environments if the aim is yield certainty and not yield maximisation.

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Appendices

Appendix 4.1 Grain yield and kernel endosperm modification means for genotypes

Entry	Pedigree	Grain yield t ha^{-1}	Kernel endosperm modification score (1-5)
1	SC527Q	7.29	2.96
2	SC633	7.56	2.29
3	SC721	8.49	2.19
4	CZL03016/CML144	7.33	2.78
5	CZL03016/CML159	4.53	2.39
6	CZL03016/CML175	6.60	2.44
7	CZL03016/CML176	5.64	2.47
8	CZL03016/CML181f	6.72	2.38
9	CZL03016/CML264Q	6.86	2.54
10	CZL03016/CML492	5.98	2.14
11	CZL03016/CZL01006	6.34	2.01
12	CML144/CZL03016	7.21	2.33
13	CML144/CML159	6.24	2.44
14	CML144/CML175	6.14	2.76
15	CML144/CML176	6.76	2.66
16	CML144/CML181f	3.60	2.50
17	CML144/CML264Q	6.84	3.00
18	CML144/CML492	6.13	2.49
19	CML144/CZL01006	6.89	2.49
20	CML159/CZL03016	5.02	2.02
21	CML159/CML144	5.04	2.65
22	CML159/CML175	5.34	2.92
23	CML159/CML176	5.71	2.66
24	CML159/CML181f	5.22	2.63
25	CML159/CML264Q	6.06	3.24
26	CML159/CML492	5.89	2.70
27	CML159/CZL01006	6.24	2.46
28	CML175/CZL03016	5.64	2.59
29	CML175/CML144	6.42	2.68
30	CML175/CML159	5.83	2.79
31	CML175/CML176	5.50	3.12
32	CML175/CML181f	6.25	2.86
33	CML175/CML264Q	6.38	3.12
34	CML175/CML492	4.91	2.60
35	CML175/CZL01006	5.25	2.83
36	CML176/CZL03016	6.45	2.22
37	CML176/CML144	6.89	2.66
38	CML176/CML159	6.10	2.75
39	CML176/CML175	6.15	3.11
40	CML176/CML181f	6.51	2.70

Cont…

Appendix 4.1 cont…

Entry	Pedigree	Grain yield t/ha	Kernel endosperm modification score (1-5)
41	**CML176/CML264Q**	5.33	2.99
42	**CML176/CML492**	5.31	2.81
43	**CML176/CZL01006**	6.15	2.54
44	**CML181f/CZL03016**	6.96	2.26
45	**CML181f/CML144**	4.44	2.41
46	**CML181f/CML159**	5.79	2.46
47	**CML181f/CML175**	6.17	2.75
48	**CML181f/CML176**	7.41	2.77
49	**CML181f/CML264Q**	6.63	3.13
50	**CML181f/CML492**	6.83	2.52
51	**CML181f/CZL01006**	6.83	2.51
52	**CML264Q/CZL03016**	6.47	2.31
53	**CML264Q/CML144**	7.31	2.71
54	**CML264Q/CML159**	6.47	3.21
55	**CML264Q/CML175**	5.73	3.24
56	**CML264Q/CML176**	5.96	2.91
57	**CML264Q/CML181f**	6.55	2.91
58	**CML264Q/CML492**	6.18	2.86
59	**CML264Q/CZL01006**	6.02	2.94
60	**CML492/CZL03016**	6.09	2.37
61	**CML492/CML144**	6.12	2.44
62	**CML492/CML159**	5.90	2.78
63	**CML492/CML175**	4.66	2.82
64	**CML492/CML176**	5.11	2.66
65	**CML492/CML181f**	6.35	2.45
66	**CML492/CML264Q**	5.35	3.09
67	**CML492/CZL01006**	5.70	2.55
68	**CZL01006/CZL03016**	6.33	1.86
69	**CZL01006/CML144**	6.28	2.39
70	**CZL01006/CML159**	6.85	2.43
71	**CZL01006/CML175**	5.42	2.71
72	**CZL01006/CML176**	5.75	2.85
73	**CZL01006/CML181f**	7.02	2.34
74	**CZL01006/CML264Q**	5.99	2.65
75	**CZL01006/CML492**	5.40	2.64

Appendix 4.2 Grain yield (t ha^{-1}) results for genotypes ranked from highest to lowest.

ENTRY	Pedigree	Mean	Letter for LSD mean comparison																
3	SC721	8.49	A																
2	SC633	7.56	A	B															
48	CML181f/CML176	7.41	A	B	C														
4	CZL03016/CML144	7.33	A	B	C	D													
53	CML264Q/CML144	7.31	A	B	C	D	E												
1	SC527Q	7.29	A	B	C	D	E	F											
12	CML144/CZL03016	7.21	G	B	C	D	E	F											
73	CZL01006/CML181f	7.02	G	B	C	D	E	F											H
44	CML181f/CZL03016	6.96	G	B	C	D	E	F	I										H
37	CML176/CML144	6.89	G	B	C	D	E	F	I	J									H
19	CML144/CZL01006	6.89	G	B	C	D	E	F	I	J									H
9	CZL03016/CML264Q	6.86	G	B	C	D	E	F	I	J									H
70	CZL01006/CML159	6.85	G	B	C	D	E	F	I	J	K								H
17	CML144/CML264Q	6.84	G	B	C	D	E	F	I	J	K								H
50	CML181f/CML492	6.83	G	B	C	D	E	F	I	J	K								H
51	CML181f/CZL01006	6.83	G	B	C	D	E	F	I	J	K								H
15	CML144/CML176	6.76	G	B	C	D	E	F	I	J	K								H
8	CZL03016/CML181f	6.72	G	B	C	D	E	F	I	J	K								H
49	CML181f/CML264Q	6.63	G	B	C	D	E	F	I	J	K	L							H
6	CZL03016/CML175	6.60	G	B	C	D	E	F	I	J	K	L	M						H
57	CML264Q/CML181f	6.55	G	B	C	D	E	F	I	J	K	L	M	N					H
40	CML176/CML181f	6.51	G	B	C	D	E	F	I	J	K	L	M	N	O				H
52	CML264Q/CZL03016	6.47	G	B	C	D	E	F	I	J	K	L	M	N	O				H
54	CML264Q/CML159	6.47	G	B	C	D	E	F	I	J	K	L	M	N	O				H
36	CML176/CZL03016	6.45	G	B	C	D	E	F	I	J	K	L	M	N	O	P			H
29	CML175/CML144	6.42	G	B	C	D	E	F	I	J	K	L	M	N	O	P	Q		H
33	CML175/CML264Q	6.38	G	B	C	D	E	F	I	J	K	L	M	N	O	P	Q		H
65	CML492/CML181f	6.35	G	B	C	D	E	F	I	J	K	L	M	N	O	P	Q		H
11	CZL03016/CZL01006	6.34	G		C	D	E	F	I	J	K	L	M	N	O	P	Q		H
68	CZL01006/CZL03016	6.33	G		C	D	E	F	I	J	K	L	M	N	O	P	Q		H
69	CZL01006/CML144	6.28	G	R	C	D	E	F	I	J	K	L	M	N	O	P	Q		H
32	CML175/CML181f	6.25	G	R	C	D	E	F	I	J	K	L	M	N	O	P	Q	S	H
13	CML144/CML159	6.24	G	R	C	D	E	F	I	J	K	L	M	N	O	P	Q	S	H
27	CML159/CZL01006	6.24	G	R	C	D	E	F	I	J	K	L	M	N	O	P	Q	S	H
58	CML264Q/CML492	6.18	G	R	T	D	E	F	I	J	K	L	M	N	O	P	Q	S	H
47	CML181f/CML175	6.17	G	R	T	D	E	F	I	J	K	L	M	N	O	P	Q	S	H
43	CML176/CZL01006	6.15	G	R	T	D	E	F	I	J	K	L	M	N	O	P	Q	S	H
39	CML176/CML175	6.15	G	R	T	D	E	F	I	J	K	L	M	N	O	P	Q	S	H
14	CML144/CML175	6.14	G	R	T	D	E	F	I	J	K	L	M	N	O	P	Q	S	H
18	CML144/CML492	6.13	G	R	T	D	E	F	I	J	K	L	M	N	O	P	Q	S	H

cont…

Appendix 4.2 cont…

ENTRY	Pedigree	Mean	Letter for LSD mean comparison																
61	CML492/CML144	6.12	G	R	T	D	E	F	I	J	K	L	M	N	O	P	Q	S	H
38	CML176/CML159	6.10	G	R	T	U	E	F	I	J	K	L	M	N	O	P	Q	S	H
60	CML492/CZL03016	6.09	G	R	T	U		F	I	J	K	L	M	N	O	P	Q	S	H
25	CML159/CML264Q	6.06	G	R	T	U			I	J	K	L	M	N	O	P	Q	S	H
59	CML264Q/CZL01006	6.02	G	R	T	U			I	J	K	L	M	N	O	P	Q	S	H
74	CZL01006/CML264Q	5.99		R	T	U			I	J	K	L	M	N	O	P	Q	S	H
10	CZL03016/CML492	5.98		R	T	U			I	J	K	L	M	N	O	P	Q	S	H
56	CML264Q/CML176	5.96		R	T	U			I	J	K	L	M	N	O	P	Q	S	H
62	CML492/CML159	5.90		R	T	U			I	J	K	L	M	N	O	P	Q	S	H
26	CML159/CML492	5.89		R	T	U			I	J	K	L	M	N	O	P	Q	S	H
30	CML175/CML159	5.83	V	R	T	U			I	J	K	L	M	N	O	P	Q	S	H
46	CML181f/CML159	5.79	V	R	T	U			I	J	K	L	M	N	O	P	Q	S	
72	CZL01006/CML176	5.75	V	R	T	U	W			J	K	L	M	N	O	P	Q	S	
55	CML264Q/CML175	5.73	V	R	T	U	W			J	K	L	M	N	O	P	Q	S	
23	CML159/CML176	5.71	V	R	T	U	W			J	K	L	M	N	O	P	Q	S	
67	CML492/CZL01006	5.70	V	R	T	U	W			J	K	L	M	N	O	P	Q	S	
7	CZL03016/CML176	5.64	V	R	T	U	W	X			K	L	M	N	O	P	Q	S	
28	CML175/CZL03016	5.64	V	R	T	U	W	X			K	L	M	N	O	P	Q	S	
31	CML175/CML176	5.50	V	R	T	U	W	X				L	M	N	O	P	Q	S	
71	CZL01006/CML175	5.42	V	R	T	U	W	X				L	M	N	O	P	Q	S	
75	CZL01006/CML492	5.40	V	R	T	U	W	X					M	N	O	P	Q	S	
66	CML492/CML264Q	5.35	V	R	T	U	W	X						N	O	P	Q	S	
22	CML159/CML175	5.34	V	R	T	U	W	X						N	O	P	Q	S	
41	CML176/CML264Q	5.33	V	R	T	U	W	X							O	P	Q	S	
42	CML176/CML492	5.31	V	R	T	U	W	X							O	P	Q	S	
35	CML175/CZL01006	5.25	V	R	T	U	W	X								P	Q	S	
24	CML159/CML181f	5.22	V	R	T	U	W	X									Q	S	
64	CML492/CML176	5.11	V	R	T	U	W	X										S	
21	CML159/CML144	5.04	V		T	U	W	X										S	
20	CML159/CZL03016	5.02	V		T	U	W	X											
34	CML175/CML492	4.91	V			U	W	X											
63	CML492/CML175	4.66	V	Y			W	X											
5	CZL03016/CML159	4.53		Y			W	X											
45	CML181f/CML144	4.44		Y				X											
16	CML144/CML181f	3.60		Y															

LSD = 1.213t ha-1, t = 1.96532, EMS = 2.6675, Alpha = 0.05, DF error =444

Appendix 4.3 Ranked AMMI adjusted grain yield means (t ha^{-1}) for the seven environments

Entry	Pedigree	AR07	Rank	RA07	Rank	CH07	Rank	AR08	Rank	RA08	Rank	KR08	Rank	CH08	Rank
1	SC527Q	9.9	4	6.5	4	9.3	5	7.5	44	6.3	9	5.1	9	6.4	26
2	SC633	10.2	2	6.6	3	11.0	2	7.4	47	6.2	13	4.9	12	6.6	22
3	SC721	10.9	1	7.4	1	11.7	1	8.7	16	7.2	1	5.8	2	7.7	2
4	CZL03016/CML144	10.2	3	7.1	2	7.5	22	7.6	41	6.9	4	5.7	3	6.3	32
5	CZL03016/CML159	5.5	73	2.7	73	4.1	71	7.5	45	4.1	74	2.7	73	5.2	54
6	CZL03016/CML175	9.4	6	6.1	7	8.0	14	6.7	56	5.8	26	4.6	24	5.6	48
7	CZL03016/CML176	8.4	29	5.4	19	4.8	59	6.3	61	5.5	44	4.3	37	4.8	64
8	CZL03016/CML181f	8.5	22	5.2	34	8.8	8	8.0	32	5.6	36	4.3	39	6.6	20
9	CZL03016/CML264Q	8.2	40	5.0	46	8.8	6	8.8	15	5.8	29	4.4	34	7.1	11
10	CZL03016/CML492	7.5	60	4.7	53	4.9	58	8.3	22	5.8	30	4.5	30	6.2	33
11	CZL03016/CZL01006	9.1	8	5.5	16	9.8	4	6.0	65	5.0	63	3.7	63	5.3	52
12	CML144/CZL03016	8.4	25	5.3	25	8.6	11	9.4	6	6.3	11	4.9	13	7.6	4
13	CML144/CML159	7.6	54	4.6	59	6.8	29	8.4	21	5.6	40	4.2	44	6.5	23
14	CML144/CML175	8.2	34	5.3	27	5.8	43	7.5	43	5.8	28	4.5	27	5.8	44
15	CML144/CML176	8.5	21	5.4	22	7.8	15	8.3	23	6.0	21	4.6	21	6.7	17
16	CML144/CML181f	4.7	75	1.9	75	3.6	73	6.2	64	3.0	75	1.7	75	4.1	72
17	CML144/CML264Q	8.3	31	5.1	41	8.7	9	8.6	18	5.8	27	4.4	33	7.0	13
18	CML144/CML492	8.0	44	4.9	47	6.7	30	7.6	40	5.5	45	4.2	46	6.0	39
19	CML144/CZL01006	8.9	11	5.7	13	8.2	13	8.1	31	6.1	16	4.8	17	6.6	21
20	CML159/CZL03016	5.8	72	3.0	72	5.2	54	8.0	34	4.4	71	3.0	72	5.8	45
21	CML159/CML144	8.7	16	5.6	15	4.2	68	4.5	75	5.0	61	3.9	53	3.4	75
22	CML159/CML175	5.9	71	3.1	71	4.9	57	8.8	14	4.8	67	3.4	69	6.4	29

Cont…

Appendix 4.3 cont…

Entry	Pedigree	AR07	Rank	RA07	Rank	CH07	Rank	AR08	Rank	RA08	Rank	KR08	Rank	CH08	Rank
23	**CML159/CML176**	8.0	**45**	5.1	**42**	5.1	**55**	6.9	**55**	5.4	**50**	4.2	**42**	5.2	**55**
24	**CML159/CML181f**	7.0	**68**	4.2	**68**	4.3	**66**	7.2	**49**	5.0	**62**	3.7	**62**	5.2	**56**
25	**CML159/CML264Q**	7.2	**64**	4.3	**64**	6.2	**37**	8.5	**19**	5.5	**47**	4.1	**51**	6.5	**24**
26	**CML159/CML492**	7.6	**55**	4.8	**50**	4.7	**61**	8.0	**35**	5.7	**32**	4.5	**29**	6.0	**42**
27	**CML159/CZL01006**	8.3	**33**	5.3	**24**	5.8	**44**	7.7	**37**	5.9	**24**	4.6	**20**	6.0	**41**
28	**CML175/CZL03016**	7.8	**50**	5.0	**43**	4.6	**63**	7.0	**52**	5.5	**43**	4.3	**40**	5.3	**53**
29	**CML175/CML144**	8.5	**24**	5.3	**29**	7.6	**18**	7.6	**42**	5.6	**38**	4.3	**38**	6.1	**36**
30	**CML175/CML159**	8.3	**32**	5.1	**40**	6.7	**33**	6.5	**58**	5.2	**55**	3.9	**54**	5.2	**58**
31	**CML175/CML176**	8.2	**38**	5.1	**38**	5.6	**48**	6.0	**67**	5.1	**58**	3.9	**59**	4.6	**66**
32	**CML175/CML181f**	7.4	**62**	4.6	**60**	5.6	**49**	9.0	**11**	5.9	**25**	4.5	**28**	6.8	**15**
33	**CML175/CML264Q**	8.5	**23**	5.5	**17**	6.5	**34**	7.7	**39**	5.9	**23**	4.6	**22**	6.0	**37**
34	**CML175/CML492**	7.5	**56**	4.6	**61**	4.2	**67**	5.7	**70**	4.7	**68**	3.5	**67**	4.1	**70**
35	**CML175/CZL01006**	8.2	**41**	4.7	**55**	7.7	**16**	5.0	**73**	4.2	**72**	3.0	**71**	4.1	**71**
36	**CML176/CZL03016**	8.2	**36**	5.3	**26**	6.2	**36**	8.2	**25**	6.0	**18**	4.7	**18**	6.4	**28**
37	**CML176/CML144**	7.9	**46**	5.2	**31**	6.0	**41**	9.8	**2**	6.6	**5**	5.2	**8**	7.5	**5**
38	**CML176/CML159**	8.7	**17**	5.9	**12**	4.5	**65**	7.1	**51**	6.1	**14**	5.0	**10**	5.4	**50**
39	**CML176/CML175**	9.1	**9**	5.9	**10**	6.7	**31**	6.2	**62**	5.6	**37**	4.5	**31**	5.1	**60**
40	**CML176/CML181f**	8.4	**28**	5.4	**20**	6.7	**32**	8.1	**29**	6.0	**19**	4.7	**19**	6.4	**31**
41	**CML176/CML264Q**	7.1	**65**	4.3	**65**	4.6	**64**	7.2	**50**	5.1	**60**	3.8	**61**	5.3	**51**
42	**CML176/CML492**	6.9	**70**	4.0	**70**	5.1	**56**	7.4	**46**	4.9	**64**	3.6	**66**	5.5	**49**
43	**CML176/CZL01006**	9.4	**5**	6.3	**5**	5.8	**45**	6.0	**66**	5.9	**22**	4.8	**15**	4.8	**63**

Cont…

Appendix 4.3 cont..

Entry	Pedigree	AR07	Rank	RA07	Rank	CH07	Rank	AR08	Rank	RA08	Rank	KR08	Rank	CH08	Rank
44	**CML181f/CZL03016**	8.2	**39**	5.2	**30**	7.4	**23**	9.3	**8**	6.3	**10**	4.9	**11**	7.4	**7**
45	**CML181f/CML144**	4.8	**74**	2.2	**74**	3.3	**75**	8.3	**24**	4.1	**73**	2.7	**74**	5.6	**47**
46	**CML181f/CML159**	8.4	**26**	5.2	**35**	6.8	**28**	6.2	**63**	5.1	**56**	3.9	**58**	4.9	**62**
47	**CML181f/CML175**	6.9	**69**	4.2	**69**	5.8	**46**	9.3	**7**	5.7	**35**	4.3	**41**	7.0	**12**
48	**CML181f/CML176**	8.7	**14**	5.9	**9**	6.8	**26**	9.9	**1**	7.0	**3**	5.7	**4**	7.8	**1**
49	**CML181f/CML264Q**	7.7	**51**	4.9	**48**	6.4	**35**	9.3	**9**	6.1	**15**	4.8	**16**	7.2	**10**
50	**CML181f/CML492**	7.6	**52**	4.7	**56**	7.5	**20**	9.8	**3**	6.1	**17**	4.6	**23**	7.6	**3**
51	**CML181f/CZL01006**	8.8	**13**	5.9	**11**	6.2	**38**	8.5	**20**	6.5	**7**	5.3	**6**	6.7	**18**
52	**CML264Q/CZL03016**	7.3	**63**	4.6	**57**	5.3	**52**	9.7	**4**	6.2	**12**	4.8	**14**	7.3	**8**
53	**CML264Q/CML144**	8.9	**10**	6.2	**6**	6.1	**39**	9.5	**5**	7.1	**2**	5.9	**1**	7.4	**6**
54	**CML264Q/CML159**	8.2	**37**	5.1	**39**	7.5	**21**	8.0	**33**	5.7	**34**	4.4	**35**	6.4	**30**
55	**CML264Q/CML175**	8.1	**43**	5.4	**23**	4.1	**69**	6.9	**54**	5.8	**31**	4.6	**25**	5.2	**57**
56	**CML264Q/CML176**	7.5	**58**	4.6	**58**	5.7	**47**	8.1	**30**	5.5	**41**	4.2	**43**	6.1	**35**
57	**CML264Q/CML181f**	7.9	**49**	5.3	**28**	4.6	**62**	9.3	**10**	6.6	**6**	5.3	**7**	7.0	**14**
58	**CML264Q/CML492**	8.2	**35**	5.7	**14**	3.5	**74**	8.1	**28**	6.5	**8**	5.3	**5**	6.0	**40**
59	**CML264Q/CZL01006**	7.6	**53**	4.8	**51**	5.3	**51**	8.1	**26**	5.7	**33**	4.4	**32**	6.1	**34**
60	**CML492/CZL03016**	7.1	**66**	4.3	**66**	5.9	**42**	8.9	**13**	5.6	**39**	4.2	**45**	6.7	**16**
61	**CML492/CML144**	7.9	**47**	4.8	**49**	6.8	**27**	7.7	**38**	5.4	**51**	4.1	**48**	6.0	**38**
62	**CML492/CML159**	7.0	**67**	4.2	**67**	5.3	**53**	8.6	**17**	5.5	**42**	4.2	**47**	6.4	**25**
63	**CML492/CML175**	7.4	**61**	4.4	**63**	4.1	**70**	5.2	**72**	4.5	**70**	3.3	**70**	3.8	**74**
64	**CML492/CML176**	7.5	**57**	4.7	**54**	3.8	**72**	6.3	**60**	5.1	**57**	3.9	**56**	4.6	**67**

Cont…

Appendix 4.3 cont…

Entry	Pedigree	AR07	Rank	RA07	Rank	CH07	Rank	AR08	Rank	RA08	Rank	KR08	Rank	CH08	Rank
65	**CML492/CML181f**	7.9	**48**	4.8	**52**	7.6	**19**	8.1	**27**	5.5	**46**	4.1	**50**	6.4	**27**
66	**CML492/CML264Q**	7.5	**59**	4.6	**62**	4.8	**60**	6.7	**57**	5.1	**59**	3.8	**60**	5.0	**61**
67	**CML492/CZL01006**	9.1	**7**	6.0	**8**	5.5	**50**	5.3	**71**	5.5	**49**	4.4	**36**	4.2	**69**
68	**CZL01006/CZL03016**	8.5	**20**	5.1	**37**	8.7	**10**	7.0	**53**	5.2	**53**	3.9	**55**	5.8	**46**
69	**CZL01006/CML144**	8.3	**30**	5.0	**44**	8.4	**12**	7.2	**48**	5.2	**54**	3.9	**57**	5.9	**43**
70	**CZL01006/CML159**	8.7	**15**	5.2	**32**	10.1	**3**	7.8	**36**	5.5	**48**	4.1	**52**	6.6	**19**
71	**CZL01006/CML175**	8.8	**12**	5.4	**21**	7.1	**24**	4.7	**74**	4.7	**69**	3.5	**68**	3.9	**73**
72	**CZL01006/CML176**	8.6	**19**	5.2	**36**	7.7	**17**	5.7	**68**	4.8	**66**	3.6	**65**	4.7	**65**
73	**CZL01006/CML181f**	8.4	**27**	5.2	**33**	8.8	**7**	8.9	**12**	6.0	**20**	4.6	**26**	7.2	**9**
74	**CZL01006/CML264Q**	8.7	**18**	5.4	**18**	6.9	**25**	6.3	**59**	5.3	**52**	4.1	**49**	5.1	**59**
75	**CZL01006/CML492**	8.2	**42**	5.0	**45**	6.0	**40**	5.7	**69**	4.8	**65**	3.6	**64**	4.5	**68**

5 Determination of normal endosperm maize pollen contamination levels that can occur in QPM without loss of nutritional superiority

Abstract

The feasibility of the coexistence of quality protein maize (QPM) and normal endosperm maize varieties without the total loss of QPM nutritional superiority through normal endosperm maize pollen contamination was evaluated. Using physical mixtures of pure QPM grain and contaminated QPM grain a total of twelve treatments were set up to simulate some of the different levels of normal endosperm maize pollen contamination. Included were control treatments of both QPM and normal endosperm maize. Eight replications per treatment were produced per environment. The Quality Index (QI) value of 0.80 was considered as the limit below which contaminated QPM grain ceases to be nutritionally superior to normal endosperm maize grain. Chemical analysis and determination of QI values for the different samples was conducted at CIMMYT's Soil and Plant Analysis Laboratory (SPAL). The reduced maximum likelihood (REML) analysis for QI values revealed significant differences among the treatments at each individual site ($p < 0.0001$). Homogeneity test of site QI variances was significantly different ($p < 0.05$) and, therefore, the sites could not be combined during analysis. The linear regression of the QI treatment means at each site against the levels of contamination produced parallel and highly significant linear plots ($p<0.0001$) with different y—intercepts ($p<0.01$). The y-intercepts were equivalent to the QI of QPM without contamination. Thus the two different sites produced different initial nutritional values for the same variety. The nutritional superiority of contaminated QPM grain was maintained up to 15.3% and 31.9% level of contamination for moisture stressed environment and optimum environment, respectively. The values indicated the levels of normal endosperm maize pollen contamination that could be tolerated before the QPM variety lost its nutritional superiority at each site. The limit to the threshold for contamination that could be tolerated before the nutritional value of QPM was equivalent to normal endosperm maize was dependent on environmental conditions during crop growth. Moisture stressed environments lowered both the QI value of the pure QPM treatment and the level of normal endosperm maize pollen contamination that could be tolerated before total loss in nutritional superiority.

5.1 Introduction

Maize is one of the staple cereal crops in sub-Saharan Africa. However, maize is nutritionally deficient in two essential amino acids, tryptophan and lysine (Bressani, 1992; Mertz et al., 1964; Vasal, 2001). Consequently the sub-Saharan Africa human population (mostly weaning infants and the poor) whose diets are solely dependent on maize is predisposed to malnutrition that gets manifested as general stunting, wasting, and underweight. In the worst cases the malnutrition problem degenerates into kwashiorkor[8]. FAO (2006) statistics identify sub-Saharan Africa as one of the regions critically affected by malnutrition despite the development and existence of quality protein maize (QPM) technology.

The mutant *opaque-2* gene that confers high levels of both tryptophan and lysine in quality protein maize (QPM) is recessive to the wild type allele that exists in normal endosperm maize (Mertz et al., 1964). Thus the nutritional superiority of normal endosperm maize pollen contaminated QPM grain is adversely affected due to the dominance of the wild type allele found in normal maize. Lauderdale (2000) reported that the recessive nature of the gene that confers high lysine and tryptophan in QPM, opaque-2 gene, is a drawback to QPM variety dissemination and adoption efforts. The pertinence of the issue was further highlighted by Vasal (2002) who encouraged more basic studies and development of strategies to minimise contamination of QPM crops by normal endosperm maize pollen under coexistence in farmers' fields.

In the past, the thrust aimed for absolute isolation of QPM crops from normal endosperm maize crops to prevent pollen contamination. Several strategic options have potential to either prevent or mitigate the effects of normal endosperm maize pollen contamination in QPM crops but currently each option has its own limitations: apomixis, feasibility is still a dream (Perotti et al., 2004); α-zein gene family silencing through antisense inhibition (Huang et al., 2006) is not widely acceptable due to genetic modification (Krivanek et al., 2007); standard seed maize production isolation guidelines (Beck, 2004; Havazvidi, 1990) are not easy to manage in the smallholder farming sector mainly due to both shortage of arable land and the prevalent desire to plant the bulk of maize crops with the

[8] **Kwashiorkor** is protein calorie malnutrition which can lead to infant morbidity and mortality. It disables the immune system such that the child is susceptible to a host of infectious diseases. The term is from Ivory Coast where it means the deposed child (weaned off) Source: MedicineNet.com. 1998. Webster's New World Medical Dictionary [Online]. Available by MedicineNet.com http://www.medterms.com/script/main/art.asp?articlekey=4124 (posted 12 October 2008).

first rains in the smallholder farming sector; and blanketing an area or community with QPM varieties is also not acceptable as it takes away the farmer's right to grow varieties of their choice (Lauderdale, 2000). In many cases, farmers have a tendency to grow more than one variety, and they will not readily agree to stop growing their accustomed varieties. Therefore, one of the practical solutions is to develop strategies that allow for coexistence of QPM crops and normal maize crops in the smallholder farming sector.

The coexistence strategy offers a lot of promise because according to Cordova (2000), anecdotal evidence indicated that foreign pollen contamination of QPM crops by normal endosperm maize crops is far less than previously thought. Basing on Twumasi-Afriye et al. (1996a and 1996b) study in Ghana, Vivek et al. (2008) concluded that coexistence of a QPM crop and a normal endosperm maize crop next to each other does not completely render the entire harvest non-QPM grain, but rather induces some levels of contamination ranging from 0% to11%. At these levels of contamination, QPM grain samples were found to be nutritionally superior to normal endosperm maize grain. The nutritional superiority of contaminated QPM grain was found to exist up to 20% inclusion of normal endosperm maize grain in a physical mixture (Ahenkora et al., 1999). The nutritional benefits of QPM are limited to the farm level when farmers grow maize for their own consumption and not when the QPM is sold to the centralised marketing organisations.

Although the stability of QPM cultivars for both agronomic and quality performance was reported by Pixley and Bjarnason (2002), according to Vivek et al. (2008) many skeptics still doubt whether the expression of QPM quality attributes (which include lysine and tryptophan content, and the Quality Index[9)] is consistent across environments and varieties. Lack of stability in the expression of quality traits could mean different environments have different thresholds to normal endosperm maize pollen contamination before nutritional superiority is lost. Weiland (1992) reported xenia to be influenced by the environment. Xenia is the immediate effects of foreign parent pollen on nonmaternal tissue of the kernel (Kiesselbach, 1960). The process of normal endosperm maize pollen contamination of QPM varieties is similar to xenia. Therefore, it is relevant that empirical investigations on the level of contamination at which the nutritional worth

[9] **Quality Index:** The tryptophan content to protein content ratio expressed as a percentage. Source: Vivek, B.S., A.F. Krivanek, N. Palacios-Rojas, S. Twumasi-Afriye, and A.O. Diallo. 2008. Breeding quality protein maize (QPM): Protocols for developing QPM cultivars. CIMMYT, Mexico, D.F.

of contaminated QPM grain ceases to be superior to normal endosperm maize grain be conducted with a set of different varieties grown in a different environment.

The objective of the study was to determine the level of normal endosperm maize pollen contamination that can occur in quality protein maize grain without loss of nutritional superiority. The hypothesis tested was that quality protein maize varieties can tolerate some contamination by normal endosperm maize pollen without losing nutritional superiority.

5.2 Methods and materials

5.2.1 Germplasm

The varieties used in the experiment were C3728, a white endosperm experimental QPM hybrid variety from Seed Co Zimbabwe Ltd, and PHB30B50, a yellow normal endosperm maize hybrid from Pioneer Hi-Bred International. Both varieties are three way hybrids with similar flowering dates and adapted to the test environments.

5.2.2 Experimental design and management

5.2.2.1 Field contamination experiments

The varieties were grown at CIMMYT Harare station (1409 masl, 17º43' S, 31º01' E), and Agricultural Research Trust (ART) farm (1480 masl; 17º43' S, 31º05' E) during the 2006/7 cropping season. Rainfall total at ART farm was 529mm and 582mm for CIMMYT Harare. An additional 67mm of irrigation water was applied at planting to ensure good germination at ART farm. The ART farm site was planted on the 5th of November 2006 and the CIMMYT Harare site on the 30th of December 2006. Standard cultural practices for growing maize were followed at both sites. The soils at both ART farm and CIMMYT Harare are reddish clay (fersiallitic; Salisbury 5E series). The application rates for fertilisers were 175 kg N-65 kg P-20 kg K ha-1 at ART farm, and 166 kg N-56 kg P-28 kg K ha-1 at CIMMYT Harare. The targeted plant population densities were 53 000 plants per hectare achieved by a spacing of 0.75m between the rows and an effective spacing of 0.25m in the row at both sites.

The two different varieties (C3728 and PHB30B50) were grown on the same block at each of the two locations as a pollen contamination study with rows of plants of the yellow PHB30B50 surrounding the white C3728 crop. At physiological maturity ears with

pure yellow PHB30B50 F_2 grain and contaminated C3728 F_2 grain were harvested for preparation of grain samples for laboratory analyses.

5.2.2.2 Laboratory contamination experiments

Physical mixtures of pure QPM grain and contaminated QPM grain were constituted in various proportions ranging from 50% to 100% QPM to represent some of the possible different levels of pollen contamination (see Table 5.1).

Table 5.1 Constitution of treatments of normal endosperm, QPM, and contaminated QPM

Treatment	Normal %	QPM%	Contaminated QPM%
1	100	0	0
2	0	100	0
3	0	95	5
4	0	90	10
5	0	85	15
6	0	80	20
7	0	75	25
8	0	70	30
9	0	65	35
10	0	60	40
11	0	55	45
12	0	50	50

The lowest level of normal endosperm maize pollen contamination used was 5% with incremental levels of 5% up to 50% level of normal endosperm maize pollen contamination. Treatments 1 and 2 were controls representing normal endosperm maize and pure QPM grain, respectively. The CIMMYT Soil and Plant Analysis Laboratory (SPAL) protocols required that each sample be made up of 20 to 50 kernels (Vivek et al., 2008). In each of the 12 treatments, a single kernel was assumed to be equivalent to 5% and therefore a total of twenty kernels were needed to make up each treatment. Two contaminated ears, each with enough kernels to constitute two replications of all the eleven QPM treatments (at least 230 white kernels and at least 110 yellow kernels) were selected from each of the two blocks grown per location in the summer season of 2006/7. Thus four ears were selected from ears harvested from ART farm and another four ears were also selected from ears harvested from the crop grown at CIMMYT Harare in the 2006/7 cropping season. Thus a total of eight ears each with enough kernels (both yellow and white) to constitute all the eleven QPM kernel containing treatments were used. The constitution of two complete replications with grain from a

single ear was done so as to reduce as much as possible the level of resultant residual errors.

5.2.2.3 Laboratory quality tests

Grain samples constituting the twelve treatments in Table 5.1, with two replications per each of the eight ears were subjected to quality analysis at the CIMMYT's Soil and Plant Analysis Laboratory (SPAL) in Mexico. Whole grain samples were analysed for both nitrogen and tryptophan using CIMMYT's SPAL protocols (Vivek et al., 2008). According to the protocols protein content is estimated using sample nitrogen content. Sample nitrogen content in the CIMMYT – SPAL protocols is determined using the Technicon Autoanalyser II method (Vivek et al., 2008) whilst for tryptophan determination the Opienska-Blauth et al. (1963) method modified by Hernandez and Bates (1969) was used.

The traits of interest were nitrogen and tryptophan content, the derived protein content and the corresponding Quality Index (QI) for each sample. The QI was calculated as tryptophan content to protein content ratio in a sample expressed as a percentage (Vivek et al., 2008). According to Vivek et al. (2008), the selection criteria for whole grain samples is that tryptophan should be higher than 0.075% whilst the QI should be higher than 0.80. These selection criteria were used to determine the threshold contamination at which grain from normal endosperm maize pollen contaminated QPM crops ceases to be superior to normal endosperm maize grain i.e. the level of contamination that gave a QI value of 0.80. The level of 0.80 was determined in collaboration with animal nutritionists (Vivek, personal communication).

5.2.3 Statistical analyses

An analysis of variance of the physical mixtures experiment was conducted in SAS 9.1.3 using the "Proc Mixed model (REML statement)" (SAS, 2004). In the model environments and replications within environments and ears within environments were considered random effects, while treatments (contamination levels) were considered fixed effects. Before a combined analysis could be done, tests for homogeneity of variances following the Levene's test, and Welch's test were conducted using the GLM procedure in SAS. A linear regression of the relationship between mean QI values and the pollen contamination level for the eleven QPM containing treatments was conducted.

The corresponding pollen contamination level for a QI threshold value of 0.80 was interpolated using the regression equation for the fitted line for each site. The slopes and the intercepts of the fitted regression lines were compared and tested for differences using a dummy variable regression conducted with Proc GLM in SAS to test for homogeneity of regression lines (SAS Workshop, n.y.).

5.3 Results

5.3.1 Tests for homogeneity of variances

Results for SAS 9.1.3 for homogeneity of residual variances for both ART farm and CIMMYT Harare are given in Tables 5.2, 5.3 and 5.4.

Table 5.2 ANOVA for homogeneity of ART farm and CIMMYT Harare sites variances

Source	DF	Sum of Squares	Mean Square	F-Value	Probability
Site	1	0.158279	0.1582788	15.92	<.0001
Error	189	1.878916	0.00994135		

Table 5.3 Levene's test for homogeneity of ART farm and CIMMYT Harare sites variances

Source	DF	Sum of Squares	Mean Square	F-Value	Probability
Site	1	0.00151	0.00151	5.57	0.0193
Error	189	0.0511	0.00027		

Table 5.4 Welch's test for homogeneity of ART farm and CIMMYT Harare site variances

Source	DF	F-Value	Probability
Site	1	15.87	<.0001
Error	173.8		

The ANOVA test for homogeneity of variances was highly significant at p=<0.0001, whilst Levene's test was significant at p<0.05 and Welch's test was highly significant at p=<0.0001. Thus all the three tests for homogeneity of variances indicated that the variances for the two sites were significantly different and therefore the two sites could not be pooled for a combined analysis. Therefore, further analysis proceeded separately for each site.

5.3.2 Analysis of variance

The F values of 12.71, and 29.17 for the treatments (fixed effects) from the mixed REML model with 76 and 77 error degrees of freedom at ART farm and CIMMYT Harare respectively, were both significantly different from zero (P<0.0001) at both sites (Table 5.5).

Table 5.5 Estimates for Q.I. treatment means and the standard errors recorded at ART farm and CIMMYT Harare station

Contamination level by normal maize	Treatment	ART Farm Estimate	LSD ART	CIMMYT Harare Estimate	LSD CIMMYT Harare
100% (normal maize check)	1	0.57	J	0.60	I
0% (QPM check)	2	0.92	K	0.86	A
5%	3	0.87	KLM	0.83	AB
10%	4	0.86	KLM	0.84	AB
15%	5	0.89	KL	0.81	CB
20%	6	0.86	KLM	0.78	CD
25%	7	0.85	KLM	0.76	ED
30%	8	0.83	LMN	0.71	GF
35%	9	0.78	NO	0.72	EF
40%	10	0.76	NOP	0.73	EF
45%	11	0.74	OP	0.67	H
50%	12	0.74	P	0.68	GH

LSD for CIMMYT Harare was 0.0389 and for ART farm were 0.0765 when treatment 1 was involved and 0.0740 among the rest of the ART farm treatments.

At ART farm standard errors were 0.037 for treatment 1 versus the rest of the treatments and 0.036 when excluding treatment 1. At CIMMYT Harare standard error was 0.020 among all the treatments. Treatment means with the same letter in the LSD column were not significantly different at p = 0.05.

5.3.3 Comparison of ART farm treatment means

The QI of normal endosperm maize grain sample was significantly different (P <0.0001) from the QI values of all the other eleven QPM grain samples. The QI value of the uncontaminated QPM grain sample was highly significantly different (P<0.01) from QPM samples with 30% to 50% contamination with normal endosperm maize. However, the QI of the uncontaminated sample (100% QPM grain) was not significantly different (P>0.05) from the QPM samples with 5% to 25% contamination.

5.3.4 Comparison of CIMMYT Harare treatment means

At CIMMYT Harare the QI value of the QPM treatment was significantly different (p < 0.05) from QPM samples with 15% to 50% contamination but was not significantly different to QPM samples with 5% and 10% contamination. The general trend of the treatment means for QI was similar to what was observed at ART farm. However, the threshold value was obtained with 15% contamination at CIMMYT Harare, while at ART farm it was observed that the QI value (0.83) was above the threshold at 30%.

5.3.5 Linear regression analyses

The linear regression of QI against levels of normal endosperm maize pollen contamination in experimental variety C3728 are presented in Table 5.6. The F-values for the linear regression of QI against percent contamination were highly significant (P<0.001) at both sites (Table 5.6). The linear regression explained more than 90% of the variation at both sites (Figure 5.1).

Table 5.6 Linear regression of C3728 QI against PHB30B50 pollen % contamination at ART farm and CIMMYT Harare during 2006-7 cropping season

Source	DF	ART Farm		CIMMYT Harare	
		SS	MS	SS	MS
Regression	1	0.035163	0.035163 ***	0.039661	0.039661 ***
Residual	9	0.003861	0.000429	0.002361	0.000262
Total	10	0.039024		0.042022	

Key ** = significance at 0.001 probability

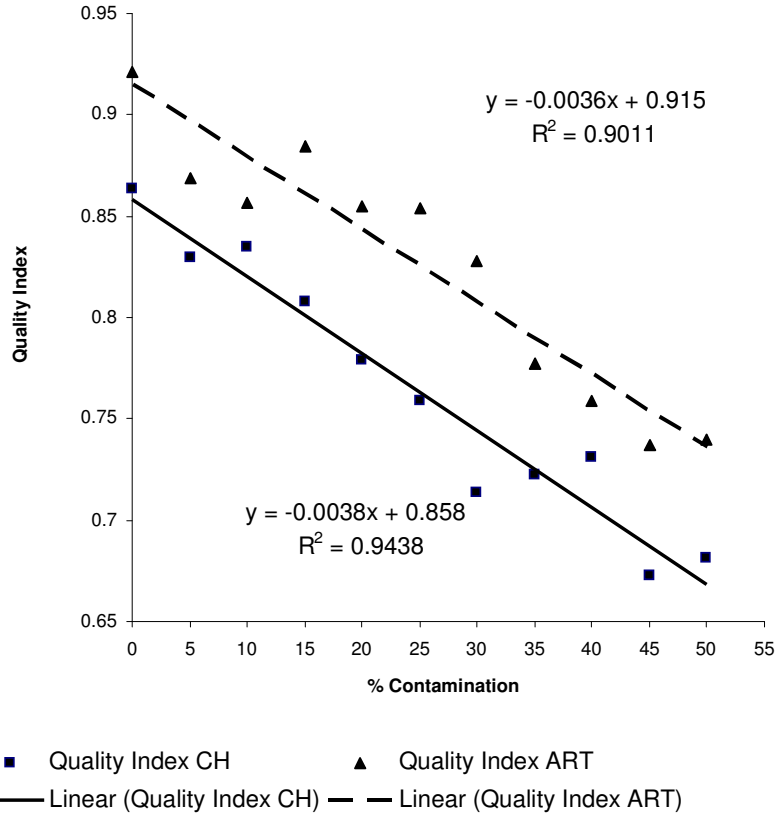

Figure 5.1 Fitted and observed plots of Quality Index against different levels of contamination in a C3728 QPM crop contaminated by PHB30B50 pollen at both ART farm and CIMMYT Harare (CH) during the 2006-7 cropping season

5.3.6 Comparison of regression intercepts

Results of the dummy variable regression conducted in SAS using PROC GLM (SAS Workshop, n.y.) showed that the intercepts (0.915 and 0.858) were significantly different ($p<0.01$) (Table 5.7).

Table 5.7 Dummy variable regression for test of homogeneity of regression lines

Source	DF	Sum of Squares	Mean Square	F-Value	Probability
Locn	2	4.945618	2.472809	7154.1	<.0001
Cont*Locn	2	0.074823	0.037412	108.24	<.0001
Intercepts	1	0.005078	0.005078	14.69	0.0012
Slopes	1	0.0000677	0.0000677	0.2	0.6635
Lines	2	0.021477	0.010739	31.07	<.0001
Error	18	0.00622169	0.00034565		

5.3.7 Comparison of regression slopes

The two fitted regression lines were parallel and this was confirmed by an F-test that revealed that the slopes were not significantly different ($p>0.05$) (Table 5.7). The two regression lines were also found to be highly significantly different ($p<0.0001$) (Table 5.7).

5.3.8 Interpolation of normal maize pollen contamination thresholds

According to Vivek et al. (2008), the whole kernel threshold value for QI below which breeding lines are discarded is 0.80. Therefore, interpolation for the pollen contamination threshold for the ART farm site was as follows:

$y = -0.0036x + 0.915$ and y was given as 0.80 and there was need to solve for x

$x = 31.9444\ \% \pm 7.222\%$ at 95% confidence interval.

The CIMMYT Harare site calculation is presented below:

$y = -0.0038x + 0.8581$ and y was given as 0.80 and there was need to solve for x

$x = 15.2895\% \pm 5.4473\%$ at 95% confidence intervals.

The upper limit for the normal endosperm maize pollen contamination at a QI of 0.8 was 20.7% for the CIMMYT Harare site whist the lower limit for the ART farm site for the same QI value was 24.7%. Thus the 95% confidence bands for the two regression lines did not overlap indicating that the threshold values to levels of pollen contamination for the two sites were distinct from each other.

5.4 Discussion

The highly significant F-values for the treatments source of variation at both ART farm and CIMMYT Harare indicated that there were differences in QI among the different treatments. However, not all the treatment means were different from each other. As expected all the QI values for all the QPM grain samples were significantly different from the normal endosperm maize grain samples at both sites. However according to Vivek et al. (2008) all the QPM containing treatments with QI values below 0.80 would not qualify as QPM samples and would not be selected for advancement in a breeding programme. Although samples with QI below 0.80 were statistically different from the QI of normal endosperm maize grain samples they are considered to be as good as normal endosperm maize in nutritional value. The data indicated that QPM grain samples with 5% to 30% contamination by normal endosperm maize pollen at ART farm would be considered to be of superior nutritional value to the normal endosperm maize grain samples. However, at CIMMYT Harare, contamination levels above 15% resulted in below threshold QI values. The major differences between the two environments were fertiliser rates, planting dates, soil moisture management. The fertiliser rates were higher at ART farm by 9kg N-11kg P ha^{-1} but 8kg K ha^{-1} lower, whilst planting dates were earlier at ART farm by seven weeks, and the rainfall totals were higher at CIMMYT Harare but most of it did not benefit the crop due to late planting. The ART farm crop was not moisture stressed during both flowering and grain filling phases whereas the CIMMYT Harare crop was moisture stressed. Hence, the threshold pollen contamination levels for the two environments without loss in QPM nutritional superiority over normal endosperm maize were around 15% and 30% for CIMMYT Harare and ART farm, respectively.

5.4.1 Linear regression analyses

The highly significant F-test values of the linear regressions indicated that the linear regression explained most of the variation at both sites. The rate of loss of nutritional value in the QPM hybrid grain at both sites was identical as indicated by the parallelism ($p>0.6635$) in the fitted regression lines (Table 5.7). This observation suggested that the rate of loss in nutritional value of a QPM variety due to foreign pollen contamination was constant and, therefore, could be modeled. If the rate of loss in nutritional value due to normal endosperm maize pollen contamination is a constant for a QPM variety or all QPM varieties, then the threshold to foreign pollen contamination for a crop can be determined from an equation if the y intercept and the constants are both known. The y intercept can be determined as the initial QI value for a pure QPM grain sample grown in

the environment under consideration. The constant can be estimated precisely from several evaluations of different QPM varieties under different environments. In this study only one variety was used.

The scenario in Figure 5.1 was similar to the Ellis-Roberts model for deterioration of quality traits (percent germination, viability, vigour, survival) in orthodox seed where it is assumed that the rate of deterioration of quality traits is a constant for different seed lots under similar environments (Ellis and Roberts, 1980; Ellis and Roberts, 1981). However, the Ellis-Roberts model was challenged by the findings of Tang et al. (1999) who reported different rates of deterioration of quality traits for different seed lots of maize. Nevertheless, it would be prudent to investigate further the nature of protein quality deterioration due to contamination in QPM grain of different varieties grown under different environmental conditions to establish a generalised model.

The differences in the intercepts of the fitted lines indicated that the nutritional potential of a QPM crop was highly influenced by the quality of the agronomic environment. Thus the initial nutritional value (measured by the QI) of C3728 was influenced by the quality of the environment. In the moisture stressed environment during grain filling (CIMMYT Harare), the experimental QPM variety, C3728 had a lower QI compared to the less stressed environment at ART farm.

The rate of loss of nutritional value as measured by the slopes of the regression lines was the same in both environments. Although some QPM treatments with QI values below the threshold were significantly different from the mean value for the normal endosperm maize treatment from the statistical point of view there were no expected differences in nutritional value between these QPM treatments and the normal endosperm maize treatment because according to Vivek (personal communication) the QI threshold based on a common understanding with nutritionists was set at 0.80.

The thresholds for the pollen contamination were different for the two environments and yet the two environments are separated by a distance of less than 10km and are in the same agroecological zone (Natural Region IIa). The management practices and planting dates were different. The two stations were under different management, for example more nitrogen and phosphorous were applied at ART (9kg N-11kg P ha^{-1} higher than

CIMMYT Harare). However there were 8kg K ha^{-1} lower application at ART farm. Differences in amounts of nitrogenous fertilisers applied coupled with differences in moisture management and different planting dates are likely to have led to differences in protein content. The varieties and batches of planted seed were exactly the same at both sites, but the ART farm crop was planted on the 5th of November 2006 whilst the CIMMYT Harare location was planted on the 30th of December 2006. This was a difference of seven weeks in crop growth. The 2006/7 cropping season was relatively dry in Southern Africa with total annual rainfall for ART farm recorded as 529mm and CIMMYT Harare 582mm whilst the long term averages for the two locations were 890mm and 820mm, respectively. Thus in addition to the differences in planting date, the CIMMYT Harare crop was severely moisture stressed during the grain filling period whilst the ART farm crop was not stressed. Therefore, fortuitously the CIMMYT Harare 2006/7 cropping environment simulated the moisture stressed environments which are characteristic of the smallholder farmers' cropping conditions in Zimbabwe and the rest of the Southern African region.

The environmental data set (two environments) is too small to make concrete conclusions and, therefore, it can be stated that since most smallholder communal farmers grow maize in harsh environments then QPM breeders should generally select for varieties whose regression slope is less steep and also with a relatively high y-axis intercept under optimum management conditions. This would minimise the potential loss in QPM nutritional superiority due to both normal endosperm maize pollen contamination and environmental stresses.

Twumasi-Afriye et al. (1996a) reported nutritional superiority of QPM up to 20% inclusion of normal endosperm maize in QPM and this was expected to be the equivalent of 20% pollen contamination, however, there was no measure of the QI of the contaminated QPM samples in that study. Using a threshold value of 0.80 for the QI, in the current study nutritional superiority of QPM was found to exist up to as high as 31.9% $\pm$ 7.2% levels of foreign pollen contamination when the environment was not severely stressed and the crop management was good, and as low as 15.3% $\pm$ 5.44% when the environment was moisture stressed during the grain filling period but with fertiliser application good. The differences between Twumasi-Afriye et al. (1996a) study and the current study is that in Twumasi-Afriye et al. (1996a) study, the physical mixtures of

contaminated QPM grain were constituted using maize kernels from two different varieties and as a result that did not exactly represent the constitution of normal endosperm maize pollen contaminated QPM grain. Normal endosperm maize pollen contaminated QPM grain would have all the kernels from the same variety but with some kernels pollinated by normal endosperm maize pollen. However, the finding of Twumasi-Afriye et al. (1996a) of a threshold of 20% contamination level was within the range of values obtained in this experiment.

5.4.2 Genotype x environment interaction effects

The results of the current study indicated that the QI of QPM grain was dependent on the prevailing environmental conditions during crop development. When the environment was harsh, the potential QI Index value decreased and as a result the foreign pollen contamination threshold for maintaining QPM nutritional superiority over normal endosperm maize subsequently decreased as well. Therefore, it is not advisable to work with one threshold contamination value for both high potential environments and low potential environments when giving recommendations to QPM technology users. There is need to widely test for the behaviour of the QI trait under different environments before full recommendations can be given for a QPM cultivar. There could be some QPM cultivars which lose all the nutritional superiority simply due to exposure to environmental stresses, and without being contaminated by normal endosperm maize pollen. Therefore, selection for QPM tolerance to abiotic stresses should evaluate the genotypes for the behaviour of the QI trait under different environments.

Contrary to the widely accepted belief that QPM production requires absolute isolation to remain nutritionally superior to normal endosperm maize, it was shown that C3728, an experimental QPM hybrid can tolerate contamination from normal endosperm maize pollen up to certain levels depending on the environmental conditions. When the growing conditions are harsh and moisture stressed, the nutritional potential of the QPM crop is likely to be lowered and absolute isolation should be encouraged. Where the conditions are difficult to implement absolute isolation, the aim should be to reduce levels of normal endosperm maize pollen contamination to the minimum possible. Thus the coexistence of QPM crops and normal endosperm maize crops without total loss of QPM nutritional superiority should be feasible depending on both the prevailing environmental conditions and the level of normal endosperm maize pollen contamination.

5.5 Conclusions

Two different threshold values to the loss in nutritional superiority of QPM due to normal endosperm maize pollen contamination were observed for the two different environments; 15.3% for moisture stressed environment (CH) and 31.9% for optimum environment (ART farm). Both the prevailing environmental conditions during crop growth and the inherent rate of loss in nutritional value due to environmental stresses were critical in determining the threshold to loss in nutritional superiority of a QPM crop due to normal endosperm maize pollen contamination. The potential QI values were 0.915 and 0.858 for ART farm and CH environments, respectively and were significantly different from each other. The rate of loss of nutritional value for the C3728 QPM grain produced from the two environments was the same. The similarity in the rates of nutritional loss due to pollen contamination for the two environments suggested that a QPM variety's rate of nutritional loss due to normal endosperm maize pollen contamination could be the same in all environments. Coexistence of QPM crops and normal endosperm maize crops without total loss of nutritional superiority is feasible depending on the prevailing environmental conditions and the level of pollen contamination. Further studies on the behaviour of the QI trait at varying levels of pollen contamination in different genotypes, under different environments representing both optimal and suboptimal growing conditions need to be conducted.

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6 Geostatistical analysis of quality protein maize (QPM) crops contamination with pollen from adjacent normal endosperm maize fields.

Abstract

The patterns and levels of pollen contamination on a white QPM variety by a yellow normal endosperm maize variety were studied using ordinary kriging (OK), a geostatistical procedure. Nine 0.21ha blocks of a QPM crop with a perimeter of yellow normal endosperm maize were grown for three cropping seasons (2005/6, 2006/7 and 2007/8) at two sites. Samples of five ears were randomly selected from each of the 160 plots in each block. However, in 2005/6 the number of plots was only 40. The total number of yellow kernels per plot multiplied by 100 and divided by the total number of both yellow and white kernels per plot was the observed level of normal endosperm maize pollen contamination. Ordinary kriging was used to estimate the levels of normal endosperm maize pollen contamination at unsampled points. Both prediction and error surfaces were produced for each of the nine blocks using the best OK model selected on the basis of having the smallest root mean square error (RMSE) after several trial and error attempts. The experiments are indicated in brackets, and the final model (with least RMSE) for each experiment were stable (QCS20061), pentaspherical (QCS200711), exponential (QCS200712, QCS200721, QCS200722, QCS200821, and QCS200822), rational quadratic (QCS200812), and J-Bessel (QCS200812). On average more than 50% of the crop in each experiment had less than 20% normal endosperm maize pollen contamination levels. Both high levels of contamination and high prediction errors occurred towards the edges of the blocks. The arithmetic average levels of contamination for experiments (QCS200711, QCS200712, QCS200721 and QCS200722) that had samples analysed for determination of the contamination threshold to nutritional superiority were below the normal endosperm maize pollen contamination thresholds of 15.3% (CIMMYT Harare) and 31.9% (ART farm). It was concluded that the levels of contamination were far less than previously thought to threaten the nutritional superiority of QPM over normal endosperm maize. Hence, QPM and normal endosperm maize crops can coexist without fear of total loss of QPM nutritional superiority. Furthermore ordinary kriging was successfully used for the first time in visualising the spatial distribution of foreign pollen contamination in fields of QPM crops.

6.1 Introduction

Maize is one of the staple crops in sub-Saharan Africa (FAO, 1995) and is nutritionally deficient in two essential amino acids, tryptophan and lysine (Bressani, 1992; Mertz, 1994; Vasal, 2001). This predisposes the sub-Saharan Africa human population whose diet is solely dependent on

maize to malnutrition that gets manifested as general stunting and in the worst-case scenario develops into *kwashiorkor*[10]. FAO (2006) statistics identify sub-Saharan Africa as one of the regions critically affected by malnutrition despite the development and existence of quality protein maize (QPM) technology. Although the nutritional benefits, of QPM cultivars (high in lysine and tryptophan levels) to monogastric animals and humans have been demonstrated (Vasal, 2002), their widespread use remains low. Krivanek et al. (2007) estimated that 200 000 hectares of QPM were being grown during the period 2003-5. This indicates a low level of QPM adoption considering that annual maize area in sub-Saharan Africa was estimated as 22 million hectares by FAOSTAT (2004).

The reluctance to grow QPM varieties is partly due to the fear that the nutritional benefits of a QPM variety can potentially disappear when they coexist with normal endosperm maize varieties. This is because the *opaque-2* gene allele in QPM is recessive to the corresponding wild type allele found in normal endosperm maize such that when pollinated by normal endosperm maize pollen, QPM is adversely affected. Currently normal endosperm maize varieties dominate the areas planted to maize crops whilst absolute isolation of QPM crops is difficult. The high pressure on land resulting in small differently owned plots adjacent to each other makes it difficult to implement isolation procedures. Since absolute physical and or temporal isolation of QPM crops from normal endosperm maize crops is difficult to implement in the smallholder farming sector of sub-Saharan Africa, co-existence of QPM and normal endosperm maize crops remains to be the way forward and it is therefore pertinent to determine the characteristic pattern and precise levels of normal endosperm maize pollen contamination in fields of QPM crops at flowering.

Literature on studies of normal endosperm maize pollen contamination of QPM crops is scarce and most of the recommendations are based on either seed production practices or results from studies on coexistence of transgenic crops and normal endosperm maize. Confronted by unavailability of specific information on normal endosperm maize contamination in QPM crops, Twumasi-Afriye et al. (1996a) conducted an experiment in Ghana to estimate the level of yellow normal endosperm maize pollen contamination in a white endosperm QPM crop. The QPM crop

[10] **Kwashiorkor:** is protein calorie malnutrition which can lead to infant morbidity and mortality. It disables the immune system such that the child is susceptible to a host of infectious diseases. The term is from Ivory Coast where it means the deposed child (weaned off) Source: MedicineNet.com. 1998. Webster's New World Medical Dictionary [Online]. Available by MedicineNet.com http://www.medterms.com/script/main/art.asp?articlekey=4124 (posted 12 October 2008).

was conventionally grown and completely surrounded by a yellow normal endosperm maize crop of the same maturity. The normal endosperm maize crop and the QPM crop were allowed to cross freely at flowering. Details of separation distances between the proximal rows of the two different crops were not given but it was observed that severe contamination occurred within the nearest 12m from the normal endosperm maize crop. The highest level of contamination in Twumasi-Afriye et al. (1996b) experiment was 11% (Vivek et al., 2008), but the question to be asked is "Is this level of contamination typical of all environments and conditions?"

Although Twumasi-Afriye et al. (1996a) study managed to estimate the average level of normal endosperm maize pollen contamination and also observe that severe contamination was within the first 12m from the normal endosperm maize crop, the study did not establish in a single realisation, the pattern and distribution of normal endosperm maize pollen contamination in the QPM fields, and yet this is important in fully understanding and predicting the characteristics, and extent of foreign pollen contamination in QPM crops.

The spatial description of a variable or phenomenon occurring in a random manner can be achieved through geostatistical methods, particularly kriging. Kriging is a technique from the Gaussian branch of statistics. The kriging technique makes use of the linear least squares estimation procedure (Tonkin and Larson, 2002) for optimal interpolation of variables with a spatial distribution. The advantage of kriging is that it is a robust method that reliably provides the best linear unbiased estimator, minimum estimation variances and optimal interpolation (Dubrule, 1983; Dubrule, 1984; Laslett et al., 1987; Oliver and Webster, 1990; Royle et al., 1981). Kriging has been used for spatial analysis in agriculture mostly for crop yield estimates (Oliver and Webster, 1990) and spatial variation of soil properties (Laslett et al., 1987; McBratney and Webster, 1986). Amongst the kriging procedures, ordinary kriging is the most used because it is the most common form of kriging and it is the conventional default (Subcommittee D18.01, 2004) and furthermore it assumes a constant but unknown mean, and only requires the existence of enough observations to be able to estimate the semi-variogram (Webster and Oliver, 2001). This makes ordinary kriging the geostatistical method of first choice.

There are no known reports on the application of kriging in the study and analysis of spatial variation of cross pollination by yellow normal endosperm maize pollen in a white endosperm

QPM maize crop. This is the first time that kriging has been adapted for decision making in the dissemination of QPM varieties. The generation of graphical information on characteristic patterns and precise levels of foreign pollen contamination in QPM crops would help extension agents, agronomists, seed scientists, and breeders, in advising farmers on applicable strategies when pursuing household nutritional security through QPM dissemination, adoption and utilisation.

The objective of this study was to estimate the average levels and patterns of foreign maize pollen contamination in QPM crops coexisting with normal endosperm maize varieties. The hypothesis was that the average levels of foreign pollen contamination in fields of QPM crops are far below what is thought and the pattern of pollen contamination in QPM crops coexisting with normal endosperm maize can be estimated through kriging.

6.2 Methods and materials

6.2.1 Trial sites and cultural procedures

Experiments were conducted during the seasons of 2005/6 at ART farm (1480 m a.s.l.; 17º43' S, 31º05' E), and for both 2006/7 and 2007/8 at both ART farm and CIMMYT Harare Research Station (1409 m a.s.l., 17º43' S, 31º01' E). Long term annual average rainfall totals for ART farm was 800mm and for CIMMYT Harare was 820mm. Rainfall totals for the ART farm cropping seasons were 1016mm, 529mm, and 837.5mm for 2005/6, 2006/7 and 2007/8, respectively. Supplementary irrigation applied to germinate the crops at ART farm was 22mm, 67mm and 66mm in the 2005/6, 2006/7 and 2007/8 cropping seasons, respectively. The CIMMYT Harare site received 582mm, and 978.3mm of rainfall in the 2006/7 and 2007/8 cropping seasons, respectively. There was no supplementary irrigation applied on the CIMMYT Harare crops as these were planted with the rains after the beginning of the rainy season in both cropping seasons.

In the 2005/6 cropping season, the ART farm site was planted on the 26th of November 2005 whilst in the 2006/7 cropping season the ART farm site was planted on the 5th of November 2006 and the CIMMYT Harare site on the 30th of December 2006. The 2007/8 crops were planted on the 16th of November 2007 and 31st of December 2007 for the ART farm site and the CIMMYT Harare site, respectively.

The soils at both ART farm and CIMMYT Harare are reddish clay (described as *fersiallitic* and derived from *epidiorite Salisbury 5E series*). The application rates for fertilisers were 175 kg N-65 kg P-20 kg K ha^{-1} at ART farm, and 166 kg N-56 kg P-28 kg K ha^{-1} at CIMMYT Harare. The targeted plant population densities were 53000 plants per hectare achieved by a spacing of 0.75m between the rows and a spacing of 0.25m in the row at both sites. The crops were grown using standard, and optimal cultural practices for maize production which included hand weeding and use of herbicides to control weeds throughout the season.

6.2.2 Estimation of contamination levels

An experimental white endosperm QPM cultivar (VH05954 or C3728) was grown on a rectangular, 0.21ha measuring 50m by 42m. The QPM cultivar was completely surrounded by a yellow normal endosperm maize cultivar (SC506 or PHB30B50 and or SC608). Thus the two crops (white endosperm QPM and yellow normal endosperm) were distinct but adjoining as indicated in Figure 6.1.The perimeter of yellow normal endosperm maize crop band was at least 10m wide around the 0.21ha of white endosperm QPM crop at CIMMYT Harare.

In all circumstances the two different crops, yellow normal endosperm maize, and white endosperm QPM were chosen so as to maximize flowering overlap, hence, they were expected to flower on the same dates. Both the shape and area planted to the white endosperm QPM crop were assumed to be representative of both average sizes and shapes of most maize crops fields found in the smallholder farming sector in SSA. The details on the crops grown are presented in Table 6.1. The ART farm QPM crops flowered in synchrony with 2.5ha, 24.58ha, and 19.19ha perimeter of a yellow normal endosperm maize crop in the 2005/6, 2006/7 and 2007/8 cropping seasons, respectively. At CIMMYT Harare, the area of the perimeter of yellow normal endosperm crops that flowered in synchrony with the white endosperm QPM crop in each of the two seasons was 0.48ha.

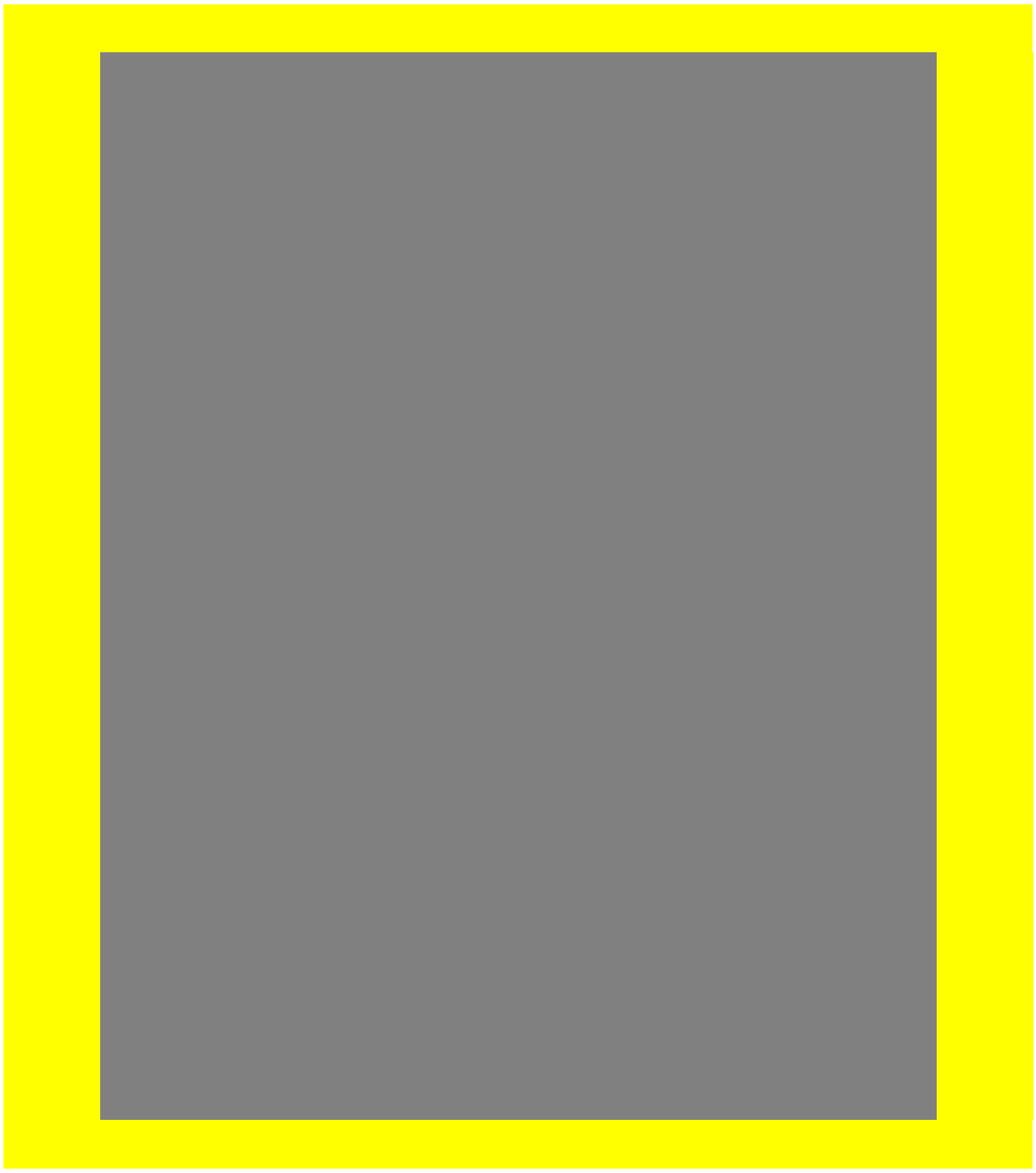

Figure 6.1 Relative positions of the white endosperm QPM crop (grey area) to the yellow endosperm normal maize crop (yellow area)

In the 2006/7 season two rectangular blocks were planted at ART farm. However, one of the blocks at ART farm was surrounded by the yellow normal endosperm maize crop on three sides only (Table 6.1). Standard maize growing practices were applied throughout the growing period.

Table 6.1 Details of seasons and crops

Season	Location	Number of blocks	Sides of block with yellow normal endosperm maize	White QPM variety and Area (Ha)	Yellow normal endosperm maize variety(ies) and Area (Ha)
2005/6	**ART farm**	1	4	VH05954 (0.21 Ha)	SC506 (2.5 Ha)
2006/7	**ART farm**	1	4	C3728 (0.21 Ha)	PHB30B50 (24.58 Ha)
2006/7	**ART farm**	1	3	C3728 (0.21 Ha)	PHB30B50 (24.58 Ha)
2006/7	**CIMMYT Harare**	2	4	C3728 (0.21 Ha)	PHB30B50 (0.48 Ha)
2007/8	**ART farm**	2	4	C3728 (0.21 Ha)	PHB30B50 (0.39 Ha) and SC608 (19.19 Ha)
2007/8	**CIMMYT Harare**	2	4	C3728 (0.21 Ha)	PHB30B50 (0.48 Ha)

Key: Variety VH05954 = CML144/CML159//Obatampa-SR

The 2005/6 experiment had a total of 40 plots relatively positioned as indicated in Figure 6.2. Thus the 5m length plots were located at alternate positions interspaced at 10 metres as shown in Figure 6.2. There were 40 plots in the block. The length of each plot was 5m and there were 5 plots in each row. Eight rows were selected for harvesting from a total of 56 rows. Adjacent harvested rows were interspaced at 6.0m except the last two rows on northern side of the block (7 and 8) that were interspaced at 5.25m from each other.

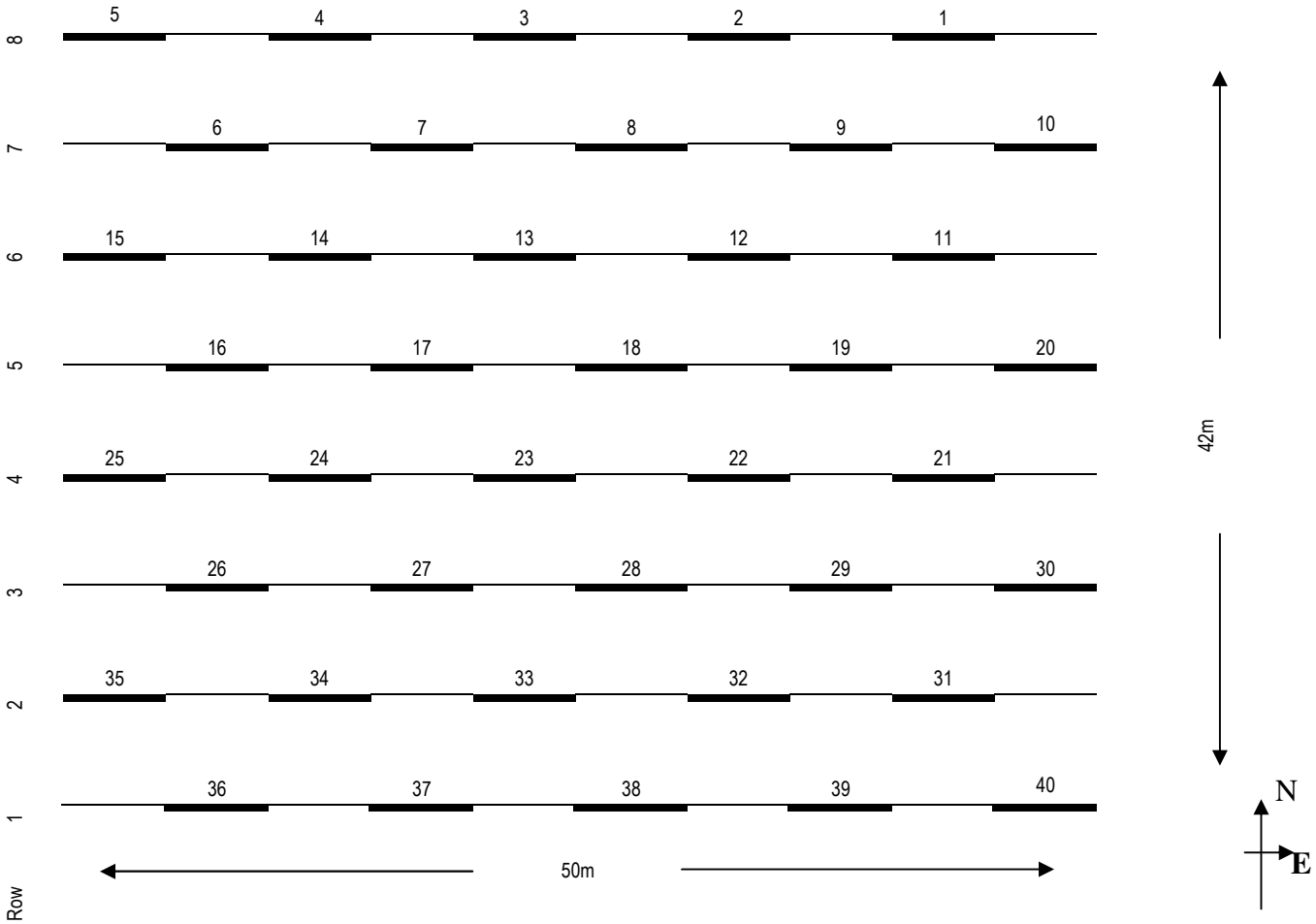

Figure 6.2 Relative positions of plots in the QPM crop grown in the 2005/6 season at ART farm Research Station in Harare

In the 2006/7 and 2007/8 crops, sixteen rows were selected for harvesting at maturity in each of the blocks and each row was divided into ten equal plots of 5m length as shown in Figure 6.3. This produced a total of 160 plots distributed among the sixteen chosen rows. There were 160 plots in each block. The length of each plot was 5m and there were 10 plots in each row. Adjacent harvested rows were interspaced at 3.0m except the two centre rows (8 and 9) that were interspaced at 75cm from each other and the last two rows on both sides of the blocks (1 and 2, and 15 and 16) that were interspaced at 2.25m from each other.

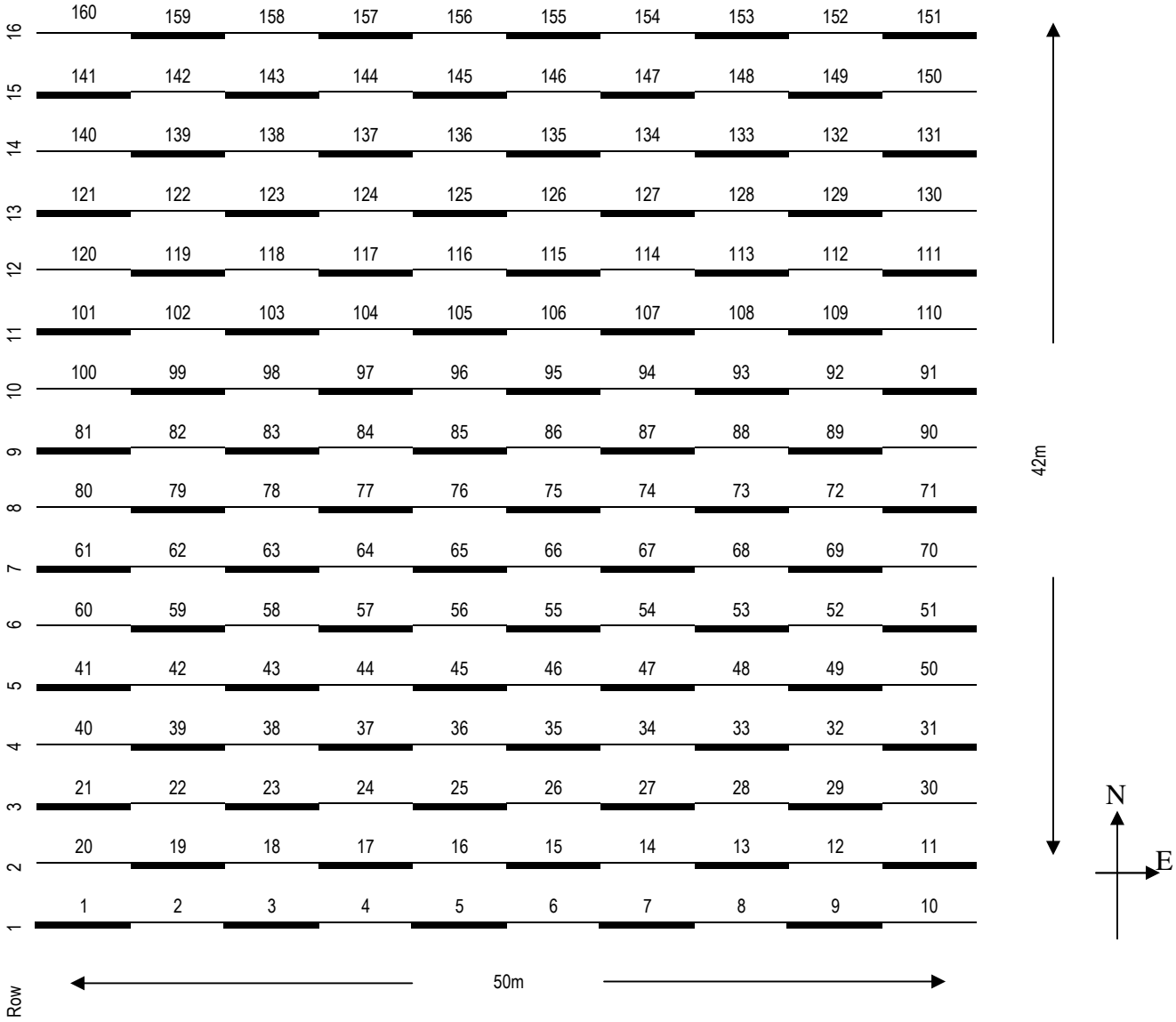

Figure 6.3 A diagram representing plots in one of the two blocks grown at CIMMYT Maize Research Station during the 2006/7 and 2007/8 cropping seasons, and at ART farm (2007/8). Direction of rows in the ART farm 2006/7 crops was oriented at 90^0 to the direction of rows in Figure 6.3

Ear harvesting was done by hand and five ears were selected randomly from each plot for kernel counting in the seed laboratory. The randomly selected five ears per plot were hand shelled individually and stored in labeled envelopes in the laboratory. The total number of kernels, the total number of yellow endosperm kernels, and the total number of white endosperm kernels were determined. The number of yellow kernels per ear multiplied by 100 divided by the total number of kernels per cob was calculated as the percent level of normal endosperm maize pollen contamination in the QPM grain from each sampled ear. An average percent contamination value for each plot was obtained by adding the contamination levels of five cobs in a plot and dividing the total by five. The arithmetic grand mean for the 160 plots was used to estimate the across field level of contamination.

6.2.3 Modeling of the spatial variation of contamination

6.2.3.1 Modeling and function selection

A theoretical variogram is used to estimate parameters that are used in kriging. In order to construct a theoretical variogram, a model is needed that can be used to determine values for any possible sampling interval within the space under consideration. Percentage values of levels of contamination in QPM crops of CML144/CML159//Obatampa (an experimental variety from CIMMYT Zimbabwe) and C3728 (an experimental maize variety from Seed Co) grown in three seasons (2005/6 to 2007/8) at two locations (ART farm and CIMMYT Harare) giving a total of nine experiments on 0.21ha blocks were studied for modeling of the pattern of normal endosperm maize pollen contamination.

Modelling for semivariances (spatial analysis) and the optimum interpolation of foreign pollen contamination in a field of QPM in this study was done using geostatistical analysis software, ArcMAP 9.2 from ESRI (Johnson et al., 2001). The observed data sets for each of the nine experiments were fitted to various different models (specified later) using the ordinary kriging procedure. According to Oliver and Webster (1990) and Ahmadi and Sedghamiz (2007) the general equation of linear kriging which is an estimation method by local weighted average weighting is:

$$Z^*(x_p) = \sum \lambda_i Z(x_i) \qquad [1]$$

Where $Z^*(x_p)$ is the kriged value at location x_p (and can be an estimate for a block of land), and λ_i are the weights associated with the data and should sum up to one to ensure there is no bias and subject to this are chosen to minimise estimation variance. $Z^*(x_i)$ is the known value at location x_i.

Amongst the kriging procedures (which include indicator kriging and co-kriging), ordinary kriging is the default and most used (Subcommittee D18.01, 2004). Therefore, the observed data was analysed using ordinary kriging and to achieve unbiased estimations in ordinary kriging the following two equations should be solved simultaneously according to Goovaerts (1997);

$$\sum_{i=1}^{n} \lambda_i \gamma(x_{i,}, x_j) - \mu = \gamma(x_{i,}, x_j) \qquad [2]$$

$$\sum_{i=1}^{n} \lambda_i = 1 \quad [3]$$

where μ is the lagrange multiplier, and $\gamma(x_{i,}, x_j)$ is the value of the variogram corresponding to a vector with origin in $x_{i,}$ and extremity in x_j (Goovaerts, 1997). Thus the spatial variation can be expressed in a mathematical form using the variogram, $\gamma(h)$. The variogram, $\gamma(h)$ is defined as one-half the variance of the difference between attribute values at all points separated by h as follows

$$\gamma(h) = \left(\sum_{i=1}^{N(h)} [Z(x_i) - Z(x_i+h)]^2 / [2N(h)] \qquad (4)$$

where Z(x) indicates the magnitude of variable, and N(h) is the total number of pairs of attributes that are separated by a distance h.

The process involves developing an experimental variogram which is used to construct a theoretical one. The linear least squares method is applied in estimating the parameters of the theoretical variogram. Therefore, the interpolation of the semivariance of any location or point within any distance in a given range can be computed using the theoretical variogram. Cross-validation of each predicted model was done by removing one datum at a time and estimating it using all the other values (Johnson et al., 2001). The Root Mean Square Error (RMSE) or square root of the average squared differences between the estimated values and the observed values were calculated for each set of conditions.

$$RMSE = ((SSEi)/n)^{1/2} \qquad [1]$$

where SSE is sum of errors (observed – estimated values), and n is the number of pairs of values of errors. The conditions or settings that were varied were presence of anisotropy[11] or absence of anisotropy (isotropy) and the lag settings which were either set to the ARCMAP 9.2 default for the loaded files or the sampling distance between the observations (5m and 10m). Lag refers to the line that separates any two sampling points. The lag has both distance and direction and so it is a vector. The function with the least RMSE for each model was obtained by trial and error under four different conditions stated above.

The most commonly used models for developing the theoretical variogram are Gaussian, exponential, spherical and (Bayraktar and Turalioglu, 2005; Isaaks and Srivastava, 1989) and linear types (Bayraktar and Turalioglu, 2005). Different data sets are best described by different models and hence several model options available in ArcMAP 9.2, a geostatistical software, were considered and these include Circular, Spherical, Pentaspherical, Tetraspherical, Exponential, Guassian, Rational Quadratic, Hole Effect, K-Bessel, J-Bessel, and Stable (Johnson et al., 2001). Theoretical variograms were fitted for each of the eleven available models and the best function for each block was selected for each of the eleven models from results of two different

[11] **Anisotropy:** A property of a spatial process where spatial dependence (autocorrelation) changes with both the distance and the direction between two locations. Isotropy: The spatial dependence changes only with the distance between two locations (direction is unimportant).Johnston, K., J.M. Ver Hoef, K. Krivoruchko, and N. Lucas. 2001. Using ArcGIS Geostatistical Analyst, GIS by ESRI, pp. 300. ESRITM, Redlands.

settings for lag and anisotropy or isotropy on the basis of the magnitude of RMSE (Gundogdu and Guney, 2007; Webster and Oliver, 1990).

6.2.3.2 Contamination threshold maps

Using the threshold values for contamination established in the experiment on nutritional value of contaminated QPM crops based on a minimum QI of 0.80 (in chapter 5), maps were drawn indicating the lower and upper 95% confidence intervals for the areas with contamination levels that did not exceed the established threshold values for each of the four 2006/7 experiments (QCS200711, QCS200712, QCS200721, and QCS200722). The pollen contamination threshold values were determined in a related experiment in Chapter 5. The percentage of grain nutritionally superior to normal endosperm maize grain was determined for the prediction surface of each block using area calculated through zonal statistics in the Raster menu of ARCMap 9.2 (ESRI®, 2001). The overall area weighted average level of normal endosperm maize pollen contamination for each block was determined assuming that the farmer was able to produce a homogenous mixture of the harvested grain from each of his/her lands. The average contamination levels for a homogenous mixture of grain from each experiment were calculated using two methods, the arithmetic average and the area weighted average.

6.2.4 Assumptions

1. The shape and size of the QPM crop area used in the study represents the majority of the crop areas found in the smallholder farming sector;
2. The pattern of QPM contamination produces a continuous surface with no open space between the maize plants both in the row and between the rows;
3. The weather in terms of wind speed and direction is representative of the events in most smallholder farming sectors;
4. The chemical and nutritional properties of the two varieties used in the study are representative of both the average QPM and average normal endosperm maize varieties;
5. There were no differences in pollen viability between the normal endosperm maize crop and the QPM crop;
6. The worst-case scenario of contamination occurs when the two crops are planted as one crop such that adjoining rows and sections of the two different crops are separated by the standard between row spacing of 0.75m and 0.25m in the row respectively and also the flowering of the two crops occurs synchronously.

6.3 Results

In all the experiments, the flowering of the QPM and the normal maize crops overlapped in a desirable manner resulting in considerable levels of contamination. Inspections at the flowering period revealed that there was good nick between the contaminant crop and the receptor crop.

6.3.1 Root mean square error values

The obtained lowest RMSE values are presented in Table 6.2. The most appropriate variogram for each experiment was chosen based on the least RMSE by trial and error procedure. In all the crops the best model had anisotropy. The exponential model was the best model in five of the nine blocks of crops (Table 6.2). The best model for the 2005/6 experiment was the stable, whilst the pentaspherical was the best in one of the ART farm 2007 crops, and the rational quadratic was the best in one of the ART farm 2008 crops. The J-Bessel model was the best in one of the two ART farm crops. All the crops at CIMMYT Harare had the best model as the exponential with anisotropy and at lag settings of 6.0 m sampling distance and 11 lags in both cropping seasons.

Table 6.2 Root mean square error values of the best model for each of the nine blocks

Lag size (m)	No. of lags	Experiment	Year	Location	Selected Model	RMSE
3.75	12	QCS20061	2006	ART	Stable	8.623
6.0	11	QCS200711	2007	ART	Pentaspherical	7.243
6.0	11	QCS200712	2007	ART	Exponential	8.772
6.0	11	QCS200721	2007	CIMMYT	Exponential	9.22
6.0	11	QCS200722	2007	CIMMYT	Exponential	9.392
6.0	11	QCS200811	2008	ART	Rational Quadratic	9.773
3.83	12	QCS200812	2008	ART	J-Bessel	11.93
6.0	11	QCS200821	2008	CIMMYT	Exponential	8.028
6.0	11	QCS200822	2008	CIMMYT	Exponential	7.154

Key: ART = ART farm, CIMMYT = CIMMYT Harare, RMSE = Root Mean Square Error,

6.3.2 Prediction and prediction error maps.

Selected prediction maps and the associated prediction error maps for percent contamination are presented in Figures 6.4 to 6.7. For the sake of brevity only a few maps of the results were presented and a graph of the summary statistics of the other maps is presented in Figure 6.10.

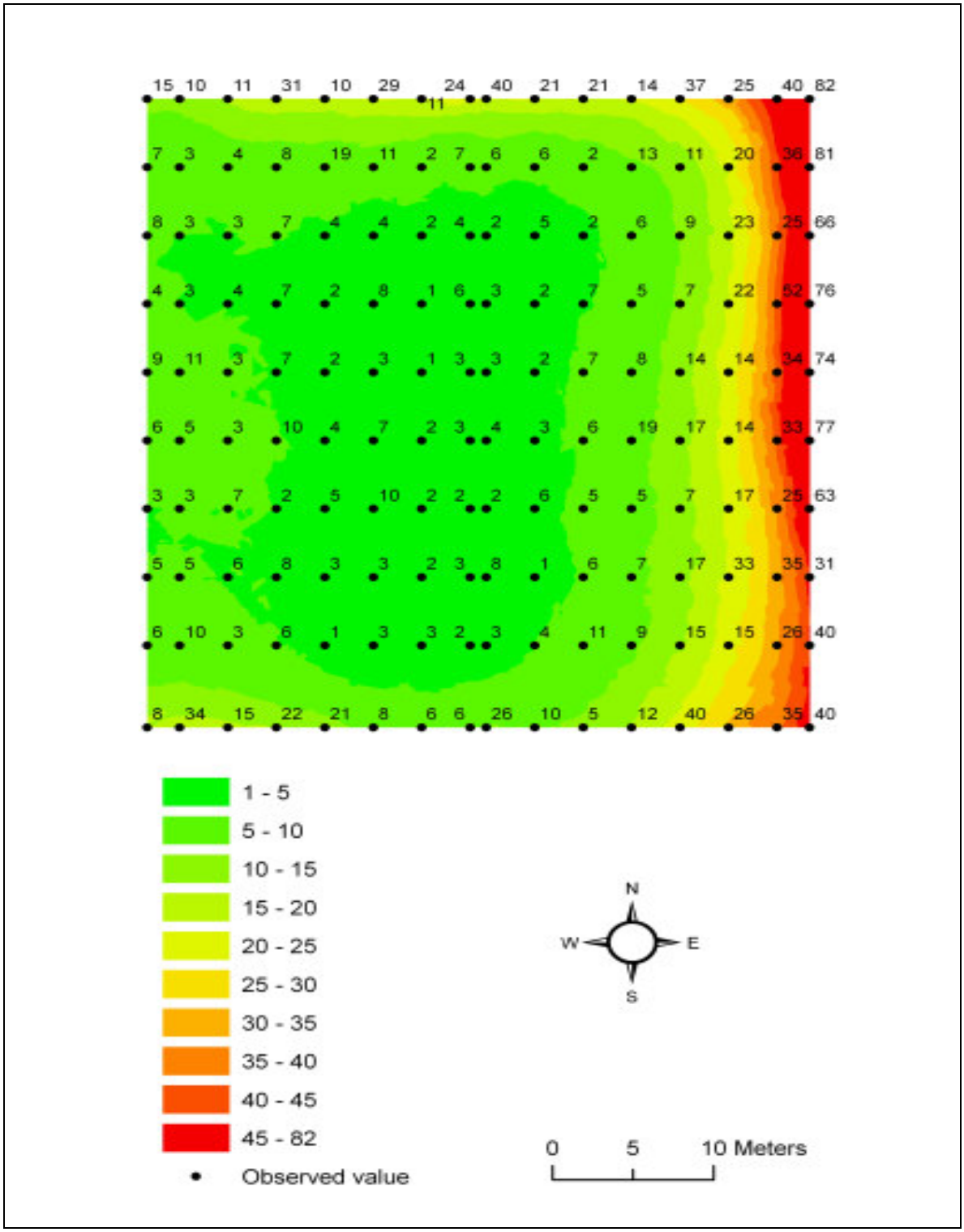

Figure 6.4 Observed and predicted values map for percent contamination in experiment QCS200711 grown at ART farm during 2006/7

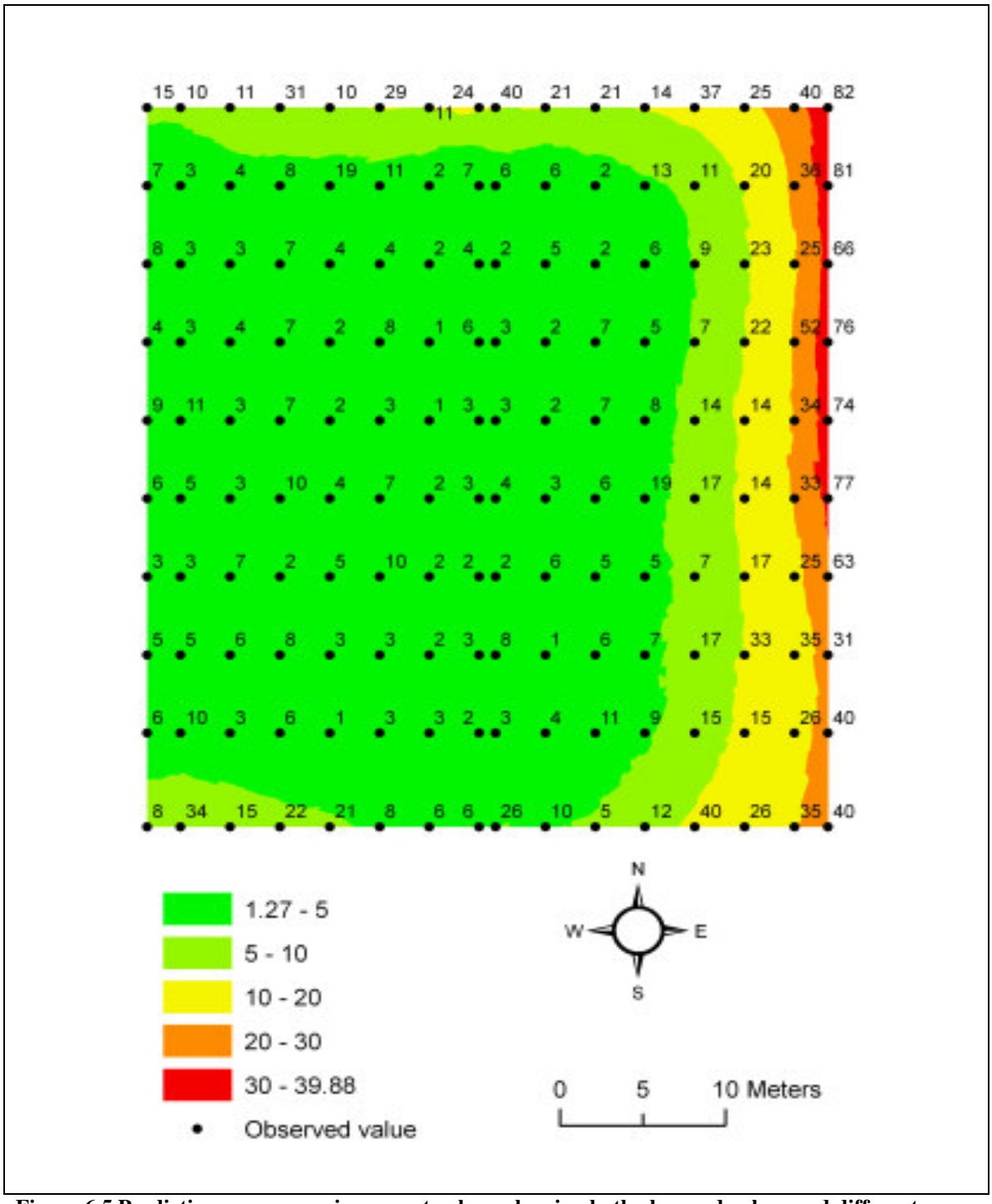

Figure 6.5 Prediction errors map in percent values, showing both observed values and different zones for prediction errors in experiment QCS200711 grown at ART farm during 2006/7

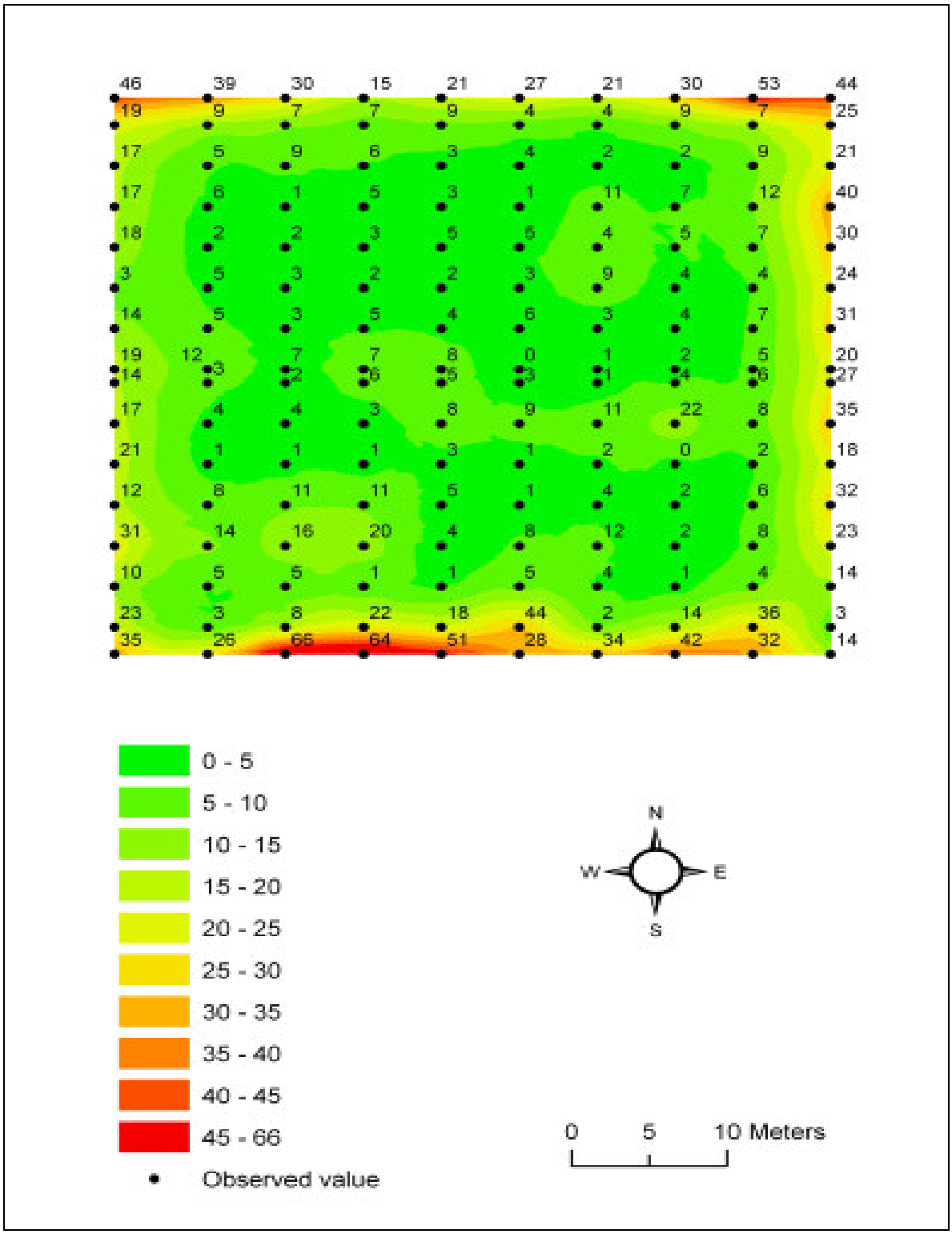

Figure 6.6 Observed and predicted values map for percent contamination in experiment QCS200721 grown at CIMMYT Harare during 2006/7

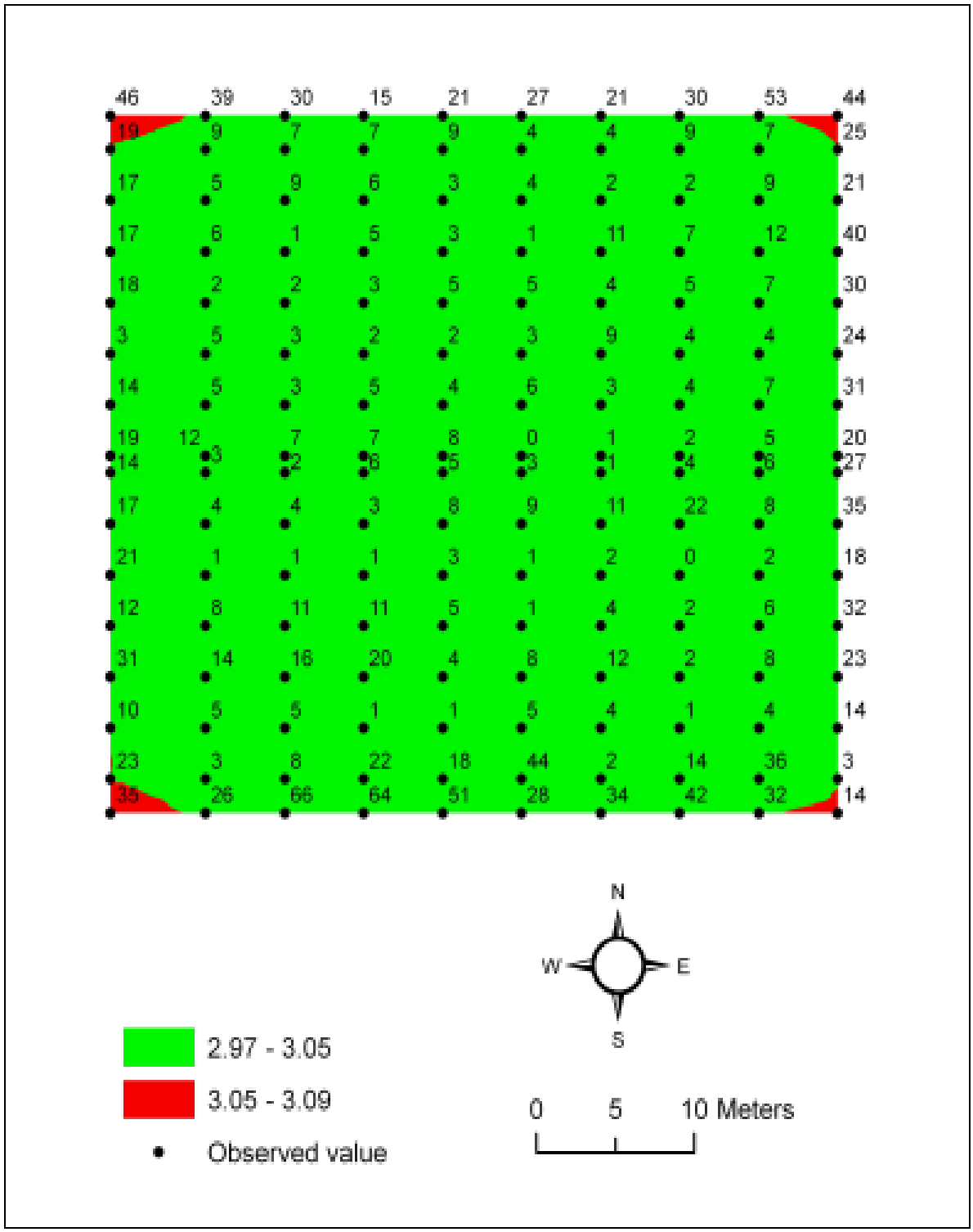

Figure 6.7 Prediction errors map in percent values, showing both observed values and different zones for prediction errors in experiment QCS200721 grown at CIMMYT Harare during 2006/7

6.3.3 Threshold maps

Threshold maps were drawn for four experiments only, QCS200711, QCS200712, QCS200721, and QCS200722 based on a minimum QI of 0.80. This is because these were the crops where the threshold levels were analysed. The threshold to contamination is not transferable from one experiment to another since it was found to be influenced by the environment in the experiment reported in Chapter 5. Only two maps Figure 6.8 and 6.9 were presented for the sake of brevity and the rest of the information was presented in Table 6.3.

Table 6.3 Area (%) with Quality Index (QI) value of at least 0.80 and above

Experiment	% Area under 95% confidence lower limit	Estimate	% Area under 95% confidence upper limit	% Area with QI below 0.80
QCS200711	87.9	92.9	95.9	4.1
QCS200712	94.8	96.5	97.5	2.5
QCS200721	62.2	82.1	91.1	8.9
QCS200722	65.6	76.8	85.7	14.2

Estimate to contamination threshold for QCS200711 and QCS200712 = 31.94 $\pm$ 7.22% and for QCS200721 and QCS200722 = 15.23 $\pm$ 5.45%

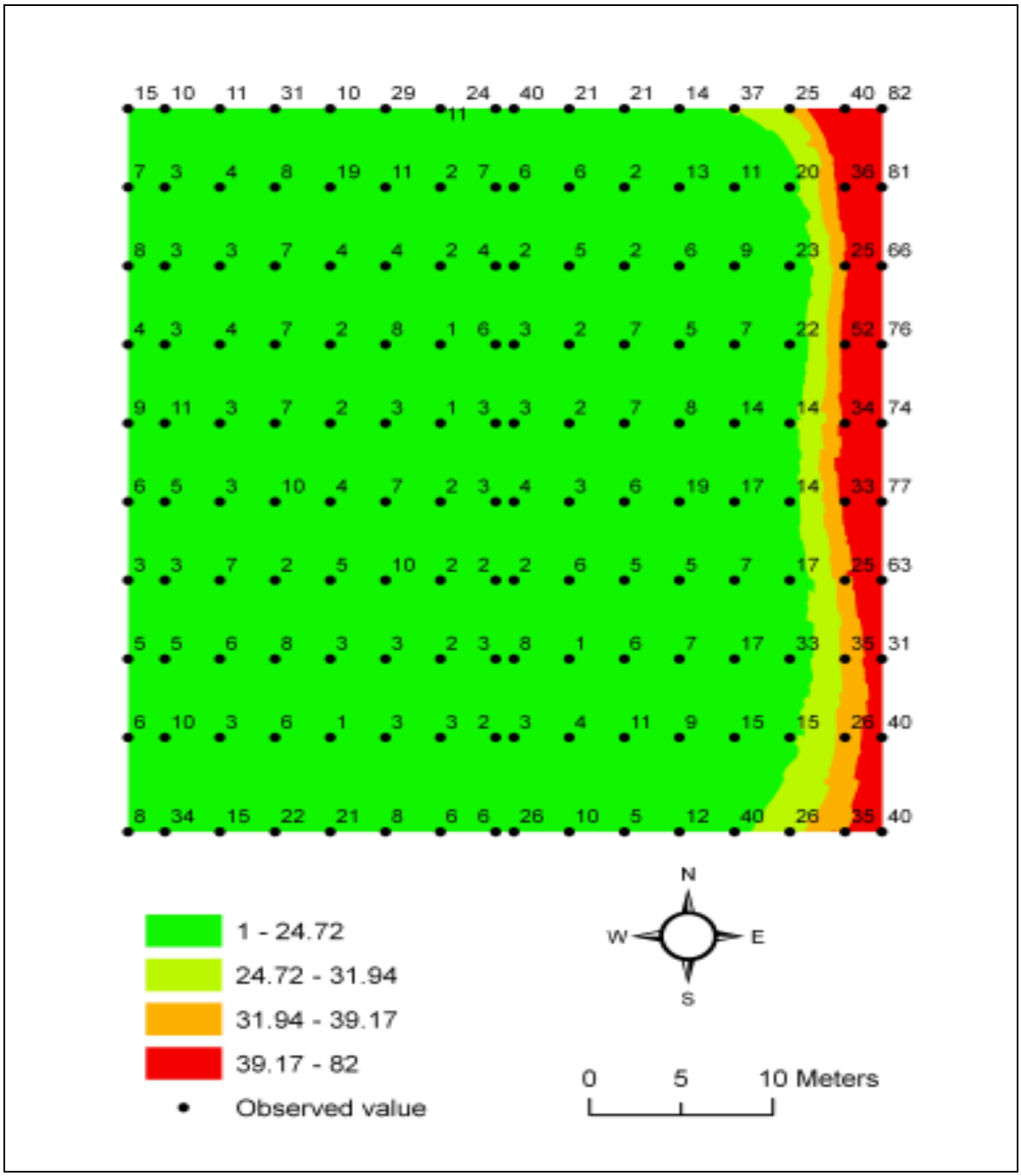

Figure 6.8 Threshold map showing observed values, zones falling under 95% confidence lower limit (24.72%), threshold limit zone (31.94%) and 95% confidence upper limit zone (39.17%) for contamination in experiment QCS200711 grown at ART farm during 2006/7

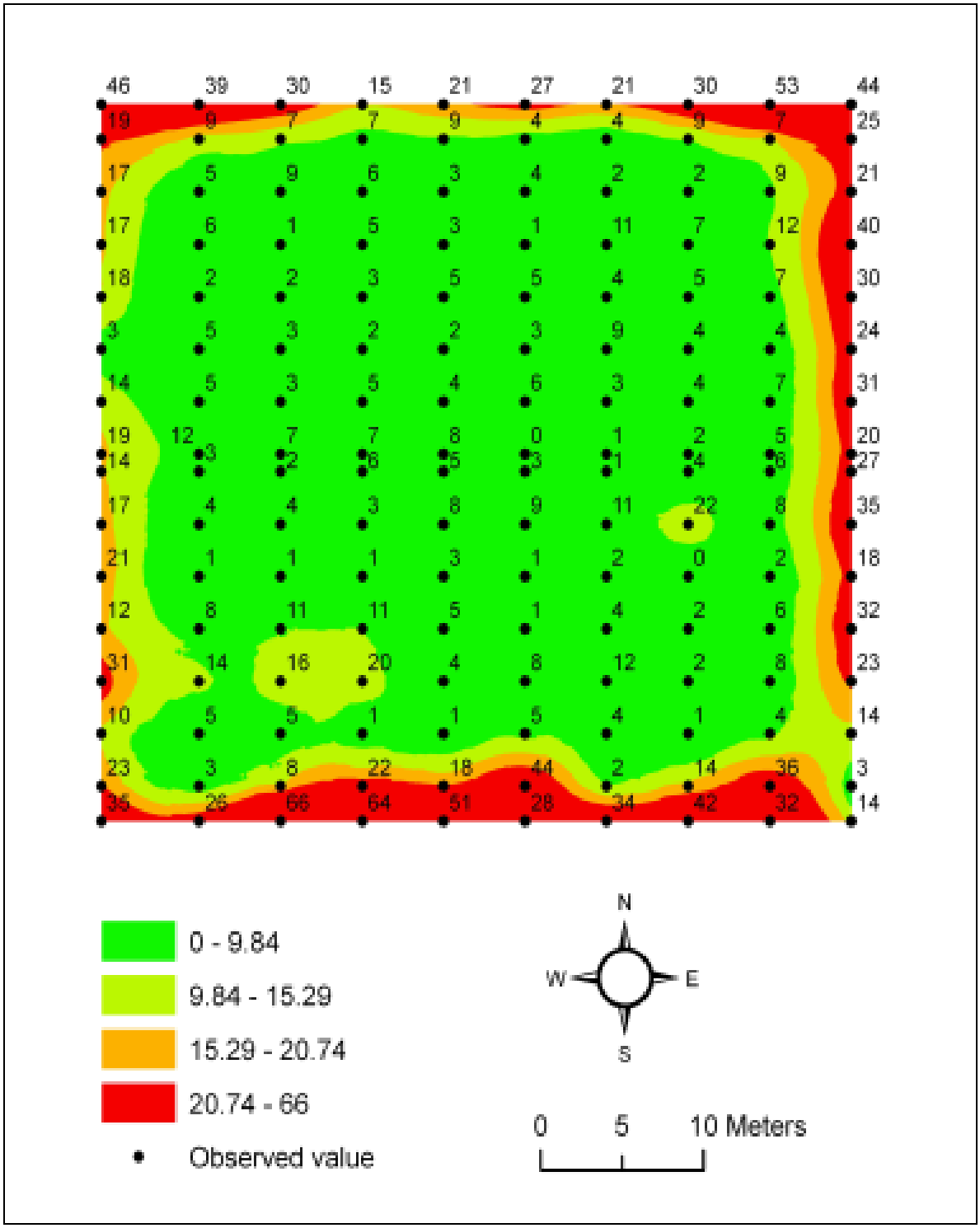

Figure 6.9 Threshold map showing observed values, zones falling under 95% confidence lower limit (9.84%), threshold limit zone (15.29%) and 95% confidence upper limit zone (20.74%) for contamination in experiment QCS200721 grown at CIMMYT Harare during 2006/7

6.3.4 Cumulative contamination zone % area

The contaminated areas for each zone or class break in the prediction maps were expressed as a percentage of the total area and in all the experiments and more than 50% of the cumulative per cent area for each experiment was found to be within the 20% pollen contamination zone upper class limit (Figure 6.10). The experiment with the highest cumulative percent (%) area under the 20% contamination zone was experiment QCS200712 and the lowest was QCS200812 and the rest of the experiments lay between these two extremes.

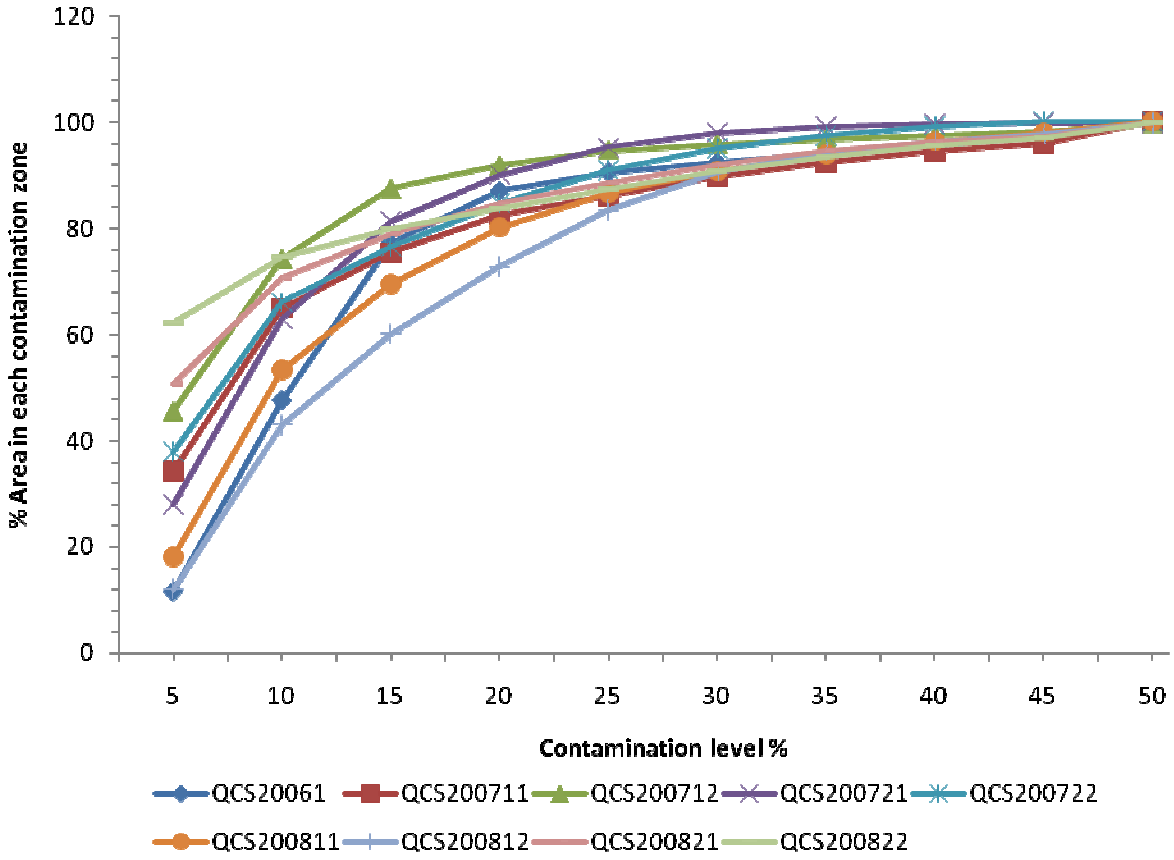

Figure 6.10 Cumulative percent area of contamination zones in different experiments

6.3.5 Average contamination levels

The average contamination levels for a homogenous mixture of grain from each experiment based on both the arithmetic averages and the area weighted averages are presented in Table 6.4. The arithmetic average contamination percent values ranged

from 11.85% to 17.92% for homogenous mixtures of grain from the experiments conducted in the three different seasons. The area weighted contamination percent values ranged from 10.8% to 17.5%. The ranges of prediction errors for CIMMYT Harare location (1.76 – 5.94%) were generally lower than those for the ART farm site (1.13 – 44.82%).

Table 6.4 Summary of contamination statistics for the different experiments

Experiment	Arithmetic average contamination %	Area weighted average contamination %	Unsampled points prediction Errors range %
QCS20061	15.85	15.3	2.25 - 34.85
QCS200711	14.11	14.2	1.27 - 39.88
QCS200712	11.85	10.8	1.27 – 26.37
QCS200721	12.48	12.2	2.97 – 3.09
QCS200722	13.31	12.6	5.61 – 5.94
QCS200811	16.45	15.7	1.13 – 32.40
QCS200812	17.92	17.5	1.40 – 44.82
QCS200821	14.91	12.3	4.08 – 4.29
QCS200822	14.58	11.7	1.76 – 1.85

The QCS200721, QCS200722, QCS200821, and QCS200822 (all CIMMYT Harare experiments) had lower prediction errors than QCS20061, QCS200711, QCS200712, QCS200811 and QCS200812 (all ART farm environments) implying that the kriging predictions were better for CIMMYT Harare experiments than ART farm experiments. The areas responsible for the high prediction errors in both ART farm and CIMMYT Harare environments were mostly found at the edges of the crop fields and relatively small (Figures 6.5 and 6.7).

6.4 Discussion

The capabilities and benefits of using ordinary kriging for estimating pollen contamination were demonstrated and the contamination patterns for each block or crop field could be visualised in a single realisation. The advantage of kriging is that the errors associated with kriging can also be spatially visualised. The prediction standard errors across the nine experiments ranged from 1.27% to 44.82%. In all the nine experiments the areas with very high prediction standard errors were very small (Figures 6.5 and 6.7) and more than 50% of the area in each experiment had prediction errors of about 10% and below. This showed that the precision of ordinary kriging was good except for the small areas at the edges of the blocks.

Most of the values of both area weighted average levels of contamination and arithmetic mean levels of contamination were above the 11% average contamination levels reported by Twumasi-Afriye et al. (1996b). The arithmetic average is the method used by Twumasi-Afriye et al. (1996b) and the averages for this study could be slightly higher because the present study was conducted under worst-case scenarios for pollen contamination. This, therefore, implies that in circumstances which are not worst case scenarios for contamination, the levels of normal endosperm maize pollen contamination would be far less than previously thought and QPM and normal endosperm maize crops can potentially coexist without serious erosion of nutritional superiority. This can be substantiated by the fact that under worst case scenario for contamination at least 50% of the area in each experiment had contamination levels of only 20% and below (Figure 6.10). This is a low level of contamination for the bulk of the area considering that normal endosperm maize pollen and QPM pollen had equal chances of fertilising the QPM silks.

The relatively high levels of normal endosperm maize pollen contamination (above 50%) that were both observed and predicted towards some of the edges of the QPM crop fields were a good indication of the fact that there was optimum overlapping of flowering periods between the QPM crops and the normal endosperm maize crops in all the experiments. In the spatial distribution of predicted levels of contamination, the highest levels of pollen contamination were found to occur mostly on either the northern or eastern side or both eastern and northern sides of the experimental blocks (Figures 6.4 to 6.7), and these are both directions of prevailing north easterly winds in Zimbabwe. Unfortunately there was no weather data recording equipment adjacent to the blocks of experiments and so the results could only be discussed in the context of the climate for the area.

The best model was not the same for the different experiments conducted between 2005/6 to 2007/8. Out of the eleven models, five gave the least RMSE values in different experiments. In each of the experimental blocks, it was not the same model that was best except for experiments QCS200712, QCS200721, QCS200722, QCS200821, and QCS200822 where the exponential with anisotropy was the best model. The experiment QCS20061 had a different sampling pattern and 75% less observations compared to each of the other eight experiments and the best model for QCS20061 was the stable with anisotropy. Thus because of the differences in both number of observations and

sampling pattern the best model for QCS20061 was not surprisingly different from the rest of the experiments.

The other eight experiments had the same number of observations and similar sampling patterns but the spatial variation of pollen contamination was explained by different models except for experiments QCS200712, QCS200721, QCS200722, QCS200821 and QCS200822 where the exponential was the best model. Experiments QCS200711 and QCS200712 were both conducted at ART farm in the same year (2006/7) but were different in the design in that QCS200712 was surrounded by source plants of the contaminating pollen on three sides only and not four. Therefore, the best model for QCS200711 and QCS200712 were expected to differ.

The two 2007/8 experiments at ART farm (QCS200811 and QCS200812) had two different models as best models for explaining the spatial variation in pollen contamination despite that the layout of the two experiments was the same. Experiment QCS200811 had the rational quadratic as the best model whilst experiment QCS200812 had J-Bessel as the best model. The spatial variation of pollen contamination in all the four experiments, QCS200721, QCS200722, QCS200821, and QCS200822 grown at CIMMYT Harare was best explained by the exponential model. The differences between the two locations, CIMMYT Harare and ART farm, was that crops planted in the former had no effective wind breaks. The CIMMYT Harare experiments were planted in areas where the crops were relatively far from wind breaks in the form of trees. Although the two sites are not very far from each other (less than 10km) the weather patterns during the flowering periods at the two sites could have been different because the planting dates for the ART farm sites were earlier by seven weeks and eight weeks in the 2006/7 and 2007/8 cropping season, respectively.

The aim of the experiment was not to get the same model explaining the spatial variability since it was not possible to replicate the draughts and troughs of the wind currents that carried pollen across each of the different crops. The position of the crop could also not be replicated in the same cropping season relative to the geographical positioning system and, hence, the weather patterns experienced in two similarly planted crops would be different even if they were planted on the same day and adjacent to each other. This potentially led to different patterns of contamination even in adjacent QPM

crops. The most important issue was to get an idea about the average levels of normal endosperm maize pollen contamination (which were found to be below 20% under worst case scenario), and be able to visualise in a single realisation the spatial distribution of normal endosperm maize pollen contamination in a QPM crop.

The threshold levels of normal endosperm maize pollen contamination based on the 2006/7 crops reported in Chapter 5 were 31.94% $\pm$ 7.222% at 95 % confidence interval and 15.2895% $\pm$ 5.4473% at 95% confidence intervals for ART farm (QCS200711 and QCS200712) and CIMMYT Harare (QCS200721 and QCS200722) experiments, respectively. Using the stricter lower 95% confidence limit for threshold levels for contamination, the percentage areas contaminated by foreign pollen beyond the threshold level were 12.1%, 5.2%, 37.8% and 34.4% for experiments QCS200711, QCS200712, QCS200721 and QCS200722 respectively. Thus at both locations it was possible to get nutritionally superior QPM grain from the 2006/7 experiments. The thresholds for the same QPM variety (C3728) were found to differ with change of growing environment in the study reported in Chapter 5. The threshold (or level of normal endosperm maize pollen contamination that could occur without loss in nutritional superiority) was 31.9% in a good growing environment (ART farm) and 15.3% in a harsher moisture stressed environment (CIMMYT Harare). Therefore, threshold levels obtained for QPM grain sampled from the 2006/7 experiments could not be safely used for either the 2005/6 or the 2007/8 experiments. In the threshold maps produced for the 2006/7 cropping season only, it was found that based on a Quality Index of 0.80 the bulk of the crop passed for QPM at both ART farm and CIMMYT Harare.

Although the average foreign pollen contamination levels (Table 6.4) were all below the 20% reported by Twumasi-Afriye et al. (1996a) as the limit based on physical mixtures it could only be said that for all the experiments the contamination levels were far less than previously thought and comments on the nutritional value of the harvested grain were restricted to the experiments of 2006/7 cropping season (commented above). This is because the nutritional value was found to depend on the particular growing conditions as reported in Chapter 5.

Despite that the designs for all the experiments represented the worst case scenarios for contamination between two independent adjacent crops, it was found that the area

weighted average levels of contamination were far less than previously thought and this agrees with what was reported by Cordova (2000). According to Burris (2001), one of the contributing factors to low levels of foreign pollen contamination is the copious amounts of desirable pollen from the QPM crop that compete with the foreign pollen for the silks and, thereby, reducing the chances of foreign pollen landing on QPM silks. Therefore, if more than 50% of the grain produced under the worst case scenario of contamination, could be classified as nutritionally superior based on 2006/7 experiments, then because farmers would operate under scenarios where they attempt to minimise normal endosperm maize pollen contamination, it means that QPM and normal endosperm maize crops can mutually coexist without the nutritional value of the whole QPM crop being adversely affected by normal endosperm maize pollen contamination alone.

The prediction error maps indicated that the magnitudes of the errors were mostly high towards the edges of the crop fields and for the bulk of the crop in each experiment the central part of the field had relatively low prediction errors. This therefore meant that kriging can be successfully used to establish the levels of contamination in a QPM crop as long as there is an accurate assessment to distinguish between the contaminated QPM grain and the pure QPM grain. The prediction error ranges for CIMMYT Harare (QCS200721, QCS200722, QCS200821, and QCS200822) were generally lower than those for the ART farm site (QCS200711, QCS200712, QCS200811 and QCS200812). Although the best models were identified in each case, the prediction errors for ART farm site could have been improved through improving the sampling pattern. A suggestion for improving the prediction at the edges of the field and reduce the magnitude of the errors was to reduce the sampling distance and increase the number of sampling points towards the edges of the fields. The prediction errors for experiments QCS20061, QCS200711, QCS200712, QCS200811 and QCS200812 could have been possibly reduced by increasing the sampling points. In general the sampling distance can be reduced and the number of sampling points increased across the whole block of experimental crop so as to reduce the errors further but when the cost factor is limiting as is always the case it would be prudent to increase the sampling points towards the edges only so as to improve the prediction at unsampled points and reduce the prediction standard errors.

The size of the block with the QPM crop was considered to be representative of the relatively small sized lands that are planted to different maize varieties in the smallholder farming sector (about a quarter of a hectare to half a hectare). However, if the size of the block with the QPM crop is increased under the same growing conditions it meant there could be lesser average levels of contamination than registered in these crops. Another important aspect to note is that if all the other conditions are held constant, then the shape of the block with the QPM crop is an influencing factor to the levels of contamination that can take place. A square block would experience less contamination relative to a rectangular block since most of the high levels of contamination occur at the edges of the field. A good example is to imagine the effect of achieving one hectare of a QPM crop from 10 rows only, compared to one hectare in a square block of 100 meters by 100 meters and both are surrounded by a normal maize crop. The one hectare based on ten rows would experience a relatively higher level of average contamination Therefore, it would be important to advise smallholder farmers that if the chances of contamination are high they should then plant their QPM crops in a relatively square shaped block.

It was observed from the prediction maps that the side of the QPM crop proximal to the direction from which the prevailing winds originated had the highest and greatest levels of contamination (Experiments QCS20061, QCS200711, QCS200712, QCS200811, and QCS200812). Therefore, it would be important to advise farmers to plant QPM crops upwind relative to the positions of normal endosperm maize crops provided they know the wind direction patterns in their area. At this stage it would be very difficult to recommend to farmers who grow open pollinated QPM varieties in an area where QPM is not the predominant crop to save seed for future plantings. However, if the maize crop in the area is predominantly QPM, it seems farmers can save seed from ears harvested from the central parts of their fields provided there are no off type plants in their QPM crops.

In 2006/7 (experiments QCS200711 and QCS200712), despite that the QPM crops planted at ART farm had a high pressure of foreign pollen due to the large adjacent areas (indicated in Table 6.1) planted to yellow normal endosperm maize contaminant crops which flowered in synchrony with the 0.21ha of QPM crop, it was still possible to find at least 87.9% and 94.8% of the QPM crop at ART farm (Table 6.3) qualifying as

nutritionally superior QPM grain from the relatively small area of 0.21ha planted to QPM. This was based on the stricter 95% confidence lower limits. Therefore, taking this into consideration, it is possible for the farmers to benefit from QPM crops that coexist with normal endosperm maize as long as they plant fresh QPM seed every cropping season and also the wind strength is moderate. It is important to note that the levels of contamination tend to be determined by the prevailing set of climatic conditions during crop growth such that if all the other conditions are held constant and wind strength and speed increases, then the expectation is that higher levels of normal endosperm maize pollen contamination might occur. The farmers who are expected to benefit from the nutritional superiority of QPM are those who do not sell their QPM grain to centralised distribution systems where the QPM nutritional benefits get diluted and lost, but those who consume the QPM grain at the farm level.

6.5 Conclusion

Different ordinary kriging models were successfully used for spatial analysis and prediction of foreign pollen contamination levels in QPM crop fields in the presence of a marker that distinguished normal endosperm maize pollen contaminated QPM kernels from pure QPM kernels. The obtained weighted average levels of foreign pollen contamination were far less than previously thought. Therefore, QPM crops can mutually coexist with normal endosperm maize crops provided the individual areas of the QPM crops are relatively large. The farmers who grow QPM can benefit as long as they consume the grain and not sell it to centralised distribution systems. Improved sampling and modeling approaches are expected to reduce the sampling errors observed in some of the experiments. Geostatistics was successfully applied as a decision making tool in the dissemination of QPM, and where absolute or near-absolute isolation is difficult then QPM varieties proponents can confidently advocate for the coexistence of QPM and normal endosperm maize crops as long as seed for future plantings is not saved from the QPM crops.

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7 Overview of research findings

7.1 Introduction

This chapter reviews the objectives and the findings of the research, and provides recommendations based on the findings and gaps identified in the study.

7.2 Research hypotheses

There were five main hypotheses investigated in this study and these are:

1) Smallholder farmers have preferences and perceptions on maize varieties and these have implications for QPM varieties breeding and dissemination

2) The inbred lines in the study were diverse for general combining ability effects, specific combining ability effects and reciprocal cross effects.

3) The experimental hybrids were both comparable to the checks and adapted to subtropical environments.

4) The contamination of QPM by normal endosperm maize pollen under coexistence is far less than previously thought.

5) Quality protein maize crops can tolerate some contamination by normal endosperm maize pollen without losing nutritional superiority.

7.3 Summary of the main findings

7.3.1 Soliciting smallholder farmer participation in the development and setting up of QPM breeding goals, objectives and dissemination strategies

The farmers identified three main types of varieties grown in their different areas which were hybrids, improved open pollinated varieties, and “Hickory King” (landrace OPV). Most farmers indicated that they preferred and grew hybrids because the hybrids were easily available but they also strongly liked “Hickory King” because of the quality of the culinary products produced by the variety. Open pollinated varieties were an alternative when the farmers failed to access seed from the suppliers.

The farmers highlighted that there were desirable attributes that they would expect to find in a QPM variety developed for them. The attributes can be categorized into agronomic and quality attributes. The agronomic attributes were as follows:

1) High grain yield potential

2) Relatively early maturity

3) Tolerance to drought stress

4) Good resistance to foliar diseases

5) Good tolerance to field pests

The quality attributes that the farmers demanded in new QPM varieties were as follows:

1) Big kernels similar to “Hickory King” kernels

2) Hard kernels similar to “Hickory King” kernels

3) Sweet taste of product similar to “Hickory King” kernels and flour

4) Weevil resistance similar to “Hickory King” kernels

5) White flour from the new variety similar to“Hickory King” flour

The farmers pointed out that although they liked the quality attributes of “Hickory King” they disliked the agronomic attributes which were late to maturity and susceptibility to foliar diseases. It was found out that the hybrids, because of their poor quality characteristics, were grown mainly for sale to the grain marketing authorities whilst “Hickory King” grain, because of its desirable attributes, was reserved for domestic consumption.

In addition the farmers also indicated their preferences for the different methods of QPM information dissemination. The most preferred method was through the local AREX officers followed by broadcasting through the radio.

The farmers revealed that AREX was the most important organisation in their maize production activities and involvement of the extension department in QPM extension activities would guarantee success. When asked to demystify the term QPM in their own words the farmers proposed the terms “Godzamhuri” which meant Family nourisher, and “Mupedzakwashi” which meant “Kwashiorkor terminator”.

7.3.2 Estimation of GCA, SCA and reciprocal effects on quality traits and grain yield among the publicly available elite CIMMYT QPM inbred

The type of gene action for the various traits studied was established. The findings were as follows:

1) Specific combining ability effects were important in the control of mainly grain yield but were also involved in the control of anthesis days and the QI trait.

2) General combining ability effects were very important in the control of protein content, tryptophan content, QI, kernel endosperm modification and anthesis days.

3) Reciprocal-cross effects were identified in anthesis days, tryptophan content, and QI but they were less important to both GCA and SCA effects in anthesis days whereas in both tryptophan and QI they were larger than SCA effects.

4) Both maternal and nonmaternal effects were important in the control of both anthesis days and QI. The nonmaternal proportions were greater than the maternal proportions in both traits.

5) Nonmaternal effects were both significant and greater than maternal effects for tryptophan content whilst maternal effects were nonsignificant.

The study identified inbred lines that can be used as potential sources of desirable attributes in a QPM breeding programme and the findings were as follows:

1) The best inbred line for GCA values for protein content, tryptophan content and QI was CML264Q and the worst line for both tryptophan content and QI was CZL03016.

2) The best inbred line for GCA values for kernel endosperm modification scores was CZL03016 followed by CZL01006. The worst line for kernel endosperm modification was CML264Q.

3) The best positive SCA value for grain yield was from the cross of CZL03016 and CML144 and the worst and negative SCA value was from the cross of CML144 and CML181f

4) The highest level of SCA value for QI was for the cross of inbred CZL03016 and CML264Q

5) The highest level of reciprocal cross effects for both tryptophan content and QI were in the cross of CML264Q and CZL03016.

6) The least reciprocal cross effects for anthesis days were between the cross of inbred CZL03016 and CZL01006.

7.3.3 Comparison of the performance of the experimental QPM genotypes against selected check cultivars, and comparison of the performance of QPM hybrids against normal endosperm maize hybrids for both grain yield levels and kernel endosperm modification scores

It is important to highlight that in all the instances both the experimental hybrids and QPM hybrids had significant sources of variation indicating availability of genetic differences in the groups. Comparisons were done both in the form of contrasts and use of least significant differences tests and the results were as follows:

1) The checks as a group performed significantly better than the group of experimental hybrids for grain yield but the best check variety was not significantly different from the top three best experimental hybrids though the best check yielded at least a ton higher than the best experimental hybrid.

2) The normal hybrids performed significantly better than the QPM hybrids for grain yield but the best normal endosperm maize hybrid was not significantly better than the top four best QPM hybrids though the yield difference was at least a ton with the normal endosperm maize hybrid on top.

3) The check hybrids were not significantly better than the experimental hybrids for kernel endosperm modification and several experimental hybrids performed better than checks and the best entry was the cross of CZL01006 to CZL03016 but it was not significantly different from the best check. However, amongst the checks, the best normal endosperm maize check was significantly better than the QPM check.

4) The normal endosperm maize hybrids were significantly better than the QPM hybrids for kernel endosperm modification but the genotype with the most desirable score was a QPM hybrid CZL01006/CZL03016.

7.3.4 Investigation of the adaptation of QPM genotypes for grain yield and kernel endosperm modification under different subtropical environments.

The findings from the AMMI ANOVA and biplots were as follows:

1) AMMI2 was suitable for grain yield and AMMI1 was suitable for kernel endosperm modification

2) The seven environments were diverse in their influence on the genotypes despite that they were created from the two cropping seasons and four sites using the location by year factor.

3) The genotypes and environments were more variable leading to high levels of interaction with Spearman Rank correlation coefficient of -0.61 ($p<0.0001$) for grain yield.

4) The best yielding genotypes according to AMMI1 biplot were SC721, SC633 and SC527 followed by the cross of CML144 to CZL03016 and all interacted positively with environment AR07 (ART farm 2007). However, in AMMI2 biplot, generally most of the genotypes were unstable and all were far from the origin and had specific adaptations to the different environments.

5) The lowest yielding genotypes interacted positively with environment KR08 (Kadoma Research Centre, 2008)

6) The most stable genotype with no specific adaptation for grain yield was the cross of CML176 to CML181f which was found at the origin in both AMMI1 and AMMI2 biplots.

7) The best genotype for kernel endosperm modification interacted positively with the AR07 (ART farm 2007) environment whilst the worst genotypes interacted positively with the KR08 (Kadoma Research Centre 2008) environment.

7.3.5 Determination of normal endosperm maize pollen contamination levels that can occur in QPM without loss of nutritional superiority

Normal endosperm maize pollen contaminated QPM was found to be nutritionally superior to normal endosperm maize maize grain but with different levels of superiority for different growing conditions as follows:

1) The relatively stress free environment of ART farm had a higher potential for nutritional value as indicated by the QI value of 0.915 compared to a QI value of 0.858 for the severely stressed CIMMYT Harare site.

2) The rate of nutritional value loss was both the same and significantly linear under the two different environments.

3) The level of normal endosperm maize pollen contamination that could be tolerated under a stressed environment (15.3% at CIMMYT Harare) was much lower than the level that could be tolerated by a relatively stress free crop (31.9%) at ART farm before total loss of nutritional superiority.

7.3.6 Estimation of the average levels and patterns of foreign maize pollen contamination in QPM crops coexisting with normal endosperm maize varieties.

The findings were as follows:

1) Kriging was successfully used to predict the spatial characteristics of the levels of contamination in QPM crops coexisting with normal endosperm maize crops.

2) The prediction maps indicated relatively higher levels of contamination at the edges and much lower levels at the central part of QPM crop fields.

3) The average contamination levels for all the QPM crops were below 20%, estimated using both arithmetic and area weighted averages.

4) Thus the contamination levels were far less than previously thought considering that this was a worst-case scenario based study

5) The highest levels of foreign pollen contamination were experienced in the direction of prevailing winds (The North East direction)

6) At least more than 50% of the crop in each experiment had pollen contamination levels below 15%.

7) In the two experiments (QCS200711 and QCS200712) grown at ART farm a relatively stress free environment, 87.9% and 94.8% of the crop area passed for QPM, respectively, based on thresholds from the study in Chapter 5. The stricter 95% lower confidence limit was used in determining the areas.

8) In the two experiments (QCS200721 and QCS200722) grown at CIMMYT Harare a relatively stressed environment, 62.2% and 65.6% of the crop area passed for QPM, respectively, based on thresholds from the study in chapter 5. The stricter 95% lower confidence limit was used in determining the areas.

7.4 Implications of findings to the breeding of QPM varieties

The findings from the PRA have paradigm shifting consequences on the breeding of both QPM and normal maize varieties for the smallholder farmers. The first point to make is that farmers indicated that they are knowledgeable and they know what they want in the context of their environments. Quality attributes other than protein content, tryptophan content, QI, kernel hardness and kernel endosperm modification that are emphasised in the breeding of QPM were perceived highly by the smallholder farmers. Thus in addition to the traditional QPM attributes breeders have to consider traits like kernel size, taste of products from the variety, colour of flour prepared from the variety, and resistance to weevils.

The demand by farmers for varieties with high yield potential, early maturity, tolerance to drought, resistance to foliar diseases and tolerance to pests in addition to the quality traits mentioned above gives the breeder a mammoth task in attempting to satisfy the varietal needs and preferences of the farmers. Some of the traits are already known to be negatively correlated in general such as high yield potential and early maturity. Combining these two traits with the other quality traits in the same breeding project makes the breeding process more complicated. In QPM it also seems that good modification, QI and grain yield have negative correlation. Thus the breeding process is expected to be more complex than before.

The other implication is that if QPM varieties are to be a success then smallholder farmers should be consulted throughout the breeding process so that products that are desirable to them are not sidelined in the process. Otherwise exclusion of smallholder farmers views in the breeding process risks the development of a variety that would not be acceptable to the farmers. Furthermore the partners that have positive influence on the farming communities such as AREX, Grain Marketing Board and nongovernmental organisations should also be involved in the QPM breeding and production process.

The findings from the experiment on combining abilities revealed that the set of inbred lines was diverse for the traits of interest in the development of QPM varieties. Specific combining ability effects were found to be very strong in the determination of yielding ability, and were also present in the determination of anthesis date and tryptophan content but to a lesser

extent. This meant that to be able to develop high yielding QPM varieties from this set of inbred lines, the best approach should be to develop hybrid varieties. The cross with the highest specific combining ability was CZL03016 to CML144 and this should be the starting point for developing high yielding QPM hybrids. If the specific combining ability for QI is repeatable across studies then it could be worthwhile to consider developing heterotic groups for QI.

On the other hand all the quality traits which include kernel endosperm modification, protein content, tryptophan content and QI had highly significant GCA effects. The traits can be improved by crossing complementary inbred lines and looking for transgressive segregants from the progenies. Reciprocal cross effects were significant but less important in anthesis days, but more important than SCA effects in tryptophan content and QI and, therefore, would need to be taken note of so that they do not inflate genetic effects such as SCA and GCA. Desirable scores for most kernel quality traits (protein content, tryptophan content and QI) were associated with inbred line CML264Q whilst the desirable score for kernel endosperm modification was found in CZL03016.

In the study on adaptation, the genotype with no specific adaptation was the cross of CML176 to CML181f and this cross should be central to the development of more genotypes with yield stability. The environments were diverse and it implied that when considered across seasons it was possible to get a set of widely varying environments for the screening of genotypes. On the other hand it also meant that genotypes need to be tested for relatively longer periods before certainty about their performance can be established.

The variation of nutritional potential as indicated by the QI of experimental variety C3728 for the two locations CIMMYT Harare and ART farm implied that there is need to adequately test the nutrition potential of new QPM varieties in both optimum and suboptimum growing environments before they are released to the farmers. The thresholds to foreign pollen contamination are not the same for optimum growing conditions and suboptimal conditions and so there is need to develop the tolerance levels of new varieties before they are released to the farmers.

If the rate of nutritional loss due to foreign pollen contamination is a constant for a variety (and also may be across varieties) then varieties can be screened for tolerance to foreign pollen contamination by establishing the maximum attainable QI values under optimum growing conditions. The cultivars with higher values would be the ones that can tolerate higher levels of contamination.

The average foreign pollen contamination levels for homogenous mixtures of grain from the different experiments were all below 20% under the worst-case scenario (crosspollination was encouraged to the maximum possible under natural conditions, spatial separation was the least possible for two distinct crops) for contamination. Therefore this implied that the contamination levels that can occur when there is some effort to prevent crosspollination like temporal or spatial isolation would be much lower. Therefore fear of loss of the QPM trait due to foreign pollen contamination alone should not be an obstacle to the adoption of QPM varieties.

The way forward in the development of QPM varieties for the smallholder farmers in this study would be to begin by collecting accessions of the different versions of “Hickory King” seed that is available in their communities. The accessions should be characterised in a study after which they can be used with the QPM germpalsm in this study to develop both improved QPM inbred lines, hybrids and improved open pollinated QPM varieties. Inbred recycling procedures and introgression of the “Hickory King” traits into the QPM germplasm or vice versa should precede the generation of QPM hybrids or QPM varieties.

The introgression of traits should take into account the merits and demerits of the studied inbred lines. Inbred line CML264Q would be a good source for imparting all the kernel quality traits (protein content, tryptophan content, and QI) except kernel endosperm modification which can be improved through using CZL03016 and CZL01006. The cross of CZL03016 and CML144 would be a good starting point to establish heterotic groups for yield with newly developed lines. Earliness to maturity can be obtained from CZL03016.

The evaluation of QPM test materials for both yield and quality traits (especially QI) should be done under both optimum and suboptimum conditions (representative of the farmer’s growing environments) so as to get the limits to the expression of the traits. The participation of smallholder farmers in the development of QPM varieties targeted for them should be encouraged at all stages so that the products that come out of the breeding efforts have relevance to their farming systems.

It seems there is no stakeholder interested in paying a premium for QPM. In order to encourage the adoption of QPM varieties, open pollinated varieties can be the starting point and local consumption of QPM should be encouraged. This is better compared to selling the QPM grain to marketing authorities where the QPM grain risks being mixed with normal endosperm maize grain potentially leading to the dilution of nutritional benefits.

It was found out from the study that “Hickory King” kernel quality attributes were perceived highly by the farmers. The nine inbred lines were genetically diverse for the studied traits and experimental hybrids that were comparable to normal endosperm maize checks could be obtained from the F_1 crosses among the inbred lines. The nutritional superiority of contaminated QPM grain was maintained up to 15.3% and 31.9% levels of normal endosperm maize pollen contamination for harsher and optimum growing environments, respectively. Normal endosperm maize and QPM crops can coexist without total loss of QPM nutitrional superiority.