Evaluation of South African high quality protein maize (*Zea mays* L.) inbred lines under optimum and low nitrogen conditions and the identification of suitable donor parents

By

Dimakatso Roselina Masindeni

Submitted in accordance with the requirements for the degree

Philosophiae Doctor

Faculty of Natural and Agricultural Sciences

Department of Plant Sciences (Plant Breeding)

University of the Free State

October 2013

Supervisor: Prof. M.T. Labuschagne

Co-supervisors: Dr. K. Mashingaidze and Dr. A. Van Biljon

Declaration

I declare that the thesis hereby handed in for the qualification Philosophiae Doctor in Agriculture at the University of the Free State is my own independent work and that I have not previously submitted the same work for a qualification at/in another university/faculty. I further concede copyright of the thesis to the University of the Free State.

Dimakatso Roselina Masindeni Date 25/11/2013

Dedication

I dedicate this work to my loving husband (Eric Ndou) and my lovely daughter (Thivhonali Ndou). My mother (Thokozile Getrude Masindeni) and grandmother (Roselina Phelele Masindeni) for all the sacrifices you have made in giving me a good solid foundation for a better future. My late mother in-law (Thivhonali Mmbadi) for all the sacrifices you have made in raising my husband. My late uncle in-law (Thinanungo Mmbadi) and my late grandfather (Elliot Lemosa Masiteng), I am grateful to your fatherly love and guidance, which carried me through to where I am today.

Acknowledgements

First I give honour, glory and praises to the Almighty GOD. Thank you for blessing my life and for the strength and wisdom you gave me to complete this study. Psalm 23.

Prof. Maryke Labuschagne, thank you for all your inputs, guidance and assistance with some of the statistical analyses and for your constant supervision and careful reviewing of the thesis. I am deeply grateful to you for providing helpful suggestions and comments on the draft documents leading to this thesis. Dr. Kingstone Mashingaidze, thank you for encouraging me to do the PhD, believing that I can make it, and for assisting with the trial implementation when I left the ARC-GCI. Your suggestions, inputs and guidance have made execution of this study and thesis write up practicable. Dr. Angie Van Biljon, thank you for showing me how to do the tryptophan analysis, helping me out when I was sick, and for carefully reviewing the thesis. I am very much indebted to you all for your patience, understanding and love throughout the rest of my study. I do not know what I would have done without your help and support.

Ms. Sadie Geldenhuys, thank you for the administration support and words of encouragements you provided throughout the course of my study, it really meant a lot to me. Dr. Lewis Machida thanks for all the suggestions, inputs and encouragements during my visit in Zimbabwe. Mr. Nicki Landman thanks for helping with tryptophan analyses. My fellow students who were present at the university during my laboratory analysis and thesis write up, Mr. AbduRahman Beshir, Dr. Thoko Ndhlela, Mr. Elliot Tembo, Mr. Fortunus Kapinga, Ms. Mmapaseka Malebana and Dr. Joyce Moloi thanks for inputs, words of encouragement and great Ethopian coffee, and for showing me how to do GGE biplots, it was not easy but it helped to have you beside me.

I am indebted to the National Research Foundation and ARC-GCI for funding the project. My appreciation goes to the ARC-GCI maize breeding team: Mr. Piet Hansa, Mr. Eric Ndou, Mr. Willy Ratladi, Ms. Lieketso Moremoholo, Ms. Jabulile Mkhatshwa, Ms. Hendrieta Moletsane, Mr. Popi Malgas, Ms. Maria Tlhagale, the interns and many casual workers for all the hard work during planting, harvesting and data collection of the trials.

I am deeply grateful to the Limpopo Department of Agriculture my current employer for providing me with financial support during implementation of the Limpopo trials and allowing me time off to complete my research study at the university and ARC-GCI; with special thanks to Prof. Edward Nesamvuni, Dr. Konanani Liphadzi, Mr. Richard Ramugondo and Dr. David Nthakheni for their encouragement and support. Special thanks also goes to Mr. Shadrack Batsile Dikgwatlhe who is a colleaque and great friend, for assisting in implementation and management of the Limpopo research trials while I was on maternity leave and not forgetting the help received from Mr. Richard Ngwepe, Mr. Chris Sonnekus, Ms. Mpolokeng Mokoeana, Mr. Charles Khorommbi and all farm workers at Towoomba and Tshiombo research stations during planting, harvesting and data collection. Further appreciation goes to other colleaques especially Mr. Gabriel Lekalakala, Ms. Charlotte Ledwaba, Ms. Susan Mashego and Ms. Nkateko Shihlomule for all your help, while I was away on study leave.

Further appreciation goes to all the friends I made at the ARC-GCI, Limpopo Department of Agriculture and during my stay in Bloemfontein, Ms. Lesego Kok-Ngonyama, Ms. Ndishavha Manari, Mr. Shadrack Dikgwatlhe, Ms. Makhotso Lekhooa, Ms. Beaulah Millicent Kruger, Ms. Sanari Moriri and Mr. Thifhindulwi Malala, for all your help, encouragement and prayers during the course of my study with special thanks to the Ngonyama and Manari families for welcoming me into your homes.

The Masindeni/Masiteng family (mother, grandmother, sisters, brother, cousin, nieces and nephews) thank you for your prayers, love and support. To all my in-laws, especially Vho-Mudzunga, Vho-Ndidzeni and Ntsundeni: *“ndi a livhuwa ngamaanda”*, for everything.

Last but not least, to my husband and daughter thank you for being my rock. Eric my love your patience, understanding, guidance, inputs, encouragement, support and love gave me the strength not to give up hope, in tough times. I am grateful to you for being a mother and father to our daughter throughout my study. I am glad to know I can always count on you, honestly I do not think I would have pulled it off without you. Thivhonali my angel,

sorry I was not always there for you during your early childhood, especially when you went through tough times (the hand burn) in your life. I hope what I achieved here will make you to be proud of me and I can make up for the time lost. I love you guys very much.

Table of contents

List of tables

List of figures

Abbreviations and symbols

ADT	Adaptability
AEA	Average environment axis
AMMI	Additive main effects and multiplicative interaction
ANOVA	Analysis of variance
ARC-GCI	Agricultural Research Council-Grain Crops Institute
CH	Cedara optimum nitrogen
CIMMYT	International Maize and Wheat Improvement Center
CL	Cedara low nitrogen
cm	Centimetre(s)
CML	CIMMYT maize line
CP	Cross-pollination
CPD	Cross-pollination differences
Cum	Cumulative
CV	Coefficient of variation
EH	Endosperm hardness
Env	Environment
F_1	First filial generation
F_2	Second filial generation
FAO	Food and Agriculture Organisation of the United Nations
G	Gram(s)
G	Genotype
GCA	General combining ability
GEI	Genotype and environment interaction
GGE	Genotype and genotype by environment interaction
ha	Hectare(s)
HG	Heterotic groupings
IPCA	Interaction principal component analysis
Kg ha^{-1}	Kilogram per hectare
KZN	KwaZulu-Natal

L. ha^{-1}	Litre per hectare
L x T	Lines x Testers
LAN	Lime ammonium nitrate
LowN	Low nitrogen
LP	Limpopo
LSD	Least significant difference
LT	Lowland tropics
M	Metre(s)
masl	Metres above sea level
Max	Maximum
MDG	Millennium Development Goals
mha	Million hectares
Min	Minimum
ml	Millilitre
mm	Millimetre(s)
MS	Mean squares
N	Nitrogen
NAM	Nested association mapping
NRC	National Research Council
ns	Non-significant
NW	Northwest
OptN	Optimum nitrogen
OPVs	Open-pollinated varieties
PCA	Principal component analysis
PH	Potchefstroom optimum nitrogen
PL	Potchefstroom low nitrogen
QI	Quality index
QPM	Quality protein maize
R	Cross fertilisation effect
RCPE	Relative cross fertilisation effect
RDA	Recommended daily allowance

Rep in E	Replication in environment
Rn	Average total rainfall
S	Milligrams of sample
SA	South Africa
SAGL	Southern African Grain Laboratory
SCA	Specific combining ability
SP	Self-pollination
SREG	Site regression
ST	Subtropics
STTW	Subtropical temperate warm
Syn	Synthetic
T	Average temperature
t ha^{-1}	Ton per hectare
Tn	Average minimum temperature
Tryp	Tryptophan
TSH	Tshiombo optimum nitrogen
TSL	Tshiombo low nitrogen
TWH	Towoomba optimum nitrogen
TWL	Towoomba low nitrogen
Tx	Average maximum temperature
Var	Variation
%	Percent
o2	Opaque-2
^{o}C	Degrees celcius

Chapter 1

General introduction

Maize (*Zea mays* L.) is the most widely used cereal grain for both human and animal nutrition, throughout the world. In 2011 it was the most produced grain crop with a production of 883.46 million ton (FAOSTAT, 2011). Overall the United States was the highest producer with South Africa (SA) ranking 9th in the world, thus being the African continent's biggest producer. Maize is a staple food crop for the majority of the population in SA and it is ranked first in terms of production, yield and consumption. The crop is produced by both commercial and subsistence farmers throughout the country, with the Free State (41.7%), Northwest (27.2%) and Mpumalanga (18.6%) province contributing the majority of the produce in 2010 (DAFF, 2011a). The crop is used for both human food and livestock feed. During 2011 the production and consumption was 10.68 and 9.96 million ton, respectively (BFAP, 2011; DAFF, 2011b). Consumption trend of maize between 2005 and 2011 shows a steady increase from 8 to 10 million ton (USDA GAIN, 2012), which shows the importance of the crop in the country. The country produces both white and yellow maize; with yellow maize mostly fed to animals. Currently about 50% of the white maize is for human consumption, while 40% and 10% are for animal consumption and industrial uses, respectively.

Maize is an important source of proteins and carbohydrates and it plays a vital role in the diet of humans and accounts for about 50-60% of the dietary protein in poor communities (Showemimo, 2004). Dietary protein is the primary source of the nine essential amino acids and provides nitrogen (N) for the synthesis of the 11 non-essential amino acids. Although it serves as an important source of proteins and carbohydrates, it has limited amounts of two amino acids, tryptophan and lysine, that are essential for human growth and development (Bressani, 1992; Knabe *et al.*, 1992; Vasal, 1999). Lack of these essential amino acids usually results in malnutrition, which mostly has a negative effect on young children, and women who are pregnant or lactating (Pixley and Bjarnason, 2002). In Africa malnutrition is prevalent and more than 10% of children younger than

five years were undernourished between the years 2005 and 2008 in most countries (FAO, 2008). Improving nutritional quality of maize is the best way to reduce the occurrence of malnutrition diseases such as pellagra and kwashiorkor (NRC, 1988).

Maize protein quality research has received relatively little attention from breeders, mainly due to lack of funding and the difficulty involved in breeding for improved nutritional quality. Research on maize nutritional quality started in the early 1960's when a soft opaque-2 maize mutant was discovered (Vietmeyer, 2000). Opaque-2 maize was found to have an *o2* gene that results in high lysine and tryptophan content (Mertz *et al.*, 1964). The maize with high lysine looked and tasted just like normal maize though the yields were about 10% lower. It was also susceptible to pests and diseases due to its soft chalky endosperm and inability to dry quickly (Vietmeyer, 2000; Krivanek *et al.*, 2007). During those periods efforts were made mainly by the International Maize and Wheat Improvement Center (CIMMYT) researchers to focus on maize nutrition. Drs. Evangelina Villegas and Surinder Vasal around 1986 managed to develop a better product from the soft opaque maize with good agronomic traits and high lysine and tryptophan content. They named this product quality protein maize (QPM) because amongst other things it had improved nutritional quality, and performed better or comparable to normal maize with regard to yield, appearance, and disease and pest resistance (Vasal *et al.*, 1993a; CIMMYT, 2000; Vasal, 2000; Vietmeyer, 2000; Krivanek *et al.*, 2007; Vivek *et al.*, 2008). These researchers won the World Food Prize in 2000 in recognition of their accomplishments.

Compared to normal maize, QPM has about 70-100% more lysine and tryptophan (Bressani, 1991; 1992; Prasanna *et al.*, 2001; Sofi *et al.*, 2009). The protein's biological value is associated with the digestibility and metabolism of the essential amino acids it contains. The amount of protein needed in the diet to supply the essential amino acid requirements is small when the protein's biological value is high. QPM's biological value is very high and has been found to be comparable to cow's milk. It was found that children fed QPM showed the same growth as those fed modified cow milk formula (Graham *et al.*, 1990). Superior grain protein quality is only expressed when endosperm

tissue is homozygous recessive (*o2o2*) (NRC, 1988). The increased tryptophan and lysine content can be better utilised because of the improved essential amino acid balance (Pixley and Bjarnason, 2002; Mbuya *et al.*, 2011). QPM cultivars can help to reduce protein deficiencies in areas where maize forms a large proportion of the diet (Bressani, 1992). Many researchers have reported recovering of children with malnutrition when fed with QPM (Graham *et al*., 1980; NRC, 1988; Akuamoa-Boateng, 2002) and increased weight in animals (Gevers, 1974; Onimisi *et al.*, 2009).

The development of QPM was a great effort to counteract the effects of malnutrition in areas where maize is a staple. Several organisations have been actively involved in development of maize with improved nutritional quality. CIMMYT has been the most dedicated in terms of resources allocated to research and progress made thus far, even during the times when other organisations abandoned research on improving maize nutrition. Their researchers have developed a number of QPM inbred lines, hybrids and open-pollinated varieties which are used throughout the world today and are making a great impact in many breeding programmes. In SA, the Agricultural Research Council's Grain Crops Institute (ARC-GCI) carried out most of the breeding which resulted in the development of maize with improved quality during the same period as CIMMYT; however the research stopped in 1995 and was restarted in 2005. During both periods quite a number of maize genotypes with high lysine and later with added improved kernel hardness were developed, tested and released, while other germplasm were introduced into the country to improve the breeding programme. It is evident today that the development of QPM was an important research milestone which brought hope in the fight against malnutrition (Batik, 2000; Vietmeyer, 2000).

QPM was only grown by four countries in 1977 (Sofi *et al.*, 2009) and by 2000 about 11 countries; and it was estimated that the area would increase from 1 to 3.5 million hectares (mha) in 2003 (Batik, 2000). The economists' expectations were met because in 2003 there were more than 23 countries growing QPM on an area of more than 3.5 mha (Sofi *et al.*, 2009). The area of QPM production in sub-Saharan Africa is around 200 000 ha with about 17 countries growing the crop. QPM is very popular in Ghana and Uganda and this

is reflected by the area currently allocated to the crop at 71 250 and 46 717 ha, respectively (Krivanek *et al.,* 2007). In SA the production of QPM is mainly done by small-scale farmers who mainly grow the crop for subsistence. Currently there is little or no information on studies that have been done on QPM germplasm for genotype and environment interaction (GEI), stability in low N conditions, donor identification, and pollination effects in SA and worldwide.

Maize variety trials are mainly planted in open pollinated fields and usually pollen from one maize plant can have an immediate effect on the yield and quality traits of the other plants. The immediate effect sometimes referred to as xenia, usually results in increased yield, weight, seed size or is shown by an immediate colour change. Abdulai (2005) reported an increased seed size up to 1.97 g per 100 kernels from a large seeded population and a decrease in seed size up to 9.88 g per 100 kernels from a small seeded population compared to 30.95 g per 100 kernels from open pollinated hybrids in a cross-pollination experiment. This research project is important because most of the studies on pollination effects were done on normal maize with limited information on QPM.

In SA, the contribution by small-scale farmers to the total maize production is small mainly due to size of the area allocated to the crop and the stressful conditions the crops encounter. Abiotic stress is the most harmful factor affecting the growth and productivity of crops worldwide. The two most important abiotic stress factors limiting maize production are poor soil fertility and drought (Lafitte and Edmeades, 1988; Beck *et al.,* 1996; Bänziger and Lafitte 1997; Bänziger and Diallo 2004).

Poor soil fertility caused by limited use of N fertilisers by farmers does not only affect yield and related traits in maize, it also has a negative effect on the protein quantity and quality. The use of fertilisers is limited amongst small-scale farmers generally because of its high cost, and these impacts on food security and economic growth. Therefore to imitate the environments in which farmers grow their maize, a method was developed whereby N is depleted in the soil so that researchers can test newly developed materials and select promising lines which can perform well under farmers' conditions (Bänziger and Lafitte, 1997). Poor soil fertility in QPM has been found to affect yield, protein,

endosperm and the tryptophan in grain and protein. According to Ngaboyisonga (2008) N deficiency reduced protein quantity, the levels of tryptophan in the grain and endosperm modification of QPM inbred lines by 29%, 20% and 75%, respectively. He concluded that QPM germplasm planted under N and water deficiency conditions are likely to be rejected for human consumption because of the higher proportion of soft and chalky kernels present. The reason for rejection is based mainly on the fact that the kernels are easily damaged by pests which results in yield reduction. In SA soft grain would also be rejected by millers because of low extraction rates. That is why it is important to test QPM lines under stress environments and select good germplasm to use in the development of superior products that will be available for use by small-scale farmers in SA.

Maize germplasm is grown in a wide array of environments in the world, though in most cases, when tested across several locations it encounters GEI. It is a restricting phenomenon to breeders; because it results in germplasm performance differences from one environment to another and reduces genetic progress in plant breeding programmes (Kaya *et al.,* 2006). This phenomenon, when it is significant, results in the need to evaluate the germplasm for stability in different environments. Maize researchers in SA have dedicated a lot of research time on stability of agronomic traits. QPM research, on the other hand, has concentrated mostly on stability of both agronomic traits and protein and endosperm quality traits in stress and optimum environments. However currently there is more information documented on agronomic traits (Vasal *et al.,* 1993b; Pixley and Bjarnason, 2002; Gissa 2008; Machida, 2008; Ngaboyisonga, 2008) than protein and endosperm quality traits (Vasal *et al.,* 1993b; Pixley and Bjarnason, 2002; Ngaboyisonga, 2008).

When developing QPM varieties, three or more donors are often used in order to convert normal maize germplasm to QPM (Vivek *et al.,* 2008). Presently, the ARC-GCI has QPM germplasm, however there is still little information on which specific QPM inbred lines to use in the breeding programme, resulting in the current use of three donors when converting normal maize germplasm. QPM inbred lines vary in the levels of tryptophan

concentration and kernel modification, and this usually has an impact on the way germplasm is converted. It is important to separate QPM germplasm into different classes in terms of their ability to transfer tryptophan and kernel traits during the conversion process. Researchers in CIMMYT managed to classify some of the QPM inbred lines in three categories which are poor, moderate and good for tryptophan content and agronomic traits, while other researchers have documented studies on QPM donors (Gissa, 2008; Vivek *et al.,* 2008; Machida, 2008). Some of the CIMMYT germplasm were included in this study to see how they react when used as donors to normal South African maize recipients and their reaction to different environments.

This study is important to the QPM breeding programme in SA since QPM donors have not been classified in the ARC-GCI germplasm. This information will be useful to breeders as it will reduce resources needed to convert normal lines into QPM lines. Thus the total number of QPM donors used to convert normal maize genotypes to QPM will be reduced by identification of good QPM donors. The good QPM donors identified will be readily available to be used in the breeding programme to develop QPM varieties in a cost effective manner. Studies on QPM hybrids were done for grain yield and grain quality traits (Machida, 2008; Ngaboyisonga, 2008; Machida *et al.,* 2010; Mutimaamba *et al.,* 2010). Few of the studies were looking at grain yield and grain quality traits.

Research objectives

The objectives of this study were as follows:

(1) To investigate the effect of pollen parents on tryptophan concentration in QPM inbred lines;

(2) To analyse GEI and do stability analysis of QPM inbred lines for kernel hardness, protein, tryptophan, oil and starch concentration under low and optimum N conditions;

(3) To compare the performance of QPM genotypes to normal maize genotypes for grain quality traits and investigate the relationship between grain traits under low and optimum N conditions; and

(4) To estimate general combining ability and specific combining ability of South African QPM inbred lines and the identification of good donors for grain quality traits.

References

Abdulai MS (2005) Effect of pollen source on seed size of hybrid maize. Ghana Journal of Agricultural Science 1: 187-191.

Akuamoa-Boateng A (2002) Quality Protein Maize: Infant Feeding Trials in Ghana. Ghana Health Service, Ashanti, Ghana, pp. 1-17.

Bänziger M and Diallo AO (2004) Progress in developing drought and N stress tolerant maize cultivars for eastern and southern Africa. In: DK Friesen and AFE Palmer (Eds.), Proceedings of the 7th Eastern and Southern Africa Regional Maize Conference, 5-11 February 2002. CIMMYT/KARI, Nairobi, Kenya, pp. 189-194.

Bänziger M and Lafitte HR (1997) Efficiency of secondary traits for improving maize for low nitrogen target environments. Crop Science 37: 1110-1117.

Batik RA (2000) Villegas and Vasal win world food prize for developing quality-protein maize. Diversity 16: 2.

Beck D, Betran FJ, Banziger M, Ribaut JM, Willcox M, Vasal SK and Ortega A (1996) Progress in developing drought and low soil nitrogen tolerance in maize. In: D Wilkinson (Eds.), Proceedings of 51st Annual Corn and Sorghum Research Conference, Chicago, Washington, pp. 85-111.

BFAP (2011) The South African agricultural baseline. Bureau for Food and Agricultural Policy. South Africa, pp. 13.

Bressani R (1991) Protein quality of high-lysine maize for humans. Cereal Foods World 36: 806-811.

Bressani R (1992) Nutritional value of high lysine maize in human. In: ET Mertz (Eds.), Quality protein maize. American Association of Cereal Chemists, St. Paul, Minnesota, USA, pp. 205-224.

CIMMYT (2000) CIMMYT in 1999-2000. Science and Sustenance. Mexico, D.F.: CIMMYT, 74 pp.

DAFF (2011a) Crops and Markets. Available on the internet from: http://www.daff.gov.za/docs/statsinfo/CropMarketsQ2_2011.pdf/ [Date Accessed: 08 August, 2012].

DAFF (2011b) Trends in Agricultural Sector. Available on the internet from: http://www.nda. agric.za/docs/statsinfo/Trends_2011.pdf/ [Date Accessed: 08 August, 2012].

FAO (2008) Available on the internet from: http://www.fao.org/hunger/en/ [Date Accessed: 08 August, 2012].

FAOSTAT (2011) Available on the internet from: http://www.faostat.fao.org/site/567/ [Date Accessed: 29 January, 2013].

Gevers (1974) The development of high lysine maize. In: JG Du Plessis, CO Crogan, HC Kuhn and MC Walters (Eds.), Proceedings of the first South African Maize Breeding Symposium, Highveld Region, Potchefstroom, South Africa, pp. 18-27.

Gissa DW (2008) Genotypic variability and combining ability of quality protein maize inbred lines under stress and optimal conditions. PhD Thesis, University of the Free State, Bloemfontein, South Africa.

Graham GG, Glover DV, De Romaña GL, Morales E and MacLean WCJ (1980) Nutritional value of normal, opaque-2 and sugary-2 opaque-2 maize hybrids for infants and children. 1. Digestibility and utilization. Journal of Nutrition 110: 1061-1069.

Graham GG, Lembcke J and Morales E (1990) Quality protein maize and as the sole source of dietrary protein and fat for rapidly growing young children. Pediatrics 85: 85-91.

Kaya Y, Akçura M and Taner S (2006) GGE-Biplot analysis of multi-environment yield trials in bread wheat. Turkish Journal of Agriculture 30: 325-337.

Knabe DA, Sulliven JS, Burgoon KG and Bockholt AJ (1992) QPM as a swine feed. In: ET Mertz (Eds.), Quality protein maize. American Association of Cereal Chemists, St. Paul, MN, USA, pp. 225-238.

Krivanek, AF, De Groote H, Gunaratna NS, Diallo AO and Friesen D (2007) Breeding and disseminating quality protein maize (QPM) for Africa. African Journal of Biotechnology 6: 312-324.

Lafitte HR and Edmeades GO (1988) An update on selection under stress: Selection criteria. In: B Gelaw (Eds.), Second Eastern, Central and Southern African Regional Maize Workshop. The College Press, Harare, Zimbabwe, pp. 309-331.

Machida L (2008) 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. PhD Thesis, University of KwaZulu-Natal, Pietermaritzburg, South Africa.

Machida L, Derera J, Tongoona P and MacRobert J (2010) Combining ability and reciprocal cross effects of elite quality protein maize inbred lines in Subtropical environments. Crop Science 50: 1708-1717.

Mertz ET, Bates LS and Nelson OE (1964) Mutant gene that changes protein composition and increases lysine content of maize endosperm. Science 145: 279-280.

Mbuya K, Nkongolo KK and Kalonji-Mbuyi A (2011) Nutritional analysis of quality protein maize varieties selected for agronomic traits in a breeding program. International Journal of Plant Breeding and Genetics 5: 317-327.

Mutimaamba C, Lungu D and MacRobert J (2010) Combining ability analysis of quality protein maize (QPM) and non-QPM inbred lines for kernel quality and some agronomic characteristics. Second RUFORUM Biennial Meeting 20-24 September 2010, Entebbe, Uganda.

NRC (1988) Quality Protein Maize. National Academy Press, Washington, D.C. USA, pp. 41-54.

Ngaboyisonga C (2008) Quality protein maize under stress environments: gene action and genotype × environment effects. PhD Thesis, University of Nairobi, Kenya, Nairobi.

Onimisi PA, Omage JJ, Dafwang II and Bawa GS (2009) Replacement value of normal maize with quality protein maize (*Obatampa)* in broiler diets. Pakistan Journal of Nutrition 8: 112-115.

Pixley KV and Bjarnason MS (2002) Stability of grain yield, endosperm modification and protein quality of hybrid and open-pollinated quality protein maize (QPM) cultivars. Crop Science 42: 1882-1890.

Prasanna BM, Vasal SK, Kassahun B and Singh NN (2001) Quality protein maize. Current Science 81: 1308-1319.

Showemimo FA (2004) Analysis of divergence for agronomic and nutritional determinants of quality protein maize. Tropical and subtropical Agroecosystems 4: 145-148.

Sofi PA, Wani S, Rather AG and Wani SH (2009) Quality protein maize (QPM): Genetic manipulation for nutritional fortification of maize. Journal of Plant Breeding and Crop Science 1: 244-253.

USDA GAIN (2012) South Africa Grain and Feed Annual Report. Global Agricultural Information Network Report, 16 February 2012. Available on the internet from: http://gain.fas.usda.gov/Recent%20GAIN%20Publications/Grain%20and%20Feed%20Annual_Pretoria_South%20Africa%20-%20Republic%20of_2-16-2012.pdf [Date Accessed: 08August, 2012].

Vasal SK (1999) Quality Protein Maize Story. In: Improving Human Nutrition Through Agriculture: The Role of International Agricultural Research: International Food Policy Research Institute Workshop held at the International Rice Research Institute, October 5-7, Los Banos, Philippines. Available on the internet from: http://www.ifpri.cgiar.org/sites/default/files/publications/vasal.pdf [Date Accessed : 08 August, 2012].

Vasal SK (2000) The quality protein maize story. Food and Nutrition Bulletin 21: 445-450.

Vasal SK, Srinivasan G, Pandey S, Gonzalez FC, Crossa J and Beck DL (1993a) Heterosis and combining ability of CIMMYT's quality protein maize germplasm: I. Lowland tropical. Crop Science 33: 46-51.

Vasal SK, Srinivasan G, Pandey S, Gonzalez FC, Crossa J and Beck DL (1993b) Heterosis and combining ability of CIMMYT's quality protein maize germplasm: II. Subtropical. Crop Science 33: 51-57.

Vietmeyer ND (2000) A drama in three long acts: The story behind the story of the development of quality-protein maize. Diversity 16: 29-32.

Vivek BS, Krivanek AF, Palacios-Rojas N, Twumasi-Afriyie S and Diallo AO (2008) Breeding Quality Protein Maize (QPM): Protocols for Developing QPM Cultivars. Mexico, D.F.: CIMMYT, 50pp.

Chapter 2

Literature review

2.1 Maize: Importance, chemical composition and nutritional value

In SA maize constitutes about 70% of grain production and covers about 60% of the cropping area (Akpalu *et al.,* 2009). It is a staple food to the majority of the rural communities, with a *per capita* consumption of more than 100 kg per year, providing 22.7 g protein and a total of 889 kcal per day (FAOSTAT, 2009). Many of these communities rely on protein from maize and other plant sources as they cannot afford animal protein. South Africa is ranked third after Lesotho and Zimbabwe in maize *per capita* consumption.

The Southern African Grain Laboratory (SAGL, 2011) reported that South African maize on average contains 3.9% oil, 8.7% protein and 72.1% starch. The value ranges over years between 2.8-5.8%, 6.1-12.7% and 58.3-77.0% for oil, protein and starch content, respectively (Table 2.1). The nutritional quality of normal maize is extremely poor in some of the essential amino acids when it is compared to QPM. FAO (1992) reported that opaque-2 maize and QPM had 96.8% and 82.1% casein compared to 32.1% casein in normal maize. Protein of QPM in general contains about 50% and 30% more tryptophan and lysine, respectively, than normal maize (Prasanna *et al.,* 2001).

QPM looks and performs equal or better than normal maize for endosperm hardness, yield and chemical composition, except that normal endosperm is deficient in some essential amino acids. Duarte *et al.* (2004) studied QPM hybrids, obtained through conversion of normal inbred lines, together with elite QPM hybrids plus normal endosperm maize and observed that all the QPMs performed better for protein quality and similar for grain yield relative to normal endosperm maize. Converted QPM hybrids also had lower grain density relative to the normal version. According to the FAO/WHO (1991) the reference level for tryptophan in QPM maize is 1.1 g 100 g^{-1}, while Vivek *et al.* (2008) indicated a threshold value of 0.075% when analysing the whole grain.

Table 2.1 Chemical composition of South African maize from 2001/02 to 2010/11

Year	Oil%			Protein%			Starch%		
	Av	Min	Max	Av	Min	Max	Av	Min	Max
2001/02	4.2	3.0	5.5	8.9	6.7	11.6	71.5	58.3	74.7
2002/03	4.1	3.0	5.4	9.2	7.2	11.7	71.6	62.5	75.9
2003/04	4.0	3.5	4.6	9.1	7.9	10.2	71.1	70.2	72.6
2004/05	3.9	2.9	4.7	8.8	6.5	12.0	71.3	68.9	74.3
2005/06	4.0	3.2	5.0	8.4	6.4	10.4	71.2	69.5	73.4
2006/07	3.7	2.8	4.8	9.4	6.9	12.7	73.0	70.1	75.2
2007/08	3.8	2.9	4.8	8.5	6.6	10.9	72.1	69.9	75.0
2008/09	3.8	2.9	5.1	8.3	6.2	10.6	72.7	70.7	74.8
2009/10	4.0	3.3	5.8	8.3	6.5	10.1	72.9	70.6	75.4
2010/11	3.9	2.8	4.6	7.9	6.1	9.8	73.8	71.9	77.0

Av=Average; Min= Minimum; Max=Maximum (SAGL, 2011)

2.2 Malnutrition and QPM benefits

Malnutrition refers to insufficient (under-nutrition), excessive (over-nutrition) or imbalanced consumption of one or more nutrients resulting in under-nutrition or over-nutrition (UNICEF, 2006). Protein energy deficiency which relates to insufficient and inadequate intake is one of the most important forms of malnutrition that is common in Africa (Maletnlema, 1992; WHO, 1999). The biggest problem facing Africa is that the majority of people are consuming large amounts of cereals, especially maize, as staple foods without adequate supplementation with other protein sources. Malnutrition affects most people living in rural areas, who are poor and rely mostly on maize, that has low levels of tryptophan and lysine, for food. South Africa is no exception to this problem, because malnutrition is mostly encountered in rural areas where the poorest of the communities are located. Improvement of maize nutrition is an important foundation to assist in the fight against malnutrition. During the year 2000 at the Millennium Summit, 189 countries including 147 heads of state and governments signed the Millennium Development Goals (MDGs) declaration and committed themselves to reduce malnutrition by 2015 (MDG, 2011). However, in 2010 most countries were still far from reaching their MDGs. Ghana, Cameroon and Ethiopia have made a lot of progress in reaching the MDGs, with most of the developing countries still lagging behind (MDG, 2011). In 2010 the estimated number of undernourished people was around 925 million,

with developing countries accounting for 98% of this figure (Figure 2.1; FAO and WFP, 2010). For sub-Saharan Africa, where maize is a staple food, about 239 million people are affected, with Asia and the Pacific having the largest number of undernourished people.

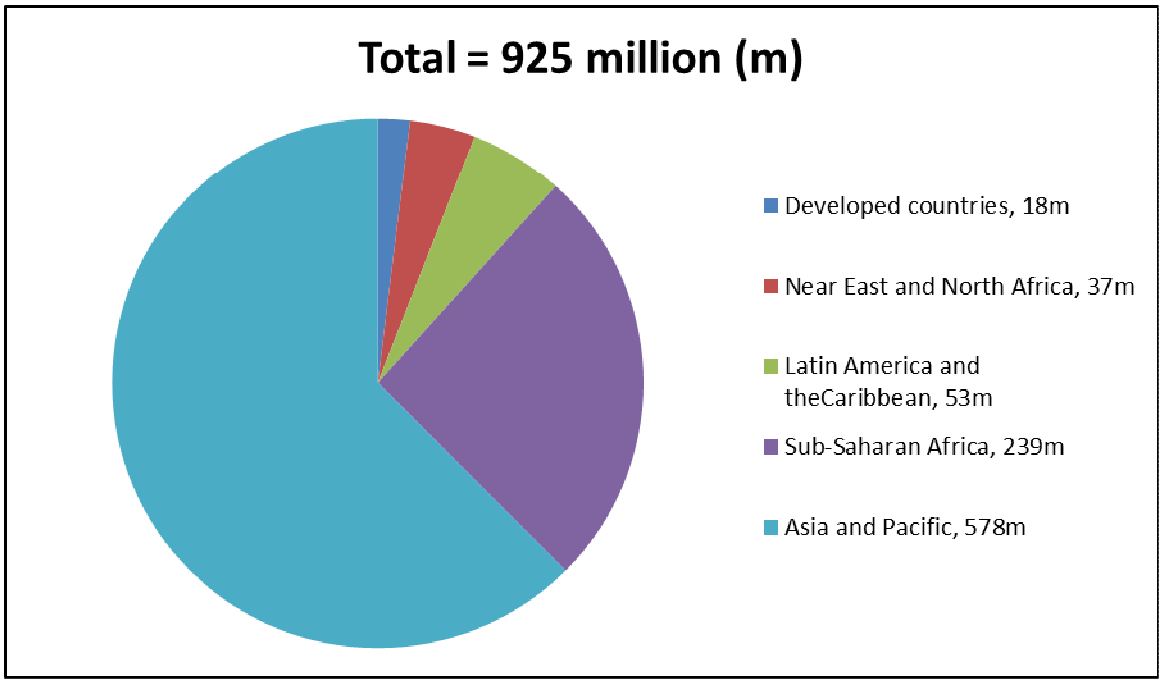

Figure 2.1 Estimation of undernourished people in the world (FAO and WFP, 2010)

Children under five years of age in the developing countries are 13% more likely to die than those in developed countries, and sub-Saharan Africa accounts for about half the deaths of those children in the developing world (UNDP, 2008). Yearly about 530 000 women die due to maternal death and 99% of these deaths occur in the developing world, with 56% of the deaths in sub-Saharan Africa (WHO, 2012). Malnutrition is one of the factors that increase the incidence of maternal deaths by 80%. The statistics of the years 2006 to 2010 show that in SA about 9%, 5% and 24% of under-fives were suffering from moderate and severe underweight, wasting and stunting respectively, according to the World Health Organisation (UNICEF, 2010). UNICEF (2012) reported that under-nutrition contributes to more than a third of the under-five's deaths globally. Still, under these circumstances, the use of bio-fortified foods that can assist in the reduction of these conditions is limited or does not exist in most developing countries. Increasing the levels of tryptophan and lysine is thus essential for improving the livelihoods of people affected by protein deficiencies.

Several researchers have been involved in determining the impact of QPM in human and animal nutrition (Graham *et al.,* 1989; Akuamoa-Boateng, 2002; Onimisi *et al.,* 2009; Sofi *et al*., 2009; Mbuya *et al.,* 2011). According to Graham *et al.* (1980; 1990) the energy and protein needs of infants and children can be adequately met with a diet exclusively based on QPM. Clark *et al.* (1977) reported that children who were suffering from Kwashiorkor and fed a diet with opaque-2 maize, recovered. According to Graham *et al.* (1980) QPM, with regard to nurturing growth in recovering malnourished children, is 50% more effective than normal maize. Bhargava *et al.* (2003) assessed the nutritional quality of QPM and normal maize in pre-school children aged between 1-3 years old. The diet had one third of recommended daily allowance (RDA) of calories and protein, with skimmed milk included as a control measure. QPM based diets were better than the control. Children fed with a QPM based diet gained 117% weight compared to those fed with normal milk based and standard diet, who gained 112% and 43% weight respectively. It was also reported that the height, head and mid-arm circumferences of the children fed on the QPM based diet were better than the group fed on a skimmed milk diet. Nuss and Tanumihardjo (2011) indicated that consuming QPM results in a 40% reduction in maize intake to meet protein requirements when compared to normal maize. Their study found that approximately 100 g QPM is required for children to maintain adequacy of lysine, while for adults nearly 500 g is required. Krivanek *et al.* (2007) indicated that the effect of using QPM in animal feed can easily be calculated in monetary terms since QPM can provide a cheaper alternative than normal maize in obtaining balanced animal feeds. Gevers (1995) highlighted that QPM silage in SA may have an economical and nutritional value as compared to normal maize in the feeding of dairy animals.

QPM can be an important tool that can assist in reaching the MDGs number one, four and five that deal with halving hunger by reducing undernourishment to less than 5% of the population, improving the lives of young children affected by malnutrition by reducing the child mortality and under-nutrition levels; and finally improving maternal health. Developing countries which are behind in reaching millennium goals can learn a lot from Ghana which is growing QPM comprising 90% of seed sales since its inception (Sallah *et al.,* 2003), and incorporating QPM in their maize diet in order to reduce child mortality, maternal death and hunger. QPM is not a cure to everything but can be used as one of the

tools, which help when used with other methods. Therefore, any efforts by society to either alleviate poverty or improve the nutritional value of crops should be supported by national governments and businesses.

2.3 Nitrogen deficiency

2.3.1 Low nitrogen in African soils and factors affecting use of fertilisers

Poor soil fertility in Africa is a widespread problem, especially as more land is being cultivated for crop production (Henao and Baanante, 1999). N deficiency occurs mainly due to unavailability or low N availability in the soil and it has been cited as an environmental stress reducing endosperm hardness and protein quality in QPM (Gissa, 2008). Cultivation of crops results in a significant reduction of N in the soil (Prinsloo, 1988; Du Toit and Du Preez, 1995; Du Preez *et al.*, 2011). Lafitte and Edmeades (1988) reported that N deficiency is the largest limiting factor in more than 20% of arable land in Africa.

In African soil, N becomes depleted due to limited or no application of fertilisers, soil erosion and leaching; and as the population increases more soils are affected because farmers are growing crops in new areas to meet production demand (Henao and Baanante, 1999). These researchers indicated that for maintaining current production more than 80 kilogram per hectare (kg ha^{-1}) N is required in South African soils without depleting available nutrients. Commercial farmers are capable of applying more than the required rate to their crop to improve crop productivity, unlike small-scale farmers, who in many cases cannot afford this.

Fertiliser application in sub-Saharan Africa, which can be used to improve quality and productivity of crops, is much lower at 9-10 kg ha^{-1} (Henao and Baanante, 1999; Molden, 2007) as compared to 100 kg ha^{-1} in South Asia, 73 kg ha^{-1} in Latin America and over 250 kg ha^{-1} in Western Europe and North America (Molden, 2007). The prices of fertiliser in Africa are twice to six times the world average (Pinstrup-Andersen *et al.*, 1999) which often tend to lead to low N application because crops are mostly grown by resource poor rural farmers who cannot afford the expensive fertiliser. According to Odhiambo and

Magandini (2008) 75% of farmers in one district of SA indicated that fertiliser is too costly and 48% said they do not have sufficient funds to buy it. Another factor which is associated with low or lack of use of fertiliser is accessibility because most of the farmers are in rural areas far away from cities and it will again cost them to travel to major cities to purchase the product. About 50% of the farmers interviewed had easy access to fertilisers and concluded that there is a need to make fertiliser more accessible to farmers by establishing strategically placed depots (Odhiambo and Magandini, 2008).

2.3.2 Effects of nitrogen on maize genotypes for grain quality traits and benefit of tolerant cultivars

Growth and development of maize plants are affected by variation in N supply (McCullough *et al.,* 1994). Laubscher (1981), McMullan *et al.* (1988) and Carr *et al.* (1992) reported that soil nutrient variation can cause differences in grain quality and yield. In areas where fertiliser use is minimal, genetic approaches to developing and selecting superior genotypes that can perform well under low N environments are crucial. A major setback facing maize genotype performance under low N environments is that researchers were developing and testing improved cultivars under optimum environments (Muza *et al.*, 2004), before taking it to farmer's conditions, in so doing leaving small-scale farmers vulnerable to poor soil fertility and unable to contribute to the economy. There are a large number of QPM cultivars developed and registered in SA for high input agriculture. Currently there are no cultivars developed for tolerance to low N in SA. Therefore it is essential to evaluate available QPM germplasm for tolerance to low soil N. Existence of genetic variation in the germplasm will allow development of low N tolerant QPM cultivars. Improved cultivars that tolerate low soil fertility will help maize farmers to obtain better yields and grain quality.

Exposing experimental cultivars to low N environments during selection and evaluation will results in cultivars that perform well under farmer's conditions. Under low N environments QPM genotypes perform differently due to the existence of genetic variability for tolerance to stress (Mosisa *et al.,* 2007; Gissa, 2008; Ngaboyisonga *et al.,* 2008). Mosisa *et al.* (2007) and Bello *et al.* (2012) found that genotype variation was significant for tryptophan in the protein and grain, protein content in the grain and protein

quality under low and optimum N levels. N deficiency results in poor endosperm modification by producing soft kernels on the maize cob as compared to high N conditions (Ngaboyisonga *et al.*, 2006; 2009; Gissa, 2008; Wegary *et al.*, 2011). However, Wegary *et al.* (2011) observed that even under low N conditions QPM still maintained acceptable protein quality and endosperm hardness. Under low N grain protein and tryptophan content were lower relative to optimum N conditions (CIMMYT, 2003). Sabata and Mason (1992) indicated that increased soil N levels resulted in increased grain protein content and decreased kernel breakage susceptibility in maize. N levels and grain protein were shown to be positively correlated (Oikeh *et al.*, 1998). Duarte *et al.* (2005) in a study with four levels of N found that high N levels increased protein, hardness and reduced breaking susceptibility. Genotypes had a larger influence than N environments. Li *et al.* (2011) found significant genotypic variation across low N, across high N and across both high and low N sites. Under low N conditions protein concentration was reduced. For starch and oil, genotype variation was greater than GEI and environment effects across high and low N conditions, while for protein concentration environmental effects and GEI was larger than genotypic variation. They concluded that examining genotypes under low and high N is of great importance. Zaidi *et al.* (2009) observed that grain protein, lysine and tryptophan contents decreased by 17.0%, 12.5% and 15.6% respectively under a low N environment.

2.4 Evaluation of genotype, environment and genotype by environment interaction effects using analysis of variance (ANOVA), additive main effects and multiplicative interactions (AMMI) and genotype and genotype by environment interaction (GGE) biplots

Production environments may vary due to factors such as rainfall, soil fertility, season, temperature and soil types. The environments used in trials can be described, for example, by different years, locations and fertilisation levels. These variables might play an important role in differences in expression of the genotypes, resulting in GEI, however sometimes the genotypic variation might be more important than environmental variation, resulting in small or no GEI. Plant breeders are concerned by GEI, because during cultivar development, it is essential to understand the interaction of the genotypes within particular environments in order to determine the stability of those genotypes. A genotype

is stable when it is able to perform consistently across a broad range of environments (Annicchiarico, 2002). Multi-location trials are conducted for various agronomic and grain quality traits in order to identify superior genotypes across a wide range of environmental conditions. The inconsistency in the performance of genotypes in a wide range of environments is known as GEI. Beck *et al.* (1991) reported that when genotypes are grown under a wide range of environments and outside their usual adaptation zone, the occurrence of large GEI is expected. Large GEI makes it difficult for the identification of better performing genotypes. The GEI is of practical significance when the ranking of genotypes varies among environments; this is known as crossover interactions (Crossa and Cornelius, 1997; Russell *et al.*, 2003).

The most important aim of a breeder is to develop genotypes such as inbred lines, hybrids and open-pollinated varieties (OPVs) that are adapted to a wide range of environments; however the occurrence of large GEI reduces the chances of making the most accurate choice of the best cultivar(s) for the end-user. It is important for breeders to evaluate different types of cultivars under various environments for grain quality and other traits valuable to the end users. Significant GEI allows breeders to further assess the adaptability and overall stability of the genotypes across different environments. Breeders are striving to identify superior inbred lines to be used as parents in the development of better cultivars, in so doing they resort to testing these materials over different environments to measure their superiority. It does not matter about the types of materials used in a study because GEI is usually present, whether cultivars are pure lines, single-crosses, double-crosses, S1 lines or any other breeding material (Dabholkar, 1999). It is regarded as a differential expression of genotypes across environments (Crossa *et al.*, 1990; Basford and Cooper, 1998), and complicates selection of genotypes for broad adaptation. It needs to be investigated and analysed properly so that its nature and causes are clearly understood. A genotype which is consistent over a range of environments has general adaptation while the one which is consistent over a limited range of environments has specific adaptation. The best way to create a widely adapted cultivar is to increase its tolerance to different stress factors (Ramagosa and Fox, 1993). The analysis of GEI in this study would assist in revealing the patterns of adaptation of QPM inbred lines for grain quality traits in low N environments.

There are a number of statistical procedures that can be useful in measuring the presence of GEI in trials and stability of genotypes to the environments, such as ANOVA (Steel and Torrie, 1980), linear regression (Finlay and Wilkinson, 1963; Eberhart and Russell, 1966), principle component analysis (PCA) (Vargas *et al.*, 1999), the environmental variance (Lin *et al*., 1986), Shukla's stability variance (Shukla, 1972), Wricke's ecovalence (Wricke, 1962) and multivariate methods such as GGE and AMMI (Crossa, 1990; Yan *et al.*, 2000; Akçura *et al*., 2005). There is limited information on the GEI, adaptation and stability of QPM germplasm in the world, especially using AMMI and GGE biplots for grain quality traits. Few researchers have reported significant GEI for protein, endosperm hardness and tryptophan in QPM (Pixley and Bjarnason 2002). Hohls *et al.* (1995b) reported significant GEI on grain yield of QPM. This is the only reported study done on South African QPM inbred lines in the country and it did not evaluate grain quality traits. The methods for determining existence and extent of GEI on various crops are discussed below.

2.4.1 Analysis of variance

ANOVA is mostly used in the assessment of cultivars in trials which result in two-way data of genotype effects and environment effects (additive) and GEI (non-additive) effects. However, the model explains only a small percentage of the variation in the GEI and does not show stability (Zobel *et al.,* 1988; Samonte *et al*., 2005). In other words it is able to detect only the existence of the GEI. Therefore AMMI and GGE biplots will be used to further explore and understand the extent of GEI and stability.

QPM cultivars have been mainly evaluated using ANOVA for grain quality traits in single and combined environments (Pixley and Bjarnason, 2002; Worku *et al.,* 2007; Zaidi *et al.,* 2009; Wegary *et al.,* 2011), with most results showing significant genotype, environments and GEI effects. Vasal *et al.* (1993) reported highly significant GEI for endosperm hardness and other yield related traits, in a study with diallel crosses of 10 QPM populations tested across locations. Taghouti *et al.* (2010) evaluated durum wheat cultivars adapted to different environments for quality traits and encountered significant genotype and environment effects and GEI for all measured quality traits. They found that for protein the environment and the genotype effects were higher than GEI effects while

for vitreousness the environments and GEI effects were higher than the genotype effect. For both protein and vitreousness environment had a larger effect than the genotype, indicating that these traits are controlled more by the environment than the genotype. It was concluded that in order to determine the protein of a cultivar, multi-location trials are necessary since the trait was influenced more by the environment than genotype and GEI. For traits that are not easily influenced by environment it is not necessary to start with multi-location trials, the genotypes can be planted in single locations before good performers are taken to multi-location trials. Dandeech and Joshi (2007) reported on a study of 74 maize genotypes, planted in four environments for determination of GEI and stability, that genotype, environment and GEI effects were highly significant for protein, starch and oil concentrations, except for the environment effect for starch content in a pooled ANOVA.

2.4.2 Additive main effects and multiplicative interaction analyses

The AMMI model is a statistical tool that has been developed to further analyse and understand GEI patterns. The analysis has been reported to be suitable for depicting adaptive responses and is useful in understanding complex GEI (Gauch and Zobel, 1989; Crossa, 1990; Gauch and Zobel, 1997). The AMMI model combines both classical ANOVA and PCA into a single model with additive and multiplicative parameters (Zobel *et al.,* 1988; Shafii and Price, 1998; Pinnschmidt and Hovmoller, 2002). The model separates the additive variance from the interaction variance and applies PCA to the interaction portion from the ANOVA analysis to extract a new set of coordinate axes that account more effectively for the interaction patterns (Zobel *et al.,* 1988; Shafii and Price, 1998; Thillainathan and Fernandez, 2001). In clarification of GEI, AMMI summarises patterns and relationships of genotypes and environments (Crossa, 1990).

Furthermore statistical model results from AMMI analysis are plotted in a graph showing the main and interaction effects for both genotypes and environments on the same scatter plot, with the noise rich residual discarded and the data separated into a pattern rich model to gain accuracy (Gauch and Zobel, 1996). The AMMI graph is used to visualise the adaptability (average performance across localities) and stability (consistent performance across environments) of various genotypes. In the AMMI graph the

differences in main effects are indicated along the abscissa while the differences in interaction are indicated along the ordinates. The interpretation of this is that the genotypes that are located close to the abscissa are relatively stable, while the ones that are located far away from the abscissa are unstable because they interact with the environments. AMMI analysis was considered to be the best model compared to linear models (Gauch and Zobel, 1988). Gauch (1988) described AMMI as a powerful tool to explore and understand GEI. The AMMI model was recognised by Gauch (1993) to be more effective and efficient in handling both the main effects and GEI in multi-location trials than other statistical models. Wallace *et al.* (1993) stated that for various measured traits in different environments AMMI analysis is capable of separating and quantifying GEI effects.

Studies using the AMMI model have been reported by various researchers on various traits across different environments (Worku *et al.,* 2007; Zaidi *et al.,* 2009; Taghouti *et al.,* 2010; Castillo *et al.,* 2012). The AMMI model has been proved to be a valuable tool for graphically showing adaptive responses (Gauch and Zobel, 1989; Gauch, 1993). Worku *et al.* (2007) used the AMMI model to evaluate the effects of GEI and stability in 14 CIMMYT maize hybrids and two commercial hybrids from Seed Co Ltd across nine environments. The AMMI analysis was able to identify significant genotypes, environments and GEI. The interaction principle component axes (IPCA) 1 and 2 explained 37.3% and 28.6% of the interaction sum of squares, respectively; together accounting for most of the interaction (65.9%). Hybrids with specific adaptation to either high or low N environments were also identified. Zaidi *et al.* (2009) found significant GEI for protein in the grain, lysine in protein and agronomic traits. Their stability results indicated that tryptophan and lysine contents were the most stable traits and grain protein and yield were most unstable. Most of the genotypes were stable for tryptophan, while most of the genotypes were unstable for grain protein.

In a recent study on yield and quality parameters of spring wheat cultivars Castillo *et al.* (2012) found that IPCA1 and 2 accounted for GEI variation of 71.89% in AMMI, however the method was not better when compared to site regression (SREG) which accounted for 81.08% of the GEI variation. The methods were able to identify cultivars with good yield, high quality and stability across environments, however SREG was the

most effective and efficient method to identify the best performer in terms of yield, quality and stability. Taghouti *et al.* (2010) evaluated durum wheat cultivars adapted to different environments for quality traits and encountered significant GEI effects for all the measured traits. The AMMI analysis showed that the first two IPCA axes were highly significant for protein and vitreousness, and together accounted for more than 70% of the GEI variation for all traits. Overall, genotypes showed differences in degree of stability for various measured traits and within genotypes and there was variability for stability, showing that between traits genetic factors for GEI varied. The method was able to select a genotype with high and stable quality. AMMI for starch content showed that environment, genotype and GEI were significant and highly significant respectively (Parkes, 2011). The analysis further indicated that IPCA1 (76.5%) and IPCA2 (23.5%) axes explained 100% of the total GEI variation and was able to identify stable genotypes. For oil content the first two IPCA were significant and accounted for more than 80% of GEI variation (Mekonnen and Mohammed, 2009).

2.4.3 Genotype and genotype by environment interaction biplot analysis

The GGE biplot is the graphical representation of the genotype main effect and GEI of multi-location trials for visual evaluation of genotypes and test environments and identification of mega-environments (Yan *et al.*, 2000). Genotypic and GEI effects are the two most important sources of variation relevant to cultivar evaluation (Yan *et al.*, 2001). The GGE biplot is based on plotting the first two interaction principal component axes (IPCAs) 1 and 2 against each other with IPCA1 on the X axis and IPCA2 on the Y axis.

There are a number of GGE biplots, which can be computed for analyses of multi-location trials data, depending on what the researcher wants to get from the data. There are those which help in cultivar evaluation, test environment evaluation and mega-environment identification (Yan *et al.*, 2000). For genotype evaluation there are mean vs stablity biplots which help identify high yielding or performing genotypes and then the stable genotypes, and mean and stability biplots which identify an ideal genotype. A genotype which ranks highest for any characteristic in all the locations used (absolute stable in performance) is regarded as an ideal genotype (Yan and Kang, 2003); however in reality such a genotype sometimes may not exist. It is important to identify such a

genotype in order to use it as a reference during genotype evaluation. In test environment evaluation there are the discriminating vs representative biplots, which are graphically represented by vectors and angles; and the discriminating and representative biplots, which are an easier visual representation of an ideal environment. The "which won where" or "what is best for what" biplot graphically shows which genotypes won in which environments and it identifies mega-environments. In a general ANOVA it is not possible to select superior cultivars for each mega environment, however GGE is able and that allows for effective exploitation of both genotype and GEI (Muungani *et al*., 2007).

The GGE biplot has been successful in identifying ideal genotypes in a study comparing seven QPM cultivars across four hill environments of Nepal, but mostly for grain yield (Upadhyay *et al.,* 2009). Badu-Apraku *et al.* (2010) used GGE biplots in evaluation of normal and QPM cultivars under stress and non-stress environments, and identified five cultivars with outstanding performance in both environments. They further identified superior genotypes for stress and others for non-stress environments. Badu-Apraku *et al.* (2011) were able to select ideal genotypes, location and identified mega-environments using various GGE biplots in evaluation of maize cultivars.

2.5 Cross-pollination effects on various traits

In maize there is data to show that crossing has an immediate effect on the yield and related traits and various grain quality traits (Krieger *et al.,* 1998; Letchworth and Lambert, 1998; Balestre *et al.,* 2007; Castañeda, 2010). Currently there is limited information on effects of pollination methods on grain yield and quality traits of QPM (Bosch *et al.,* 1978; Hossain *et al.,* 2008). Balestre *et al.* (2007) reported no significant differences between the proportion of fertilised and sampled allo-pollen and auto-pollen (self) in the maize ear and found that allo-pollen increased the mean kernel weight by 16.5 mg (gain of 4.65%). They concluded that even when there was no significant difference between the pollination methods, allo-pollen when compared to auto-pollen increased the kernel weight.

Krieger *et al.* (1998) evaluated 24 hybrids to study the effect of three pollination methods, self-pollination small-plot, open pollination small-plot, and open pollination large plot (farmer's field) at two Iowa growing locations on starch thermal properties and reported significant differences in starch gelatinisation and retro-gradation properties between the pollination methods and between locations. The study recommended the use of self-pollination when growing maize samples in small plots for research purposes due to the differences found in starch traits associated with pollination methods.

Letchworth and Lambert (1998) reported in their study of pollen parent effect on oil, protein and starch concentration in maize kernels that self-pollinated kernels had a higher protein concentration than open-pollinated kernels even though hybrid rankings showed no significant difference within pollination treatments. The results indicated that field evaluation is feasible for protein concentration under open-pollinated small plot conditions. The immediate pollen effect is sometimes referred to as xenia effect, and it usually results in increased yield, weight, seed size or is shown by an immediate colour change. Castañeda (2010) studied xenia effects on quality of a maize female inbred in production of hybrid seed and showed a significant inbred effect on yield. He found that cross-pollination with some of the inbred lines resulted in reduced yields of hybrid seed, while most of the inbred lines increased yields of the hybrid seed. Generally the findings recommended the use of open pollination for growing females to avoid loss of vigour observed with selfing.

Bosch *et al.* (1978) studied pollination effects on grain yield and quality of fertilised plants comparing self-pollination with the same QPM pollen to cross-pollination with normal maize pollen and found that for opaque-2 (QPM) treatment, yield was 12.4% higher in one hybrid ($p < 0.05$), but 3.32% and 6.55% lower in the other two hybrids. These differences in grain yield and plant traits, related with the genetic differences of the fertilising pollen, suggest that the genetic information that the pollen transferred to the embryo and to the endosperm affected not only the vigour of the seed but also the physiology of the female parent plant. Hossain *et al.* (2008) who studied the effect of genotype x pollination mode interaction on kernel modification in QPM genotypes, reported significant interaction of genotype with pollination method which suggested the importance of pollen source and its genetic constitution to confer kernel texture.

2.6 QPM donors and combining ability of lines

In a QPM breeding programme approximately three QPM donors are used to convey the high levels of tryptophan and endosperm hardness to a normal maize recipient. It would be desirable to minimise the number of worthless conversion projects. Therefore it is of great importance to study donors, in order to identify suitable donors to be used in a conversion programme and alleviate the use of many donors, thus making the breeding programme more cost effective and efficient. However, depending on the types of germplasm involved in the conversion programme it can be easy or impossible to get new QPM versions of the normal line or population. Bjarnason *et al.* (1976) observed that some F_2s of crosses between normal and opaque-2 genotypes produced more translucent to opaque kernels than the expected 3:1 ratio. This suggested that some homozygous opaque-2 kernels were modified in such a way that they were phenotypically indistinguishable from normal kernels.

CIMMYT (2004) reported findings on the identification of QPM donors in a study of F_2 seed developed through the crossing of nine QPM inbred lines to 16 normal inbred lines. They identified good and bad donors by classifying the F_2 seed for endosperm modification on a scale of 1-5 into donor types. The QPM lines were ranked according to their ability to modify kernels in crosses. According to the report, the QPM donor lines were grouped into four classes; whereby group 1 were excellent donors which combined well with most of the lines, CML144, CML181f and CML176; group 2 were good donors by combining with some lines, CML173, CML154 and CML175; group 3 were fair donors as they combined with few of the lines, CML181d, CML182; group 4 had one poor donor CML159. QPM breeders are looking for quick ways to discriminate between good and bad QPM donors in backcross programmes to prevent wasting precious time and resources. This is expected to save QPM breeders from the trouble of advancing their materials through several backcrosses before they can discard worthless crosses involving poor donors. Genotypes differ considerably in their ability to show *opaque-2* modifiers (Vasal, 1975). Mutimaamba *et al.* (2010) in a study with Zimbabwean QPM and normal inbred lines managed to identify QPM donors for the Zimbabwean breeding programme.

Kambal and Webster (1965) defined combining ability as the performance of a line in hybrid combinations. It plays an important role in selecting superior parents for hybrid combinations and in studying the nature of genetic variation because the final evaluation of inbred lines can be best determined by hybrid performance (Hallauer and Miranda, 1988). Sprague and Tatum (1942) categorised the concept of combining ability into general combining ability (GCA) which they described as average performance of lines in hybrid combinations, and specific combining ability (SCA) as the deviation of individual crosses from the average of the margins. Diallel analysis is an important tool used by breeders for partitioning of total variation of the data into SCA of each cross and GCA of each genotype; and to select superior parents for developing crosses (Hallauer and Miranda, 1988; Sughroue and Hallauer, 1997; Koutsika-Sotiriou, 1999; Shukla and Pandey, 2008).

Few studies have been published on the combining ability of South African QPM inbred lines used in the ARC-GCI QPM breeding programme for important grain quality traits (Hohls *et al.,* 1995a; 1996; Bhatnagar *et al.,* 2004). Conversely there is more available information on the combining ability of these inbred lines on resistance to grey leaf spot and grain yield traits (Gevers *et al.,* 1994; Hohls *et al.,* 1995b). In Bhatnagar *et al.* (2004); the South African lines used were tested in the US with some of the CIMMYT inbreds and US inbred lines from Texas A&M University. The research denoted highly significant GCA for kernel quality traits. Pixley and Bjarnason (1993) in a study of combining ability using 34 modified endosperm opaque-2 tropical maize inbred lines found that mean squares for GCA effects were significant for protein content, quality index (QI) and tryptophan content, whereas those for SCA effects were not significant for all the quality traits. Okello *et al.* (2005) recommended the use of QPM donors identified, which were superior in terms of GCA for foliar diseases, protein quantity and quality in the Ugandan QPM breeding programme. On the other hand Jompuk *et al.* (2007) found non-significant differences between GCA and SCA of protein content in the endosperm. Machida *et al.* (2010) studied reciprocal cross effects and combining ability of elite QPM inbred lines evaluated in subtropical environments. They reported occurrence of GCA effects for protein content, tryptophan content and kernel modification, conversely non-significant SCA effects for all the quality traits were observed except for QI. Hadji (2004) indicated significant GCA mean squares for endosperm hardness and non-significant

SCA mean squares for endosperm hardness in a study of combining ability of 10 white QPM inbred lines for various traits. Most of the combining ability analyses studies above were for crosses between QPM inbred lines. Liangming *et al.* (2010) reported that in a study of combining ability for protein, starch and oil contents in maize inbred lines both GCA and SCA were highly significant for all the measured traits. GCA mean squares were more important than SCA mean squares.

2.7 Reciprocal cross and maternal effects

Maternal effects and reciprocal effects are important components in particular when the traits are maternally determined, with grain and ear quality traits targeted (Jumbo and Carena, 2008); and are mainly analysed using diallel analysis. Reciprocal cross effect analysis helps in determining the direction of crosses for hybrids (Lopez *et al.,* 2003; Scott *et al.,* 2009). It is important to determine if these effects are present or not, because their presence bias the estimates for GCA (additive genetic variance) and SCA (non-additive genetic variance) in an unpredictable direction (Crusio, 1987; Roach and Wulff, 1987). Griffing (1956) proposed four methods to be used in diallel analyses. Of the four methods Griffing methods 1 and 3 can be used for determining reciprocal cross effects. Method 1 is used when there are data for parents, F_1 crosses and reciprocals. Method 3 on the other hand can be used when there are F_1 crosses and reciprocals. Reciprocal effects are partitioned into maternal effects and non-maternal effects (Zhang and Kang, 1997). The maternal effects are the average effects of using parents as females rather than males in their crosses expressing the maternal genotype in the phenotype (Roach and Wulff, 1987; Dhliwayo *et al.,* 2005) whilst non-maternal effects are deviations from that average; and might be caused by interaction of cytoplasmic factors and nuclear genes (Lopez *et al.,* 2003; Dhliwayo *et al.,* 2005). However, for the present study maternal effects were evaluated in a line x tester design and analysed using ANOVA for GCA and SCA.

There are various researchers who have studied reciprocals cross effects and reported the significance and presence of the effects on various traits. Jumbo and Carena (2008) in a study of elite early maturing maize population hybrids reported that reciprocal cross effects and maternal effects were not significant in maize for most of the measured traits.

In their reports they indicated the need for more research of maternal and reciprocal effects on kernel endosperm related traits given that the traits might have reciprocal and maternal effects and this might impact breeding of better quality maize. Sung (1984) made reciprocal crosses in modified opaque-2 maize inbred lines and found that the reciprocal F_1 seeds showed differences in degree of modification, zein and free amino acids and when the modified endosperm opaque-2 inbred lines were used as pollen parents, the F_1 seed showed differences from the non-modified female parent endosperm for all traits. The differences in seeds were thought to have occurred as a result of dosage and maternal effects. Vasal *et al.* (1980; 1984) reported significant reciprocal effects in kernel modification. Alika and Ojomo (1996) reported significant reciprocal effects for grain starch content and said it was an indication that cytoplasmic factors were important. Hossain *et al.* (2008) in a diallel study of QPM genotypes reported significant reciprocal effects for kernel modification and implied possible dosage effects of the endosperm modifiers.

2.8 Correlations among grain quality traits

Correlation analyses are used as a measure of the strength of association between two variables (Steel and Torrie, 1980; Hallauer and Miranda, 1988). When associations among traits are weak, it is difficult to conduct simultaneous selection between important traits, however when the relationship among traits are positive it indicates the possibility of a simultaneous selection of the traits involved. In an improvement programme breeders are interested in analysis of correlated traits, in order to know whether traits are related. Falconer and Mackay (1996) reported that correlated traits are of interest because of genetic causes of correlation through the pleiotropic action of genes, connection to changes brought about by selection and connection with natural selection.

In a QPM improvement programme it is important to select for both protein quality traits and endosperm modification and for the traits to be positively correlated. However, when endosperm hardness is negatively correlated with tryptophan it is important to determine tryptophan or lysine content in order to keep track of the concentrations (Glover, 1992). Pixley and Bjarnason (2002) reported that endosperm hardness was positively correlated with all quality traits even though the values were extremely low. Other researchers

indicated that endosperm hardness and protein content were positively correlated (Motto *et al.,* 1978; Vasal *et al.,* 1980); while Wessel-Beaver *et al.* (1985) reported that endosperm modification and protein quality are negatively correlated. Pixley and Bjarnason (2002) further supported earlier studies by various researchers (Wessel-Beaver *et al.,* 1985; Pixley and Bjarnason, 1993) that protein content is positively correlated with tryptophan content but negatively correlated with tryptophan in protein or QI. Tryptophan content on the other hand was positively correlated with QI (Pixley and Bjarnason, 2002). Gutierrez-Rojas *et al.* (2008) in a study with recombinant inbred lines reported that endosperm opacity and tryptophan content were positively correlated with some lines showing high levels of tryptophan and degree of modification showing the possibility that the two traits could be concomitantly selected. Protein content and oil content in maize have been reported to be correlated (Clark *et al.,* 2006; Dudley *et al.,* 2007); while Seiam and Khalifa (2007) reported negative correlation between the two traits. Mittelmann *et al.* (2003) studied correlation among grain quality traits with testcross populations from Family S_1 x tester and reported that oil and protein were correlated but with low values at 0.21 to 0.41. With regards to starch and protein some authors have reported that starch and protein content are negatively correlated (Clark *et al.,* 2006; Dudley *et al.,* 2007).

2.9 References

Akçura M, Kaya Y and Tanes S (2005) Genotype-environment interaction and, phenotypic stability analysis for grain yield of durum wheat in the central Anatolian region. Turkish Journal of Agriculture and Forestry 29: 369-375.

Akpalu W, Hassan RM and Ringler C (2009) Climate variability and maize yield in South Africa: Results from GME and MELE methods, IFPRI Discussion Paper No. 843, Washington, DC.

Akuamoa-Boateng A (2002) Quality protein maize: Infant feeding trials in Ghana. Ghana Health Service, Ashanti, Ghana.

Alika JE and Ojomo JO (1996) Combining ability and reciprocal effects for physico-chemical grain quality characteristics in maize. Food Chemistry 57: 371-375.

Annicchiarico P (2002) Defining adaptation strategies and yield stability targets in breeding programmes. In: MS Kang (Eds.). Quantitative genetics, genomics and plant breeding. Wallington, UK, CABI, pp. 365-383.

Badu-Apraku B, Akinwale RO and Fakorede MAB (2010) Selection of early maize inbred lines for hybrid production using multiple traits under Striga-infested and Striga-free environments. Maydica 55: 261-274.

Badu-Apraku B, Akinwale RO, Menkir A, Obeng-Antwi K, Osuman AS, Coulibaly N, Onyibe NG, Yallou GC, Abdullai MS and Didjera A (2011) Use of GGE biplot for targeting early maturing maize cultivars to mega-environments in West Africa. African Crop Science Journal 19: 79-96.

Balestre M, de Souza JC, dos Santos JB, Luders RR and Lima JL (2007) Effect of self-pollination monitored by microsatellite markers on maize kernel weight. Crop Breeding and Applied Biotechnology 7: 340-344.

Basford KE and Cooper M (1998) Genotype x environment interactions and some considerations of their implications for wheat breeding in Australia. Australian Journal of Agricultural Research 49: 153-174.

Beck DL, Vasal SK and Crossa J (1991) Heterosis and combining ability among subtropical and temperate intermediate maturity maize germplasm. Crop Science 31: 68-73.

Bello OB, Oluleye F, Mahamood J, Afolabi MS, Azeez MA, Ige SA and Abdulmaliq SY (2012) Nutritional and agronomic evaluation of quality protein maize in the southern Guinea savanna of Nigeria. Scholarly Journal of Agricultural Science 2: 52-61.

Bhargava A, Chopra N and Gupta HO (2003) Processing of quality protein maize (QPM) into dalia and processed flour and their synergistic effect on the growth of pre-school children. IFCON, Indian Agricultural Research Institute, New Delhi.

Bhatnagar S, Betran FJ and Rooney LW (2004) Combining abilities of quality protein maize inbreds. Crop Science 44: 1997-2005.

Bjarnason M, Pollmer WG and Klein D (1976) Inheritance of modified endosperm structure and lysine content in opaque-2 maize: I. Maize endosperm structure. Cereal Research Communications 4: 401-410.

Bosch L, Blanco M, Alvarez A and Blanco JL (1978) Pollination effects on the grain yield and the qualities of the fertilized plants: II. Self pollination with o2 pollen vs. cross-pollination with o2+ pollen. Maize Genetics Cooperation Newsletter 52: 122-123.

Carr PM, Jacobsen JS, Carlson GR and Nielsen GA (1992) Influence of soil and N fertiliser on performance of barley and spring wheat cultivars. Canadian Journal of Plant Science 72: 651-661.

Castañeda L (2010) Xenia Effects on Quality of Maize Female Inbred in Production of Hybrid Seeds PhD Thesis, University of Nebraska, Lincoln.

Castillo D, Matus I, del Pozo A, Madariaga R and Mellado M (2012) Adaptability and genotype × environment interaction of spring wheat cultivars in Chile using regression analysis, AMMI and SREG. Chilean Journal of Agricultural Research 72: 167-174.

CIMMYT (2003) The development and promotion of quality protein maize in sub-Saharan Africa. Progress Report Submitted to Nippon Foundation. CIMMYT, Mexico, D.F.

CIMMYT (2004) The development and promotion of quality protein maize in sub-Saharan Africa. Progress Report 2004. Submitted by the International Maize and Wheat Improvement Center (CIMMYT) to the Nippon Foundation, CIMMYT, Mexico, D.F.

Clark HE, Glover DV, Betz JL and Bailey LB (1977) Nitrogen retention of young men who consumed iso-nitrogenous diets containing normal, *opaque-2*, or *sugary-2opaque-2* corn. Journal of Nutrition 107: 404-414.

Clark D, Dudley JW, Rocheford TR and Le Deauxb JR (2006) Genetic analysis of corn grain chemical composition in the random mated 10 generation of the cross of generations 70 of IHO × ILO. Crop Science 46: 807-819.

Crossa J and Cornelius PL (1997) Sites regression and shifted multiplicative model clustering of cultivar trial sites under heterogeneity of error variances. Crop Science 37: 406-415.

Crossa J, Gauch H and Zobel R (1990) Additive main effect and multiplicative interaction analysis of two international maize cultivar trials. Crop Science 30: 493-500.

Crossa J (1990) Statistical analyses of multi-location trials. Advances in Agronomy 44: 55-85.

Crusio WE (1987) A note on the analysis of reciprocal effects in diallel crosses. Journal of Genetics 66: 177-185.

Dabholkar AR (1999) Elements of biometrical genetics. Revised ed. Concept Publishing Co., New Delhi, India.

Dandeech A and Joshi VN (2007) Stability analysis for quality traits in maize (*Zea mays* L.). Agricultural Science Digest 27: 26-29.

Dhliwayo T, Pixley KV and Kazembe V (2005) Combining ability for resistance to maize weevil among 14 Southern African maize inbred lines. Crop Science 45: 662-667.

Du Preez CC, Van Huyssteen CW and Mnkeni PNS (2011) Land use and soil organic matter in South Africa: A review on spatial variability and the influence of rangeland stock production. South African Journal of Science 107: 354-361.

Du Toit ME and Du Preez CC (1995) Effect of cultivation on the nitrogen fertility of selected dryland soils in South Africa. South African Journal of Plant and Soil 12: 73-81.

Duarte AP, Mason SC, Jackson DS and Kiehl JC (2005) Grain quality of Brazilian maize genotypes as influenced by nitrogen level. American Society of Agronomy 45: 1958-1964.

Duarte JM, Pacheco CAP, Guimarães CT, Guimarães PE and Paiva E (2004) Evaluation of high quality protein maize (QPM) hybrids obtained by conversion of normal inbred lines. Crop Breeding and Applied Biotechnology 4: 163-170.

Dudley JW, Clark D, Rocheford TR and Le Deaux JR (2007) Genetic analysis of corn grain chemical composition in the random mated 7 generation of the cross of generations 70 of IHP × ILP. Crop Science 47: 45-57.

Eberhart SA and Russell WA (1966) Stability parameters for comparing varieties. Crop Science 6: 36-40.

Falconer DS and Mackay TFC (1996) Introduction to Quantitative Genetics. 4th ed. Longman, New York, 464pp.

FAO/WHO (1991) Protein quality evaluation: Report of Joint FAO/WHO export consultant, FAO food nutrition paper; 51, FAO/WHO, Rome, pp. 19-21.

FAO (1992) Maize in human nutrition. Report Series 25, Food and Agriculture Organization, Rome, Italy.

FAO and WFP (2010) The State of Food Insecurity in the World 2010: Addressing Food Insecurity in Protracted Crises. FAO, Rome.

FAOSTAT (2009) Available on the internet from: http://faostat.fao.org/site/368/Desktop.aspx? PageID=368# ancor [Date Accessed: 07 June 2010].

Finlay KW and Wilkinson GN (1963) The analysis of adaptation in plant breeding programme. Australian Journal of Agricultural Research 14: 742-754.

Gauch HG (1988) Model selection and validation for yield trials with interaction. Biometrics 44: 705-715.

Gauch HG (1993) Prediction, parsimony and noise. American Scientist 81: 468-478.

Gauch HG and Zobel RW (1988) Predictive and postdictive success of statistical analyses of yield trials. Theoretical and Applied Genetics 76: 1-10.

Gauch HG and Zobel RW (1989) Accuracy and selection success in yield trials. Theoretical and Applied Genetics 77: 473-481.

Gauch HG and Zobel RW (1996) Optimal replication in selection experiments. Crop Science 36: 838-843.

Gauch HG and Zobel RW (1997) Identifying mega-environments and targeting genotypes. Crop Science 37: 311-326.

Gevers HO (1995) QPM breeding in Africa. In: B Larkins and ET Mertz (Eds.), Quality Protein Maize: 1964-1994, Proceedings of the International Symposium on Quality Protein Maize, 1-3 December 1994. EMBRAPA/CNPMS, Sete Lagoas, Brazil, pp. 217-229.

Gevers HO Lake JK and Hohls T (1994) Diallel cross analysis of resistance to gray leaf spot in maize. Plant Disease 78: 379-383.

Gissa DW (2008) Genotypic variability and combining ability of quality protein maize inbred lines under stress and optimal conditions. PhD thesis, Department of Plant Sciences, University of the Free State, Bloemfontein, South Africa.

Glover DV (1992) Corn protein-genetics, breeding, and value in foods and feeds. In: ET Mertz (Eds.). Quality Protein Maize. American Association of Cereal Chemists, St. Paul, pp. 49-78.

Graham GG, Placko RP and Maclean WC (1980) Nutritional value of normal, opaque-2 and sugary-2 opaque-2 maize hybrids for infants and children: 2. Plasma-free aminoacids. Journal of Nutrition 110: 1070-1074.

Graham GG, Lembcke J, Lancho E and Morales E (1989) Quality protein maize: Digestibility and utilization by recovering malnourished infants. Pediatrics 83: 416-421.

Graham GG, Lembake J and Morales E (1990) Quality-Protein maize as the sole source of dietary protein and fat in rapidly growing young children. Pediatrics 85: 85-91.

Griffing B (1956) Concept of general and specific combining ability in relation to diallel crossing systems. Australian Journal of Biological Science 9: 463-493.

Gutierrez-Rojas A, Scott MP, Leyva OR, Menz M and Betrán J (2008) Phenotypic characterization of quality protein maize endosperm modification and amino acid contents in a segregating recombinant inbred population. Crop Science 48: 1714-1722.

Hadji TH (2004) Combining ability analysis for yield and yield-related traits in quality protein maize (QPM) inbred lines. MSc Thesis, School of graduate studies, Alemaya University, Ethiopia.

Hallauer AR and Miranda JB (1988) Quantitative Genetics in Maize Breeding. 2nd ed. Iowa State University Press, Iowa, Ames. USA.

Henao J and Baanante C **(**1999) Nutrient depletion in agricultural soils of Africa: A 2020 Vision for food, agriculture, and the environment. Available on the internet from: http://www.ifpri.org/sites/default/files/publication/vb62.pdf [Date Accessed: 07 June 2012].

Hohls T, Clarke GPY, Shanahan PE and Gevers HO (1995a) Additive and non-additive genetic correlations. II. Application to data obtained from a 12 × 12 diallel cross. Biometrical Journal 37: 937-943.

Hohls T, Shanahan PE, Clarke GP and Gevers HO (1995b) Genotype x environment interactions in a 10 x 10 diallel cross of quality protein maize (*Zea mays* L.). Euphytica 84: 209-218.

Hohls T, Shanahan PE, Clarke GP, and Gevers HO (1996) Genetic control of kernel modification found in South African quality protein maize inbred lines. Euphytica 87: 103-109.

Hossain F, Prasanna BM, Kumar R and Singh BB (2008) Genetic analysis of kernel modification in Quality Protein Maize (QPM) genotypes. Indian Journal of Genetics 68: 1-9.

Jompuk P, Wongyai W, Apisitvanich S and Jampatong C (2007) Combining ability of inbred lines derived from quality protein maize populations. Kasetsart Journal of Natural Science 41: 433-441

Jumbo MB and Carena MJ (2008) Combining ability, maternal, and reciprocal effects of elite early-maturing maize population hybrids. Euphytica 162: 325-333.

Kambal AE and Webster OS (1965) Estimates of general and specific combining ability in grain sorghum. Crop Science 5: 521-523.

Koutsika-Sotiriou M (1999) Hybrid seed production in maize. In: AS Basra (Eds.). Heterosis and hybrid seed production in agronomic crops. The Haworth Press, Inc, NY.

Krieger KM, Pollak LM, Brumm TJ and White PJ (1998) Effects of pollination method and growing location on starch thermal properties of corn hybrids. Cereal Chemistry 75: 656-659.

Krivanek AF, De Groote H, Gunaratna NS, Diallo AO and Friesen D (2007) Breeding and disseminating quality protein maize (QPM) for Africa. African Journal of Biotechnology 6: 312-324.

Lafitte HR and Edmeades GO (1988) An update on selection under stress: selection criteria. In B Gelaw (Eds.), Towards Self-sufficiency, Proceedings of the Second Eastern, Central and Southern African Regional Maize Workshop, pp. 309-331.

Laubscher EW (1981) Nitrogen application, protein content and quality of wheat. Paper presented on award of FSSA Silver Medal. Fertilizer Society of South Africa Journal 2: 41-47.

Letchworth MB and Lambert RJ (1998) Pollen parent effects on oil, protein, and starch concentration in maize kernels. Crop Science 38: 363-367.

Li L, Wegenast T, Li H, Dhillon BS, C Friedrich H Lon-gin, Xu X, Melchinger AE and Chen S (2011) Estimation of quantitative genetic and stability parameters in maize under high and low N. Maydica 56: 1720.

Liangming W, Zhanxian L, Zeqiang C, Lizhu E, Xue'an H, Bo Z and Huazheng X (2010) Combining ability for protein, starch and oil contents in inbred lines of normal maize (*Zea mays* L.) Chinese Agricultural Science Bulletin 26: 179-184.

Lin CS, Binns MR and Lefkovitch, LP (1986) Stability analysis: where do we stand? Crop Science 26: 894-899.

Lopez GA, Potts BM, Vaillancourt RE and Apiolaza LA (2003) Maternal and carryover effects on early growth of *Eucalyptus globulus*. Canadian Journal of Forest Research 33: 2108-2115.

Machida L, Derera J, Tongoona P and MacRobert J (2010) Combining ability and reciprocal cross effects of elite quality protein maize inbred lines in Subtropical environments. Crop Science 50: 1708-1717.

Maletnlema TN (1992) Hunger and malnutrition: the determinant of development: the case for Africa and its food and nutrition workers. East African Medical Journal 69: 424-427.

Mbuya K, Nkongolo KK, Kalonji-Mbuyi A (2011) Nutritional analysis of quality protein maize varieties selected for agronomic characteristics in a breeding programme. International Journal of Plant Breeding and Genetics 5: 317-327.

McCullough DE, Giradin P, Mihajlovic M, Guilera AA and Tollenaar M (1994) Influence of N supply on development and dry matter accumulation of an old and new maize hybrid. Canadian Journal of Plant Science 74: 471-477.

McMullan PA, McVetty PBE and Urquhart AA (1988) Dry matter and nitrogen accumulation and redistribution and their relationship to grain yield and grain protein in wheat. Canadian Journal of Plant Science 68: 311-322

MDG (2011) Assessing progress in Africa toward the millennium development goals. Available on the internet from: http://www.nepad.org/system/files/ADFB-MDG-EN_WEB_2(2).pdf [Date Accessed: 05 February 2012].

Mekonnen Z and Mohammed H (2009) Study on genotype x environment interaction of yield in sesame (*sesamum indicum* l.). Journal of Phytology 1: 199-205.

Mittelmann A, Filho JBM, de Lima GJMM, Hara-Klein C and Tanaka RT (2003) Potential of the ESA23b maize population for protein and oil content improvement. Scientia Agricola 60: 319-327

Molden D (2007) Water for food, water for life: A Comprehensive Assessment of Water Management in Agriculture. Earthscan, IWMI, London, UK, 645p

Mosisa W, Banziger M, Erley GS, Friesen D, Diallo AO and Horst WJ (2007) Nitrogen uptake and utilization in contrasting nitrogen efficient tropical maize hybrids. Crop Science 47: 519-528.

Motto M, Lorenzoni C, Gentinetta E, Maggiore T and Salamin F (1978) Expected genetic gain for protein related traits and allocation of resources in a modified *opaque-2* synthetic. Maydica 23: 35-44.

Mutimaamba C, Lungu D and MacRobert J (2010) Combining ability analysis of quality protein maize (QPM) and non-QPM inbred lines for kernel quality and some agronomic characteristics. Second RUFORUM Biennial Meeting 20-24 September 2010, Entebbe, Uganda.

Muungani D, Setimela P and Dimairo M (2007) Analysis of multi-environment, mother-baby trial data using GGE biplots. African Crop Science Proceedings 8: 103-112.

Muza L, Waddington SR and Banziger M (2004) Preliminary results on the response of 'nitrogen use efficient' OPV and hybrid maize to N fertiliser on small-holder fields in Zimbabwe. In: DK Friesen and AFE Palmer (Eds.), Integrated Approaches to Higher Maize Productivity in the New Millennium, Proceedings of the 7th Eastern and Southern Africa Regional Maize Conference, 5-11 February 2002. CIMMYT/KARI, Nairobi, Kenya, pp. 245-250.

Ngaboyisonga C, Njoroge K, Kirubi D and Githiri SM (2006) Effects of low nitrogen and drought on grain yield and endosperm hardness of quality protein maize single cross hybrids. In: International plant breeding symposium, Mexico D.F., Mexico, pp.137.

Ngaboyisonga C, Njoroge K, Kirubi D and Githiri SM (2008) Effects of field conditions, low nitrogen and drought on genetic parameters of protein and tryptophan concentrations in grain of quality protein maize. International Journal of Plant Production 2: 137-152.

Ngaboyisonga C, Njoroge K, Kirubi D and Githiri SM (2009) Effects of low nitrogen and drought on genetic parameters of grain yield and endosperm hardness of quality protein maize. Asian Journal of Agricultural Research 3: 1-10.

Nuss ET and Tanumihardjo SA (2011) Quality protein maize for Africa: Closing the protein inadequacy gap in vulnerable populations. Advances in Nutrition 2: 217-224.

Odhiambo JJO and Magandini VN (2008) An assessment of the use of mineral and organic fertilisers by smallholder farmers in Vhembe district, Limpopo province, South Africa. African Journal of Agricultural Research 3: 357-362.

Oikeh SO, Kling JG and Okoruwa AE (1998) Nitrogen management effects on maize grain quality in the West Africa moist savanna. Crop Science 38: 1056-1061.

Okello DK, Manna R, Imanywoha J, Pixley K and Edema R (2005) Agronomic performance and breeding potential of inbred lines for improvement of protein quality of adapted Ugandan maize germplasm. African Crop Science Journal 14: 37-47.

Onimisi PA, Omage JJ, Dafwang II and Bawa GS (2009) Replacement value of normal maize with quality protein maize (Obatampa) in broiler diets. Pakistan Journal of Nutrition 8: 112-115.

Parkes EY (2011) Assessment of genetic diversity, combining ability, stability and farmer preference of cassava germplasm in Ghana. PhD thesis, Department of Plant Sciences (Plant Breeding), University of the Free State.

Pinnschmidt HO and Hovmoller MS (2002) Genotype x environment interactions in the expression of net blotch resistance in spring and winter barley varieties. Euphytica 125: 227-243.

Pinstrup-Andersen P, Pandya-Lorch R and Rosegrant MW (1999) World food prospects: Critical issues for the early twenty-first century. IFPRI, Washington DC, USA.

Pixley KV and Bjarnason MS (1993) Combining ability for yield and protein quality among modified endosperm *opaque-2* tropical maize inbreds. Crop Science 33: 1229-1234.

Pixley KV and Bjarnason MS (2002) Stability of grain yield, endosperm modification and protein quality of hybrid and open-pollinated quality protein maize (QPM) cultivars. Crop Science 42: 1882-1890.

Prasanna BM, Vasal SK, Kassahun B and Singh NN (2001) Quality protein maize. Current Science 81: 308-1319.

Prinsloo MA (1988) Effects of cultivation on the content and availability of nitrogen in selected Orange Free State soils. MSc thesis, Department of Soil Science, University of the Free State, Bloemfontein.

Ramagosa I and Fox PN (1993) Genotype x Environment interaction and adaptation. In: MD Hayward, NO Bosemark and I Ramagosa (Eds.). Plant breeding: Principles and prospects. Chapman and Hall, London, pp. 373-390.

Roach DA and Wulff RD (1987) Maternal effects in plants. Annual Review of Ecology Systems 18: 209-235.

Russell WK, Eskridge KM, Travnicek DA and Guillen-Portal FR (2003) Clustering environments to minimize changes in rank of cultivars. Current Science 43: 858-864.

Sabata RJ and Mason SC (1992) Corn hybrid interaction with soil nitrogen level and water regime. Journal of Production Agriculture 5: 137-142.

SAGL (2011) South African Maize Crop Quality Report 2010/2011Season. Available on the internet from: http://www.sagl.co.za/Portals/0/FullMaizereport2.pdf [Date Accessed: 27 August 2012].

Sallah PYK, Obeng-Antwi K, Asiedu EA, Ewool MB and Dzah BD (2003) Recent advances in the development and promotion of quality protein maize in Ghana. In: B Badu-Apraku (Eds.). Maize revolution in West and Central Africa, pp.419.

Samonte SOPB, Wilson LT, McClung AM and Medley JC (2005) Targeting cultivars onto rice growing environments using AMMI and SREG GGE biplot analysis. Crop Science 45: 2414-2424.

Scott MP, Peterson JM and Hallauer AR (2009) Evaluation of combining ability of quality protein maize derived from U.S. public inbred lines. Maydica 54: 449-456.

Seiam MA and Khalifa KI (2007) Heterosis and phenotypic correlation for oil, protein and starch content in 81 maize inbreds, hybrids and populations. Egyptian Journal of Plant Breeding 11: 209-221.

Shafii B and Price WJ (1998) Analysis of genotype-by environment interaction using the additive main effects and multiplicative interaction model and stability estimates. Journal of Agricultural, Biological and Environmental Statistics 3: 335-345.

Shukla GK (1972) Some statistical aspects of partitioning genotype-environmental components of variability. Heredity 29: 237-245.

Shukla SK and Pandey MP (2008) Combining ability and heterosis over environments for yield and yield components in two-line hybrids involving thermosensitive genetic male sterile lines in rice (*Oryza sativa* L.). Plant Breeding 127: 28-32.

Sofi PA, Shafiq A, Wani A, Rather G and Shabir HW (2009) Quality protein maize (QPM): Genetic manipulation for the nutritional fortification of maize, Journal of Plant Breeding and Crop Science 1: 244-253.

Sprague GF and Tatum LA (1942) General vs. specific combining ability in single crosses of corn. Journal of the American Society of Agronomy 34: 923-932.

Steel RG and Torrie JH (1980) Principles and procedures of statistics. McGraw-Hill, New York.

Sughroue JR and Hallauer AR (1997) Analysis of the diallel mating design for maize inbred lines. Crop Science 37: 400-405.

Sung TM (1984) Xenia effect in modified endosperm texture opaque-2 maize. Maize Genetics Co-operation Newsletter 58: 22-23.

Taghouti M, Gaboun F, Nsarellah N, Rhrib R, El-Haila M, Kamar M, Abbad-Andaloussi F and Udupa SM (2010) Genotype x environment interaction for quality traits in durum wheat cultivars adapted to different environments. African Journal of Biotechnology 9: 3054-3062.

Thillainathan M and Fernandez GCJ (2001) SAS applications for Tai's stability analysis and AMMI model in genotype x environmental interaction (GEI) effects. The Journal of Heredity 92: 367-371.

UNDP (2008) Available on the internet from: http://www.un.org/millenniumgoals/2008 highlevel/pdf/newsroom/Goal4FINAL.pdf [Date Accessed: 01 September 2009].

UNICEF (2006) http://www.unicef.org/progressforchildren/2006n4/index_undernutrition.html#note2 [Date Accessed: 07 June 2010].

UNICEF (2010) Available on the internet from: http://www.unicef.org/infobycountry/southafrica_statistics.html#87 [Date Accessed: 02 July 2011].

UNICEF (2012) The state of the world's children. UNICEF. Available on the internet from: http://www.unicef.org/iran/SOWC_2012Main_Report_EN_13Mar2012.pdf [Date Accessed: 01 May 2012].

Upadhyay SR, Gurung DB, Paudel DC, Koirala KB, Sah SN, Prasad RC, Pokhrel BB and Dhaka R (2009) Evaluation of quality protein maize (QPM) genotypes under rainfed mid-hill environments of Nepal. Nepal Journal of Science and Technology 10: 9-14.

Vargas W, Crossa J, Van F, Rami M, Sayre K (1999) Using partial least squares regression factorial regression and AMMI models for interpreting genotype and environment interaction. Crop Science 39: 955-967.

Vasal SK (1975) Use of genetic modifiers to obtain normal type kernels with the *opaque-2* gene. In High quality protein maize: Proceedings of the CIMMYT–Purdue international symposium on protein quality in maize. El Batan, Mexico, pp. 197-216.

Vasal SK, Srinivasan G, Pandey S, Gonzalez CF, Crossa J and Beck DL (1993) Heterosis and combining ability of CIMMYT's quality protein maize germplasm: I. Lowland tropical. Crop Science 33: 46-51.

Vasal SK, Villegas E, Bjarnason M, Gelaw B and Goertz P (1980) Genetic modifiers and breeding strategies in developing hard endosperm opaque-2 materials. In: WG Pollmer and RH Phipps (Eds.) Improvement of quality traits of maize for grain and silage use. Martinus Nijhoff, The Hague, pp. 37-73.

Vasal SK, Villegas E, Tang CY, Werder J and Read M (1984) Combined use of two genetic systems in the development and improvement of quality protein maize. Kulturpflanze 32: 171-185.

Vivek BS, Krivanek AF, Palacios-Rojas N, Twumasi-Afriyie S and Diallo AO (2008) Breeding Quality Protein Maize (QPM): Protocols for Developing QPM Cultivars. Mexico, D.F.: CIMMYT, 50pp.

Wallace DH, Baudoin JP, Beaver J, Coyne DP, Halseth DE, Masaya PN, Munger HM, Myers JR, Silbernagel M, Yourstone KS and Zobel RW (1993) Improving efficiency of breeding for higher crop yield. Theoretical and Applied Genetics 86: 27-40.

Wegary D, Labuschagne MT and Vivek BS (2011) Protein quality and endosperm modification of quality protein maize (*Zea mays* L.) under two contrasting soil nitrogen environments. Field Crops Research 121: 408-415.

Wessel-Beaver L, Lambert RJ and Dudley JW (1985) Genetic variability and correlations in a modified endosperm texture *opaque-2* maize population. Crop Science 25: 129-132.

WHO (1999) World Health Organisation. Management of severe malnutrition: a manual for physicians and other senior health workers. WHO. Geneva.

WHO (2012) Trends in maternal mortality: 1990 to 2010 WHO, UNICEF, UNFPA and the World Bank estimate. Available on the internet from: http://www.unfpa.org/webdav/site/global/shared/documents/publications/2012/Trends_in_maternal mortality_A4-1.pdf [Date Accessed: 27 August 2012].

Worku M, Bänziger M, Friesen D, SchulteAuf´m Erley G, Diallo AO, Vivek B and Horst WJ (2007) Protein quantity and quality, and grain yield performance of quality protein maize and normal endosperm maize under different levels of nitrogen. African Crop Science Conference Proceedings 8: 1905-1909.

Wricke G (1962) Über eine Method zur Erfassung der Ökologischen Streuberite in Feldversuchen. Z. Pflanzenzüchtg 47: 92-96.

Yan W, Cornelius PL, Crossa J and Hunt LA (2001) Two types of GGE Biplots for analyzing multi-environment trial data. Crop Science 41: 656-663.

Yan W, Hunt LA, Sheng Q and Szlavnics Z (2000) Cultivar evaluation and mega-environment investigation based on the GGE biplot. Crop Science 40: 596-605.

Yan W and Kang MS (2003) GGE biplot analysis: A graphical tool for breeders, geneticists, and agronomists. CRC Press, Boca Raton, Florida, USA.

Zaidi PH, Vasal SK, Maniselvan P, Jha G, Mehrajjudin C and Singh RP (2009) Stability in performance of quality protein maize under abiotic stress. Maydica 53: 249-260.

Zobel R, Wright M and Gauch H (1988) Statistical analysis of yield trial. Agronomy Journal 80: 388-393.

Zhang Y and Kang MS (1997) Diallel-SAS: A SAS program for Griffing's diallel analyses. Agronomy Journal 89: 176-182.

Chapter 3

Effects of pollination method on tryptophan content of 12 quality protein maize inbred lines in South Africa

3.1 Abstract

Maize is one of the most common cereal crops in SA, which produces grain for human and animal consumption due to its considerable protein content. However, its protein lacks two essential amino acids, tryptophan and lysine. Xenia effect is the immediate genetic and physiological effects which the male parent pollen can exert on the development of seed in the female parent. Xenia effects can cause significant changes in maize quality and agronomic traits. Although the changes are sometimes beneficial they are, however, detrimental when they occur in maize field trials where the accurate genotypic potential needs to be evaluated. In order to achieve the real genotypic potential, maize germplasm are usually being self-pollinated for the purpose of traits evaluation. Several researchers have studied the effects of pollination on kernel quantity and quality traits in normal maize but very few have studied this in QPM. If pollination method has no effect on kernel traits, it would not be necessary to do self-pollination in field evaluation. The objective of the study was to measure the effects of the pollen parent on tryptophan content in 12 QPM inbred lines. The inbred lines were self and cross-pollinated in the field. Hans Male was used as the pollen parent for cross-pollination. The trial was replicated three times with two row plots, where one row was self-pollinated and the other cross-pollinated. Kernels of the 12 QPM inbred lines from self and cross-pollinated rows were harvested and used for determination of the tryptophan content. This was done spectrophotometrically and statistically analysed using a paired *t*-test. Results indicated that there was no significant ($P > 0.05$) difference between the two pollination methods. The findings indicate that the QPM inbred lines can be evaluated in field trials without being self-pollinated.

3.2 Introduction

Maize is an important cereal crop, which ranks third in the world after rice (*Oryza sativa* L.) and wheat (*Triticum aestivum* L.). In SA it is preferred by the majority of the population and is the primary staple food crop. It is used as a source of protein for human and other monogastric animals (NRC, 1988; Bjarnason and Vasal, 1992). Normal endosperm maize protein is deficient in two essential amino acids, lysine and tryptophan (FAO/WHO/UN, 1985; Prasanna *et al.,* 2001). In mature maize grain the germ protein is superior in both quantity and quality (Vasal, 2000), even though the majority of the protein is in the endosperm (82%). The protein in the endosperm contains zeins; a group of four structurally distinct alcohol-soluble proteins which account for 50-70% of the endosperm protein and are rich in glutamine.

QPM can be used as a replacement of normal maize in communities where maize is used as a source of protein, because QPM has improved nutritional value. Various researchers have studied the importance of QPM for human and animal feed (pigs and broiler chickens) and have recommended it for use (Singh *et al.,* 1980; Dei, 1997; Rahmanifar and Hamaker, 1999; Krivanek *et al.,* 2007; Onimisi *et al.,* 2009; Gunaratna *et al.,* 2010). For maize to be considered QPM the lysine and tryptophan concentration in the grain must be more than doubled and this is usually expressed when the opaque-2 gene in the endosperm tissue is homozygous (Mertz *et al*., 1964; Vasal, 2000; Sentayehu, 2008; Nurit *et al.,* 2009). Tryptophan and lysine concentration in maize have been found to be highly correlated (Villegas *et al.,* 1984; Nurit *et al.,* 2009) (Figure 3.1).

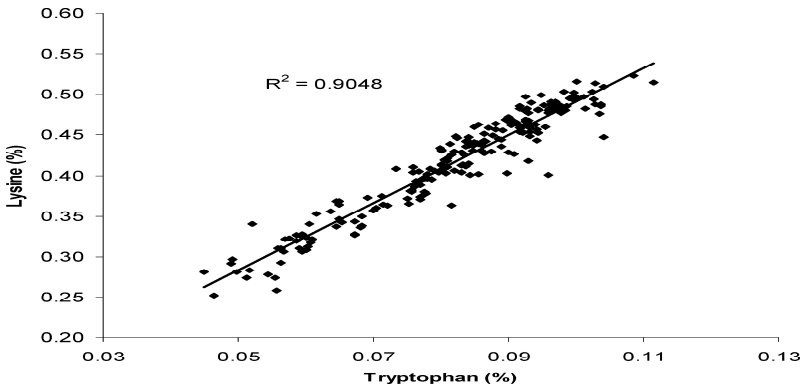

Figure 3.1 Correlation between tryptophan and lysine content in maize grain (Nurit *et al.,* 2009).

Maize is a cross-pollinating crop, which is mainly grown in open pollinated field plots without restriction of pollen interchanging from one plot to the other when evaluating germplasm for yield and quality traits. Xenia effect is considered the immediate genetic and physiological effects, which the male parent pollen can exert on the development of seed in the female parent (Castañeda, 2010**)**. The effect is immediate in maize as compared to other crops. Research on pollination methods plays an important role in determining whether maize genotypes need to be self-pollinated or just left to open-pollinate when tested for research purposes in field trials. When the effect is not significant it is not necessary to self-pollinate genotypes when planting them for research trials, but when the effect is significant it will result in a need to self-pollinate in order to determine the exact potential of the genotype and to avoid contamination. The expensive and time-consuming step of selfing germplasm during research field trials can be potentially eliminated by first conducting pollination effects studies. For evaluation of germplasm in research plots, methods such as isolation of trials in open-pollinated fields by distances and time can be used, however this is often impractical. The self-pollination and isolation methods are labour intensive, expensive and time consuming during testing stages especially where a large number of materials are to be tested.

Several researchers have studied the effects of pollination on kernel quantity and quality in normal maize (Bosch *et al.,* 1978; Bulant and Gallais 1998; Krieger *et al.,* 1998; Letchworth and Lambert, 1998; Bulant *et al.,* 2000; Weingartner *et al.,* 2002; Balestre *et al.,* 2007), however, only a few researchers studied pollination methods in QPM (Sung 1984; Pixley and Bjarnason, 1994; Hossain *et al.,* 2008). Most of the studies on normal maize endosperm have shown the importance of these effects, while in QPM few of the studies showed the importance of these effects, especially when foreign pollen is from another QPM. Since it is important to know whether the pollen from a different QPM genotype causes changes in protein quality, an investigation into this is necessary in order to understand how to proceed with the rest of the research objectives. The objective of this study was therefore to measure the effect of the pollen parent on quality traits of QPM inbred lines.

3.3 Materials and methods

3.3.1 Germplasm

Thirteen white QPM inbred lines were obtained from the ARC-GCI and CIMMYT Harare maize breeding programmes (Table 3.1). The lines having an O code after the first letter in the name were developed by Dr. Hans Gevers whilst he was at the ARC-GCI. The CML coded lines, also known as CIMMYT maize lines, are internationally accepted, and are commonly used in eastern and southern Africa for QPM development. Hans Male line which was used as the pollen source for the crosses was obtained from the Quality Seed Company in KwaZulu-Natal, SA.

Table 3.1 List of 13 QPM inbred lines used to determine the pollen parent effects on tryptophan content

Lines	HG	Origin	ADT
CML144	B	CIMMYT	LT
CML176	B	CIMMYT	LT
CML176-3	B	CIMMYT	LT
CML182	B	CIMMYT	ST
SO503W	M	ARC-GCI	STTW
SO607W	P	ARC-GCI	STTW
SO713W	P	ARC-GCI	STTW
RO421W	F	ARC-GCI	STTW
RO424W	M	ARC-GCI	STTW
RO544W	F	ARC-GCI	STTW
KO54W	F	ARC-GCI	STTW
FO215W	P	ARC-GCI	STTW
Hans Male	---	Quality seed	STTW

HG=heterotic groupings; ADT=adaptability; LT=lowland tropics; ST=subtropics; STTW=subtropical temperate warm

3.3.2 Environment

The field trial was conducted during the 2007/08 growing season, at the ARC-GCI Research station in Potchefstroom (26.7° latitude, 27.1° longitude and about 1349 m altitude). During the growing season the location received precipitation of 544.1 mm, with an average maximum and minimum temperature of 27.1^{o}C and 11.2^{o}C, respectively. An overall average temperature of 18.8^{o}C was recorded during the growing season (Table 3.2). The soil type at the Potchefstroom farm is a brownish sandy clay loam.

Table 3.2 Weather data for the growing season at Potchefstroom

Months	Tx (°C)	Tn (°C)	T (°C)	Rn (mm)
Nov	28.42	14.17	20.93	79.5
Dec	29.73	16.48	22.54	206.2
Jan	30.81	15.98	23.13	45.1
Feb	31.18	15.2	22.85	73.4
March	29.52	13.42	21.02	50.3
April	25.49	10.6	17.49	64.5
May	22.76	2.61	12.43	0
June	19.43	1.15	9.6	25.1
Average	**27.1**	**11.20**	**18.75**	**544.1**

Tx=Average maximum temperature; Tn=Average minimum temperature; T=Average temperature; Rn=Average total rainfall

3.3.3 Experimental layout

The experiment was laid out as an alpha lattice where each pair of treatments appeared together in a block once or not at all (Patterson and Williams, 1976), within a split-plot arrangement. The main-plot was aligned as the maize inbred lines and sub-plots as pollination treatments. The trial had two row plots replicated three times. Land was ploughed and disked using a tractor-drawn plough and disc harrow. Planting rows of 0.9 m apart were marked and two maize seeds per inbred line were planted per station with a jab-planter. Spacing within the row was 0.3 m between the stations in a 5 m row.

3.3.4 Pollination method

Ears of one row were cross-pollinated while those of the other row in the plot were self-pollinated. Self-pollination was done as follows: The shoot was covered in a transparent ear shoot bag before silks appeared out of the husks to prevent pollen contamination. During flowering, a brown pollination paper bag was placed on the tassel of the same maize plant for collection of pollen. This was usually done by covering the tassels in the afternoon and do pollinations mid-morning when all foreign pollen would have died. The pollen was transferred manually from the tassels and deposited on the silks of the same plant by carefully removing the transparent ear shoot bag and depositing pollen on the silks and covering the ear with the brown pollination bag. The bag stayed on the ear until full maturity to prevent contamination that could take place after self-pollination.

For cross-pollination the same method was used as for self-pollination whereby the ear shoot was covered before silk emergence. During flowering the pollen was manually transferred from the male parent to the silk of the female parents in the cross-pollination row. Ears were immediately covered with brown pollination bags after pollination and these were left until the ears were harvested.

3.3.5 Trial management

Standard cultural practices for growing maize were followed. Gaucho® (active ingredient: Imidacloprid) was applied to maize seeds before planting, to guard against soil borne insects. Thinning was done to one plant per station three weeks after emergence to maintain a plant population density of 37 037 plants ha^{-1}. Fertiliser was applied at a rate of 100 kg N ha^{-1} to all plots. The N was given as a basal application at planting as compound fertiliser 3:2:1 (32) + Zn translating to 15% N, 10.7% P, 5.3% K and 0.5% Zn. Lime ammonium nitrate (LAN) with 28% N was used for top-dressing in two equal splits at 28 and 56 days after emergence at a rate of 50 kg ha^{-1} each. Weeds were effectively controlled by herbicides and hand weeding. The pre-emergence Sorgomil Gold (active ingredient: S-metolachlor/terbuthylazine), and post-emergence Basagran (active ingredient: bendioxide) herbicides were applied at a rate of 2.6 L. ha^{-1} and 2.0 L. ha^{-1}, respectively, to control weeds. Throughout the growing season weeds were controlled by

hand weeding. Kombat (active ingredient: Carbaryl) was applied in the maize funnel of affected plants to effectively control stalk borer (*Buseola fusca*) infestation, four weeks after emergence. Irrigation was applied at planting, when the leaves showed signs of rolling and during pollination to encourage silking. The plants were left to field dry for about two weeks after reaching physiological maturity. The ears for each pollination method were hand harvested separately and then shelled for tryptophan analysis. In this study only tryptophan content was measured as lysine and tryptophan contents are highly correlated (Figure 3.1).

3.3.6 Tryptophan analysis

Twenty kernels of each inbred line of each pollination treatment, with uniform size were selected and finely ground at the ARC-GCI. The milled samples were sent to the ARC-Animal Production Institute at Irene for tryptophan analysis. Tryptophan was determined spectrophotometrically according to the method of Devries *et al.* (1980) after alkaline hydrolysis of the samples (AOAC, 1995; Method 982.30).

3.3.7 Statistical analysis

To determine the effects of cross-pollination on tryptophan concentration, a paired *t*-test was done (Steel and Torrie, 1980). From the tryptophan concentration of both self and cross-pollinated kernels, a cross fertilisation effect (R) was deduced as the relative increase of cross-pollinated tryptophan content compared to self-pollinated tryptophan content. For each inbred line, the relative cross fertilisation effect was computed as the difference between mean tryptophan content of crossed (CP) and selfed (SP) plants divided by the mean tryptophan content of selfed plants (Bulant *et al.,* 2000; Pahlavani and Abolhasani, 2006).

RCPE= [(CP - SP) / SP x 100],

Where, RCPE=relative cross fertilisation effect,
CP=mean tryptophan content of cross-pollinated plants, and
SP=mean tryptophan content of self-pollinated plants.

3.4 Results

The pollination effects were calculated by comparing the mean differences in tryptophan content between selfed and crossed seed over the different crosses and selfs. No significant (P > 0.05) differences were observed (Table 3.3). Tryptophan content mean for self-pollination, cross-pollination, cross-pollination differences and the relative cross pollination effects were 0.099, 0.098, -0.00108 and 2.7725, respectively. The pollination differences of the tryptophan content varied from -0.024 (SO503W) to 0.026 (RO544W). Relative xenia effect ranged from 29% (RO544W x Hans Male) to -24% (SO503W x Hans Male).

Table 3.3 Effects of two pollination methods on tryptophan content of 12 quality protein maize inbred lines

Inbred lines	SP	CP (x HM)	CPD (cp-sp)	RCPE
SO607W	0.111	0.099	-0.012	-10.81
CML176	0.116	0.110	-0.006	-5.17
SO503W	0.101	0.077	-0.024	-23.76
CML182	0.106	0.087	-0.019	-17.92
RO421W	0.102	0.096	-0.006	-5.88
KO54W	0.108	0.122	0.014	12.96
CML144	0.091	0.094	0.003	3.30
CML176-3	0.103	0.100	0.003	2.91
FO215W	0.093	0.102	0.009	9.68
SO713W	0.081	0.095	0.014	17.28
RO544W	0.090	0.116	0.026	28.89
RO424W	0.078	0.095	0.017	21.79
Mean	**0.099**	**0.098**	**-0.001**	**2.773**
SD	**0.012**	**0.012**		**16.126**
SEM	**0.003**	**0.004**		
P value			**0.812ns**	
***T*test**			**0.244ns**	

SP=self-pollination; CP=cross-pollination; CPD=cross-pollination differences; HM=Hans Male; RCPE=relative cross fertilisation effect**;** SEM=standard error of the mean

3.5 Discussion

The importance of xenia effect on endosperm modification, protein quantity and quality has been previously reported in both normal maize and QPM and most of the research findings are contradicting. Vivek *et al.* (2008) mentioned that in QPM when white is crossed with yellow there is an immediate visible impact at harvest on the white endosperm maize which will have yellow kernels present. This is due to the fact that the allele for yellow colour is dominant. Cordova (2000) and Machida (2008) on the other hand, studied QPM and normal endosperm maize crops growing under conditions not designed to completely prevent cross-pollination or inter-mating to determine the levels of contamination by normal endosperm maize to the nutritional quality of the QPM endosperm. They found that the pollen from the normal endosperm maize had an immediate effect on the nutritional quality of the QPM. The contamination levels to the QPM were found to be ranging from 15.3% to 31.9% and were not as high as previously expected. They concluded that QPM can be planted together with normal maize endosperm without QPM losing its entire nutritional advantage (Machida, 2008; Machida *et al.*, 2012).

Hohls *et al.* (1995) indicated that in their study they selfed some of the inbred plants to be used for endosperm modification analysis due to the fact that Wessel-Beaver and Lambert (1982) and Sung (1984) found that xenia effects significantly changed endosperm traits of normal and modified opaque-2 maize. Garcia and Souza (2002) hand pollinated their materials to control pollen within each plot in order to avoid the xenia effects. These efforts by the researchers indicate the importance of xenia effects.

Tsai and Tsai (1990) in crosses between P3732 and B73 x Mo17 found a significant ($p \leq 0.05$) cross-pollination effect causing a 20% increase in protein content and 12.4% increase in kernel weight compared to self-pollination of the P3732 genotype. Pixley and Bjarnason (1994) in a study with different QPM genotypes planted in research trials using open and self-pollination, investigated whether the pollen-parent affected the protein quality and quantity. They used six pollen treatments (four QPM inbreds, normal maize inbred and self-pollination) on the different QPM inbreds in the study. The results indicated that the pollen parent does not have an effect on protein quality traits when the

females were pollinated by a QPM male or self-pollinated. There were slight differences on kernel vitreousness for females pollinated by QPM males compared to self-pollination. Protein concentration in the grain was not affected by pollen when QPM females were crossed with normal endosperm maize. Respectively, tryptophan in grain and tryptophan in the protein were reduced by 37% and 38% and kernel modification was improved by normal endosperm maize pollen. Furthermore they indicated that for all traits measured, the interaction of males x females were not significant. They concluded that QPM germplasm can inter-pollinate in open field plots except when normal endosperm maize is involved. Their research findings were valuable for QPM research because it meant that self-pollination, which is expensive and labour intensive, will not be necessary when evaluating QPM germplasm for protein quality and kernel traits.

In this study pollen parent effects did not play a significant role in the concentration of tryptophan in QPM. Results of this study confirmed the results of Pixley and Bjarnason (1994), who studied both endosperm and tryptophan concentration and found no significant pollen parent effects and pollination methods x genotype interaction on protein quality traits. But their results were not in agreement with studies when QPM females were pollinated by normal endosperm (Machida, 2008; 2012; Vivek *et al.,* 2008). These results were different in the sense that normal endosperm maize rather than QPM was used.

3.6 Conclusions

The differences between cross and self-pollinated seeds were determined to measure the effect of cross-pollination in QPM. Tryptophan concentration of the self-pollinated inbreds did not differ significantly from the cross-pollinated material. This indicated that cross-pollination effect does not play a major role in the control of tryptophan content in QPM. The findings of this study meant that pollen from Hans Male did not have a major effect on the tryptophan concentration of the 12 inbred lines and therefore for this study it can be concluded that it is not necessary to self-pollinate QPM inbred lines when evaluating them in small research plots, since the potential of the inbreds can be accurately determined in open pollinated fields. However, in order to confirm these results additional research should be done with more than one male parent for the same

number of females. Future research may also include the effect of location with the pollination methods on tryptophan content to see the importance of pollination methods and location, since others have found significant differences when the materials were evaluated on multi-location basis (Krieger *et al.*, 1998).

3.7 References

AOAC (1995) Official methods of analysis 982.30. 16th ed. Washington, DC: Association of Official Analytical Chemists.

Balestre M, Souza JC, Luders RR and Silva NO (2007) Effect of allo-pollen in artificial crosses of white and yellow endosperm maize hybrids. Crop Breeding and Applied Biotechnology 7: 82-87.

Bjarnason M and Vasal SK (1992) Breeding of Quality Protein Maize (QPM). In: J Jarick (Eds.). Plant breeding Reviews. Vol 9. John Wiley and Sons, Inc., New York, USA, pp. 181-216.

Bosch L, Blanco M, Alvarez A, Pons A and Blanco JL (1978) Pollination effects on the grain yield and the qualities of the fertilized plants, II: self-pollination with o2 pollen vs. cross-pollination with o2+ pollen. Maize Genetics Cooperation Newsletter 52: 122-123.

Bulant C and Gallais A (1998) Xenia effects in maize with normal endosperm: I. Importance and stability. Crop Science 38: 1517-1525.

Bulant C, Gallais A, Matthys-Rochon E and Prioul JL (2000) Xenia effects in maize with normal endosperm: II. Kernel growth and enzyme activities during grain filling. Crop Science 40: 182-189.

Castañeda LA (2010) Xenia Effects on Quality of a Maize Female Inbred in Production of Hybrid Seed. PhD thesis, The Graduate School at University of Nebraska, Lincoln, Nebraska.

Cordova H (2000). Quality protein maize: improved nutrition and livelihoods for the poor. Maize Research Highlights 1999-2000, CIMMYT. Available on the internet from: http://apps.cimmyt.org/Research/Maize/results/mzhigh99-00/mrhigh99-00_qual.pdf [Date Accessed: 08 August, 2012].

Dei HK (1997) Quality - protein maize (QPM) as a feed ingredient for layer chickens. MSc thesis in Animal Science, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

DeVries JW, Koski CM, Egberg DC and Larson PA (1980) Comparison between a spectrophotometric and a high-pressure liquid chromatography method for determining tryptophan in food products. Journal of Agricultural Food Chemistry 28: 896-898.

FAO/WHO/UN **(**1985) Expert Consultation: WHO Technical Report Series No. 724. World Health Organization, Geneva. Available on the internet from: www.fao.org/docrep/003/aa040e/AA040E00.HTM [Date Accessed: 08 August, 2012].

Garcia AAF and deSouza CL Jr (2002) Phenotypic recurrent selection to improve protein quality in non-opaque maize populations. Scientia Agricola 59: 743-748.

Gunaratna NS, De Groote H, Nestel P, Pixley KV and McCabe G (2010) Evaluating the impact of biofortification: A meta-analysis of community-based studies on quality protein maize (QPM). Food Policy 35: 202-210.

Hohls T, Shanahan PE, Clarke GP and Gevers HO (1995) Genetic control of kernel modification found in South African quality protein maize inbred lines. Euphytica 87: 103-109.

Hossain F, Prasanna BM, Kumar R and Singh BB (2008) The effect of genotype x pollination mode interaction on kernel modification in quality protein maize (QPM) genotypes. Indian Journal of Genetics 68: 132-138.

Krieger KM, Pollak LM, Brumm TJ and White PJ (1998) Effects of pollination method and growing location on starch thermal properties of corn hybrids. Cereal Chemistry 75: 656-659.

Krivanek AF, De Groote H, Gunaratna N, Diallo A and Friesen D (2007) Breeding and disseminating quality protein maize (QPM) for Africa. African Journal of Biotechnology 6: 312-324.

Letchworth MB and Lambert RJ (1998) Pollen parent effects on maize kernels. Crop Science 38: 363-367.

Machida L (2008) 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. PhD thesis, African Centre for Crop Improvement, School of Agricultural Sciences and Agribusiness, Faculty of Science and Agriculture. University of KwaZulu-Natal, South Africa.

Machida L, Derera J, Tongoona P, Mutanga O and MacRobert J (2012) Geostatistical analysis of quality protein maize outcrossing with pollen from adjacent normal endosperm maize varieties. Crop Science 52: 1235-1245.

Mertz ET, Bates LS and Nelson OE (1964) Mutant that changes protein composition and increases lysine content of maize endosperm. Science 145: 279-280.

NRC (1988) Quality Protein Maize. National Academy Press, Washington, D.C. USA, pp. 41-54.

Nurit E, Tiessen A, Pixley KV and Palacios-Rojas N (2009) Reliable and inexpensive colorimetric method for determining protein-bound tryptophan in maize kernels. Journal of Agricultural and Food Chemistry 57: 7233-7238.

Onimisi PA, Omage JJ, Dafwang II and Bawa GS (2009) Replacement value of normal maize with quality protein maize (Obatampa) in broiler diets. Pakistan Journal of Nutrition 8: 112-115.

Pahlavani MH and Abolhasani K (2006) Xenia effect on seed and embryo size in cotton (*Gossypiumhirsutum* L.). Journal of Applied Genetics 47: 331-335.

Patterson HD and Williams ER (1976) A new class of resolvable incomplete block designs. Biometrika 63: 83-92.

Pixley KV and Bjarnason MS **(**1994**)** Pollen-parent effects on protein quality and endosperm modification of quality protein maize. Crop Science 34: 404-409.

Prassana BM, Vasal SK, Kassahun B and Singh NN (2001) Quality protein maize. Current Science 81: 1308-1309.

Rahmanifar A and Hamaker BR (1999) Potential nutritional contribution of quality protein maize: a close-up on children in poor communities. Ecology of Food and Nutrition 38: 165-182.

Sentayehu A (2008) Protein, tryptophan and lysine contents in quality protein maize (QPM), North India. Ethiopian Journal of Health Science 18: 9-15.

Singh J, Koshy S, Agarwal K, Singh NN, Lodha M and Sethi A (1980). Relative efficiency of opaque-2 maize in growth of preschool children. Indian Journal of Nutrition and Dietetics 17: 326-334.

Steel RGD and Torrie JH (1980) Principles and procedures of statistics: a biometrical approach. McGraw-Hill, New York, 633pp.

Sung TM (1984) Xenia effect in modified endosperm texture opaque-2 maize. Maize Genetics Cooperation Newsletter 58: 22-23.

Tsai CL and Tsai CY (1990) Endosperm modified by cross-pollination maize to induce changes in dry matter and nitrogen accumulation. Crop Science 30: 804-808.

Vasal SK (2000) The quality protein maize story. Food Nutrition Bulletin 21: 445-450.

Villegas E, Ortega E and Bauer R (1984) Chemical methods used at CIMMYT for determining protein quality in cereal grains. CIMMYT, Mexico City, Mexico.

Vivek BS, Krivanek AF, Palacios-Rojas N, Twumasi-Afriyie S and Diallo AO (2008) Breeding Quality Protein Maize (QPM): Protocols for Developing QPM Cultivars. Mexico, D.F.: CIMMYT, 50pp.

Weingartner U, Kaeser O, Long M and Stamp P (2002) Combining cytoplasmic male sterility and xenia increases grain yield of maize hybrids. Crop Science 42: 1848-1856.

Wessel-Beaver L and Lambert RJ (1982) Genetic control of modified endosperm texture in *opaque-2* maize. Crop Science 22: 1095-1098.

Chapter 4

Genotype by environment interaction for grain quality traits in quality protein maize under low and optimum nitrogen conditions

4.1 Abstract

Evaluation of maize inbred lines for grain quality traits in multi-location trials usually results in GEI. The GEI is known to influence the selection of superior genotypes in breeding programmes and results in inconsistency in the performance of genotypes from one environment to another. Understanding GEI is of great importance for the identification and selection of superior genotypes. The objective of this study was to determine genotypic variation and the existence and magnitude of GEI on endosperm hardness, QI and tryptophan, protein, starch and oil content in QPM inbred lines. Twelve QPM inbred lines were grown in four locations during the 2009/10 growing season. The four locations consisted of two N levels resulting in eight environments. Data were collected on endosperm hardness, QI, protein, tryptophan, starch and oil content. Single and combined ANOVA were performed on the data for genotype, environment and GEI effects. Large genotypic differences were detected in all grain quality traits in both low and optimum N environments. For combined ANOVAs genotypes, environments and GEI effects were highly significant for all traits except for endosperm hardness. AMMI and genotype main effect and GGE biplots were employed in the identification of superior genotypes and ideal environments for significant traits. The AMMI and GGE biplot analyses were more efficient in extracting the total GEI variation, with both explaining more than 60% of GEI variation than ANOVA. GGE biplots explained a greater proportion of the sum of squares of the GEI, except for tryptophan content, and it is better for visualisation of environments and cultivar performance than the AMMI analysis. AMMI and GGE biplots were similar in identifying high performing genotypes for all traits except for oil concentration, and the most stable genotypes except for QI, protein and oil concentration. GGE biplots were also able to identify the ideal (high performing and stable) genotypes as KO54W, KO54W, Hans Male, SO503W and CML144 for QI, tryptophan, protein, oil and starch content respectively, for further use in QPM breeding programmes. The GGE biplots for “which-won-where” further assessed

which genotypes performed well in which environments. For tryptophan content BO163W, KO54W and SO503W were the vertex genotypes and performed similar at the Potchefstroom low N (PL) environment. Hans Male and RO559W were the best genotypes across eight environments for protein content, whilst KO54W and SO713W were the best across seven environments for QI. For oil content Hans Male was the best at Cedara optimum N (CH), Towoomba optimum N (TWH), Cedara low N (CL) and Towoomba low N (TWL), while SO503W was the best at TWL, Tshiombo optimum N (TSH), Potchefstroom optimum N (PH) and Tshiombo low N (TSL), and FO215W at PL. CML144 was the best at CH, PH, TWH, TSH, CL, PL and TSL, and BO163W at TWL for starch content. GGE biplots identified CH as the most representative and discriminating environments for QI, tryptophan and starch concentration, and TSH and PH were for protein and oil concentration, respectively. AMMI biplots identified the highest yielding environments as TWH for tryptophan and protein concentration, TSH for QI and starch concentration and PH for oil concentration. The results were expected because all the environments were representative of the optimum N conditions.

4.2 Introduction

In SA maize covers about 60% of the cropping area and constitutes more than 70% of grain production (Akpalu *et al.,* 2008). It plays an important role for food, feed and industrial purposes and is mainly comprised of 70-75% starch, 8-10% protein and 4-5% oil (Boyer and Hannah 1994; Mukharib, 2006). Its importance increases with improvement of grain quality characters such as endosperm hardness, protein, starch, oil and tryptophan concentration for which some the South African maize industry is extremely strict. Public and private maize breeding programmes up to this point have mainly focused on these traits, because improving grain quality provides various end users with grain that is better suited to their needs. The SAGL annually conducts maize quality surveys to determine the quality of the maize produced in the country, using various grading systems. During the 2010/11 growing season they reported that the nutritional values of South African maize were ranging between 2.8-5.8% oil, 6.1-12.7% protein and 58.3-77.0% starch (SAGL, 2011).

Environment has a major influence on different quality traits in maize genotypes, and the environmental conditions in which maize is grown vary widely. The crop is grown in areas receiving an annual rainfall of 250 to 5000 mm, from latitude 58^0 N to 40^0 S and from sea level to higher than 3000 m altitude (Paterniani, 1990; Downsell *et al*., 1996), and has spread across the world because of its high adaptability to a wide range of environments. In SA maize is grown from semi-arid to sub-tropical wet regions across the nine provinces and is highly vulnerable to changes in rainfall and temperature (Benhin, 2006; Durand, 2006). The areas differ considerably in terms of soil type and climatic conditions. Environmental factors such as soil N availability, drought, temperature and heat stress have an influence on kernel filling, kernel hardness, yield, starch, oil and protein concentration in normal endosperm maize (Kettlewell, 1996; Commuri and Jones, 2001; Hadi, 2004; Monjardino *et al.*, 2005; Terasawa *et al.,* 2008), and some of those factors have a major effect on grain quality characteristics in QPM. Documented constraints causing significant reduction and effects to nutritional quality and kernel hardness in QPM include low soil fertility and drought (Ngaboyisonga *et al.,* 2006; Gissa, 2008; Ngaboyisonga, 2008). In Africa fertiliser use is low when compared to the rest of the world, and subsistence farmers grow maize under declined soil fertility (FAO, 2005). Researchers usually develop and test breeding materials under different conditions to that of farmers, and that needs to change in order for those materials to perform better and become adaptable to farmer's conditions. In this study the performance of different QPM genotypes under different environments was investigated. Originally the ARC-GCI breeding materials were developed and tested under optimum conditions, while the reality is that many farmers are growing maize under low N conditions.

The most important objective of a breeding programme is to develop inbred lines and hybrids that have high breeding values for yield, grain quality and other agronomic characteristics. It is important for breeders to evaluate those different types of varieties under multi-environment trials for characteristics that are valuable to the different end users (Alake and Ariyo, 2012). Multi-environment trials are usually planted in the same year at different locations with similar varieties (Gauch, 1992). Genotypes respond differently to the environments causing significant GEI where the relative performance of varieties cannot be predicted from one environment to another (Kang, 2004). Large GEI has an important and problematic bearing on the breeding of better varieties in most

breeding programmes due to the fact that it is difficult to identify better performing genotypes and it reduces selection progress. In each environment the measured performance of each genotype is a measure of the environmental main effects, genotypic main effect and GEI, with the environmental effects accounting for about 80% of the total variation and genotypic effects and GEI representing the rest of variation even though they are most relevant for cultivar recommendation (Yan *et al.,* 2007). It is important for genotypes to show reliable performance for various characters when tested over a wide range of environmental conditions (Becker and Leon, 1988). Widely adapted and consistently performing genotypes across environments are desirable to breeders and farmers. A genotype is considered to be adaptable if it has a low degree of fluctuation in performance and a high mean when grown over diverse environments (Falconer, 1981). Consequently it is important to test genotypes across a wide range of environments and conduct stability analysis studies to select genotypes with stable characteristics.

Different researchers have observed significant GEI for yield and endosperm hardness traits in QPM hybrids (Hohls *et al.*, 1995; Pixley and Bjarnason, 2002; Preciado-Ortiz *et al.,* 2006; Shava, 2007; Gissa, 2008; Machida, 2008; Ngaboyisonga, 2008). In SA GEI has been reported in normal maize for yield (Laubscher, 2000; Alberts, 2004), in QPM for yield (Hohls *et al.*, 1995), wheat (Purchase, 2000; Solomon *et al.,* 2008), sunflower yield and oil characteristics (Schoeman, 2003; Van Der Merwe, 2010), sugarcane yield and estimated recoverable crystal (Ramburan *et al.,* 2011; Zhou *et al.,* 2012) and sweet-potato (Laurie, 2010).

In terms of analysing and understanding the existence and magnitude of GEI there are various methods which are helpful in revealing GEI. The most recently used methods are the AMMI model (Gauch, 1992; Gauch and Zobel, 1996) and GGE biplots (Yan *et al.,* 2000). In SA AMMI has been broadly used on maize, while GGE biplots have only been reported on sweet-potatoes (Laurie, 2010) and sugarcane (Ramburan *et al.*, 2011; Zhou *et al.,* 2012). The AMMI firstly extracts genotype effects and environment effects to predict genotypic performance in specific environments, and then the GEI (Gauch and Zobel, 1988; 1996), while GGE biplots extract genotype and GEI and no environment effects (Yan *et al.,* 2000; 2007). The GEI and stability analysis in QPM has mainly been conducted for grain yield, endosperm hardness and other agronomic characteristics, and

literature is limited for other important grain quality characteristics such as tryptophan, oil, starch and protein and QI.

There is limited literature in the South African context on the influence of genotype, environment and GEI effects on grain quality characters such as endosperm hardness; and none on protein, tryptophan, starch and oil concentration; and in most of these studies the entries were tested under only optimum conditions. It is of great importance to test QPM inbred lines in trials over varying growing locations in order to determine and understand the occurrence and magnitude of GEI. This allows breeders to identify and select superior QPM inbred lines for use in breeding programmes. The objective of this study was to evaluate QPM inbred lines for endosperm hardness, tryptophan, protein, oil and starch concentration in low and optimum N environments; and to determine the existence and understand the degree of GEI on grain quality characteristics.

4.3 Materials and methods

4.3.1 Germplasm

A total of 12 QPM inbred lines were evaluated. Six of the inbred lines were the same as those used in Chapter 3 except for BO163W, RO559W, RO550W, RO452W, CZL01005 and CML176-2. CZL01005 and CML176-2 are from CIMMYT-Zimbabwe while the other four inbred lines are from the ARC-GCI.

4.3.2 Field trials environment, design and management

The field trials were conducted during the 2009/10 growing season in four locations namely: Cedara, Towoomba, Potchefstroom and Tshiombo. Trials were planted under two N levels (low and optimum N) at each location resulting in a total of eight environments. At the low N sites N was applied at planting and no further N was given to the crop throughout the growing season. Potchefstroom is located in the Tlokwe municipality of the Northwest (NW) province and lies at -26.73^0 latitude, 27.08^0 longitude, at an altitude of 1349 m above sea level (masl), with brown sandy loam soils. Compound fertiliser 3:2:1 (32) + Zn was applied as a basal application at planting at a

rate of 100 kg N ha^{-1} to optimum N plots and 100 kg N ha^{-1} to low N environment. LAN with 28% N was used for top-dressing in two equal splits at 28 and 56 days after emergence at a rate of 50 kg ha^{-1} each only in optimum N plots. Cedara is located in the uMngeni municipality of the KwaZulu-Natal (KZN) province and lies at -29.54^0 latitude, 30.26^0 longitude, at an altitude of 1066 masl, with reddish brown clay soils. Fertiliser used was MAP 250 kg ha^{-1}at planting for optimum N environments, 30 kg ha^{-1} low N environment and LAN given at 150 kg ha^{-1} in two equal splits of 75 kg ha^{-1} for only the optimum N sites at 28 and 56 days after emergence. Towoomba is located in the Bela-Bela municipality of the Limpopo (LP) province and lies at -24.92^0 latitude, 28.33^0 longitude, at an altitude of 1143 masl, with dark black clay soils. Fertiliser was given at a rate of 100 kg N ha^{-1} for the optimum N plots and 20 kg N ha^{-1} for low N plots, as a basal application at planting as compound fertiliser 3:2:1 (32) + Zn. LAN with 28% N was used at the optimum N environment for top-dressing at 28 days after emergence at a rate of 50 kg ha^{-1}. Tshiombo is located in the Thulamela municipality of the LP province and lies at -22.80^0 latitude, 30.48^0 longitude at an altitude of 650 masl, with brown sandy loam soils. Fertiliser was applied at a rate of 150 kg N ha^{-1} to optimum N environments, 25 kg N ha^{-1} to low N plots, and the N was given as a basal application at planting as compound fertiliser 3:2:1 (32) + Zn. LAN with 28% N was used only at the optimum N plots for top-dressing at 28 days after emergence at a rate of 100 kg ha^{-1}.

A summary of the climatic conditions for each location is listed in Table 4.1. KZN, LP and NW which are the three provinces used in this study, have about 33, 23 and 11% of the small-scale farmers who produce maize for subsistence in SA, with some of the farmers in these provinces already producing QPM. Temperature and precipitation data for each location were obtained from the weather division at ARC-Institute for Soil Climate and Water in SA using geographical points of each location; and collected from the weather stations closest to the locations. For Towoomba weather data sets for November 2009 to February 2010 were missing because the weather station was stolen during that period and only replaced in March 2010.

The inbred lines were evaluated in a (0,1) alpha lattice design (Patterson and Williams, 1976), replicated three times and with a different randomisation used in each environment.

The weed management and disease control used for Potchefstroom, Tshiombo and Towoomba sites were similar to those in Chapter 3, while for Cedara, at planting Eptam Super (active ingredient: EPTC + safener), at a rate of 3l ha^{-1} was used for control of annual grasses, yellow and purple nutsedge; and during the growth season Basagran (active ingredient: Bendioxide) was used for control of annual broad leaved weeds and yellow nutsedge at 3l ha^{-1}. GLS and NLB were controlled with Punch Extra (active ingredient: Carbendazim/iflusilazole) and narrow range mineral oil at a rate of 1.5l ha^{-1}. Stalk borer was controlled with Karate EC (active ingredient: lambda-cyhalothrin) at a rate of 120 ml ha^{-1}. Other cultural practices for all the sites were similar to those in Chapter 3.

4.3.3 Tryptophan analysis

Twenty kernels of each inbred line, with uniform size were selected and then milled at the ARC-GCI. The seeds of the inbred lines were analysed at the University of the Free State, Plantbreeding biochemical laboratory for tryptophan concentration using a colorimetric method based on glyoxylic acids reaction with tryptophan in the presence of ferric chloride and sulphuric acid (Nurit *et al.,* 2009).

Chemicals and reagents were prepared for 22 samples as follows: Papain solution (1 mg ml^{-1}) was prepared daily by adding 0.065 g papain into 65 ml of 0.165 M sodium acetate. DL-Tryptophan was used as a standard and prepared in 0.1 M sodium acetate at pH 7. This was prepared weekly by dissolving 10 mg of DL-Tryptophan into 100 ml buffer and stored in a refrigerator at 4^0C. A 30 N sulphuric acid stock was prepared by adding 833.3 ml sulphuric acid (96%) slowly into a bottle with 166.7 ml of distilled water placed on ice and stirred continuously with a magnetic stirrer. It was allowed to cool to room temperature, after which distilled water was gradually added to obtain the final volume of 1000 ml in a volumetric flask. Four reagents (A-D) were prepared. A=0.332 g glycolic acid in 35 ml 7 N H_2SO_4; B=0.017 g ferric chloride added to A; C=30 N H_2SO_4 and D=35 ml C added to 35 ml B. The defatting protocol of Folch *et al.* (1957) was followed where 1 g milled sample was measured into a 50 ml falcon tube and 10 ml of a 2:1 chloroform:methanol mixture was added. The tube was shaken and left overnight in a refrigerator at 4ºC. The content of the tube was filtrated, and about 10 ml

Table 4.1 Weather data at Towoomba, Potchefstroom, Cedara and Tshiombo during the 2009/2010 growing season

Months	Locations											
	Cedara			Towoomba			Tshiombo			Potchefstroom		
	Tx	Tn	Rn	Tx	Tn	Rn	Tx	Tn	Rn	Tx	Tn	Rn
November	22.42	11.6	70.4	-	-	-	29.1	17.1	208.3	27.3	13.5	87.9
December	24.94	13.7	52.8	-	-	-	30.0	19.2	128.3	30.6	16.3	205.0
January	26.23	15.0	140.7	-	-	-	29.2	19.2	212.3	27.3	17.1	242.6
February	28.22	15.3	97.3	-	-	-	28.8	19.4	179.8	29.2	16.2	86.4
March	26.39	14.4	67.3	32.9	16.5	92.5	29.6	18.4	22.7	28.3	15.2	143.0
April	24.7	11.3	67.3	26.4	13.5	219.0	26.4	16.6	336.3	24.5	12.2	77.5
May	24.75	7.3	3.3	26.9	8.0	19.1	25.6	13.3	21.1	23.2	6.78	25.4
June	20.54	3.3	5.08	23.7	1.9	0	23.2	8.27	9.7	19.9	-0.3	0
Mean	**24.7**	**11.5**	**63.0**	**27.5**	**9.9**	**82.7**	**27.7**	**16.4**	**139.8**	**26.3**	**12.1**	**108.5**
Total Rn in season			**504.2**			**---**			**1119**			**867.8**
Latitude	**-29.5419**			**-24.92632**			**-22.80146**			**-26.73607**		
Longitude	**30.2649**			**28.33843**			**30.48139**			**27.07553**		
Alt (masl)	**1066**			**1066**			**653**			**1349**		

Tx=Average maximum temperature in °C; Tn=Average minimum temperature in °C; Rn=Average total rainfall in mm; Alt=Altitude in metres above sea level (masl)

chloroform:methanol solution was used to rinse the tube and wash the remaining fat from the samples. The washing process was done three times. The samples were left at room temperature to dry whilst in the filter papers for about an hour, from which 0.08 g sample was placed in a 15 ml falcon tube and digested using 3 ml of the freshly prepared papain solution. A control was included in the form of a blank tube with only papain solution to be used later to zero the spectrophotometer (Helios UV, Spectronic Unicam). Samples were incubated in the waterbath for 16 hours at 64^0C, and vortexed at least twice (one hour after being placed in the waterbath and one hour before being taken out). After 16 hours the tubes were taken out of the water bath and allowed to cool at room temperature. The tubes were vortexed before being centrifuged at 3600 g for 5 minutes, ensuring that the supernatant is clear. One ml of the supernatant was carefully transferred to a glass tube; and 3 ml of reagent D was added. Samples were thoroughly vortexed for 5 seconds and then incubated at 64^0C for 30 minutes to allow colour development. The samples were allowed to cool at room temperature for two hours before reading the optical density (OD) at 560 nm on a spectrophotometer.

For the standard curve dilutions, the tryptophan in 0.1 M sodium acetate (pH 7) stock solution were used to prepare a 0, 10, 15, 20, 25 and 30 μg ml^{-1} tryptophan concentration series. Of each concentration 1 ml was added into a 15 ml glass tubes, and to this reagent D (3 ml) was added. Samples were vortexed, incubated to allow colour development. After 30 min in the waterbath (64^0C) the sample were taken out and cooled at room temperature and OD_{560nm} readings were done on the spectrophotometer and these readings were used to draw a calibration curve. The slope with the unit OD x ml μg^{-1} was calculated and the percentage tryptophan was calculated from the corrected OD (OD_{560nm} sample – OD_{560nm} average papain blanks). By multiplying the corrected OD_{560nm} by a factor: hydrolysate volume/(standard curve slope x sample weight), and dividing the micrograms of tryptophan measured during sample analysis by micrograms of tryptophan added (papain solution) to each sample, and multiplying by 100 (Nurit *et al.*, 2009).

$$\%trp\ (\mu g\ \mu g^{-1}) = \frac{OD_{560mm}}{slope} \times \frac{hydrolysis\ volume}{sample\ weight} \times 100$$

4.3.4 Protein, starch, oil and quality index determination

The method used for protein, oil and starch is the same as that used by the SAGL. An Infratec 1241 Whole Grain Analyser was used to measure fat, protein and starch concentrations in the maize grain at the ARC-GCI. The analyser uses transmission absorptions; in which the constituents to be measured in the grain absorb electromagnetic radiation in the near-infrared region of the spectrum. The grain quality components are indicated on a dry base as percentage (g 100 g^{-1}). QI was determined by taking the ratio of tryptophan concentration to protein content, and expressed as a percentage.

4.3.5 Endosperm hardness

Endosperm hardness scores were recorded following the method of Pixley and Bjarnason (2002) and Vivek *et al.* (2008). The ten best ears in each plot were identified and 10 kernels taken in the middle of the ear to make a total of 1000 kernels for each plot. A light table was used for measuring degree of kernel modification. The light table is a table with the top made of acrylic or translucent glass with a fluorescent tube placed underneath the glass as a source of light. It is placed in a moderately dark room and kernels are spread on top of the glass for selection into five different classes. For this study a sample of 1000 kernels for each inbred line was sorted and scored on a scale of 1-5, where 1 is completely modified/hard and 5 is completely opaque/soft. The scores were based on appearance of kernel endosperm on the light table as follows: class 1: 100% hard translucent, 0% soft-opaque; class 2: 75% hard translucent, 25% soft-opaque; class 3: 50% hard translucent, 50% soft-opaque; class 4: 25% hard translucent, 75% soft-opaque; and Class 5: 0% hard translucent, 100% soft-opaque (Bjarnason and Vasal, 1992; Pixley and Bjarnason, 2002; Vivek *et al.*, 2008). Endosperm modification score (EMS) of a plot was measured by using the following formula:

EMS= (A*1) + (B*2) + (C*3) + (D*4) + (E*5) / A+B+C+D+E

Where: A is the total number of kernels in class 1; B the total number of kernels in class 2; C the total number of kernels in class 3; D the total number of kernels in class 4; and E total number of kernels in class 5.

4.3.6 Statistical analyses

Data were analysed using AGROBASE Gen II software (Agronomix Software Inc., 2005). Single ANOVAs were conducted for eight separate environments, with genotypes regarded as fixed effects and replications and locations as random effects. Combined ANOVA were then done for the low and optimum N environments and then across the eight N environments in order to determine the genotypic main effects, environmental effects and GEI. The proportion of variance was calculated using the sum of squares. For GEI the stability of genotypes across various environments was assessed using the AMMI model (Zobel *et al.,* 1988; Gauch and Zobel, 1996) and GGE biplots (Yan *et al.,* 2007). AMMI1 biplots were compiled using Genstat 16th Edition (Genstat, 2012); where IPCA1 is plotted against genotype and environment means. IPCA scores against both genotype and site means were done for each trait separately using the first one or two significant IPCAs. The AMMI biplots were drawn by placing the mean on the horizontal and IPCA 1 score on the vertical axis. Genotypes or environments that appear almost on a perpendicular line of the graph have similar means, while those that fall almost on the horizontal line have similar interaction patterns (Crossa, 1990). By using IPCA 1 scores of individual environments in conjunction with IPCA scores of the genotype, the adaptability of a genotype is determined by characterisation of environments. Environments were classified into low or high potential (Crossa, 1990). The GGE biplots were plotted using Genstat 16th edition (Genstat, 2012). The biplots were drawn for each trait separately using the first two significant IPCAs. IPCA2 scores were plotted against IPCA1 scores withthe IPCA1 scores on the horizontal and IPCA2 scores on the vertical axis.

4.4 Results

4.4.1 Separate analysis of variance for grain quality traits in low N environments

4.4.1.1 Endosperm hardness

The ANOVA and mean values results for four low N environments trials are presented in Table 4.2 and Table 4.3 respectively. Genotype mean squares were highly significant ($P \leq 0.01$) for Towoomba and significant ($P \leq 0.05$) for Potchefstroom, Cedara and Tshiombo. As a percentage of total sum of squares, the genotypes accounted for 54.5, 55.8, 57.5 and 61.6 of the variation in Potchefstroom, Towoomba, Cedara, and Tshiombo, respectively. Variations were observed in rankings of genotypes across the four locations, due to changes in performance of genotypes in response to the different environments. The mean values across the locations ranged between a score of 1.06-3.20 in Cedara, 1.64-3.46 in Potchefstroom, 1.67-3.25 in Towoomba and 2.14-3.75 in Tshiombo. On average Tshiombo produced the softest kernels due to high mean scores (3.75) and Cedara produced hard kernels due to the low mean scores. Hans Male ranked first in three locations namely Potchefstroom, Cedara and Towoomba; while it was second in Tshiombo. Genotypes that ranked last were KO54W, RO559W, CZL01005 and BO163W in Potchefstroom, Cedara, Tshiombo and Towoomba respectively.

4.4.1.2 Oil concentration

Highly significant genotype mean squares ($P \leq 0.01$) were observed at all the locations and 60.2-85.0% of the variance was contributed by genotypes at all four locations (Table 4.4). Variation was observed in rankings of genotypes across four locations (Table 4.5). Hans Male ranked first in both Cedara (6.0%) and Towoomba (5.7%), while SO503W ranked first in Potchefstroom (5.8%) and second in Cedara and Towoomba; while KO54W was first in Tshiombo (5.1%). The oil concentration ranged between 4.1-6.0% in Cedara, 4.6-5.8% in Potchefstroom, 4.5-5.7% in Towoomba and 3.8-5.1% in Tshiombo. In terms of average means Tshiombo yielded the lowest average oil concentration (4.7%), while Towoomba and Potchefstroom yielded the highest average oil concentration (5.3%).

Table 4.2 Mean squares from analysis of variance and proportion of variance components for endosperm hardness of 12 QPM inbred lines tested in four low N environments of South Africa

Source	DF	Potchefstroom		Cedara		Tshiombo		Towoomba	
		MS	%Variation	MS	%Variation	MS	%Variation	MS	%Variation
Replication	2	0.125	1.81	0.178	1.84	0.164	2.05	0.515	7.19
Genotype	11	0.685*	54.53	1.011*	57.51	0.893*	61.59	0.727**	55.84
Residual	22	0.274	43.66	0.357	40.64	0.264	36.36	0.241	36.97
CV (%)		20.19		25.22		17.54		19.99	

*P≤0.05; **P≤0.01; MS=Mean squares; CV=Coefficient of variation

Table 4.3 Mean values and rankings of endosperm hardness for 12 QPM inbred lines in four low N environments

Genotype	Potchefstroom		Cedara		Tshiombo		Towoomba	
	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank
SO503W	2.17	103	2.64	8	3.25	8	2.67	9
Hans Male	**1.64**	**1**	**1.06**	**1**	2.15	2	**1.67**	**1**
CML144	2.16	2	1.95	3	**2.14**	**1**	2.11	4
RO424W	2.92	10	1.76	2	2.25	3	2.04	3
FO215W	2.63	6	2.78	10	3.31	10	2.88	10
RO559W	2.66	7	3.20	12	3.48	11	2.24	5
SO713W	2.34	4	2.21	5	2.56	4	1.98	2
BO163W	2.80	9	2.96	11	3.13	7	3.25	12
CML176-2	3.09	11	2.67	9	3.06	6	2.60	8
KO54W	3.46	12	2.52	7	3.30	9	2.52	7
RO450W	2.52	5	2.21	4	2.75	5	2.27	6
CZL01005	2.73	8	2.48	6	3.75	12	3.22	11
Average	**2.59**		**2.37**		**2.93**		**2.46**	
LSD $_{(0.05)}$	**1.07**		**1.22**		**1.05**		**1.01**	

LSD=least significant difference

Table 4.4 Mean squares from analysis of variance and proportion of variance components for oil concentration of 12 QPM inbred lines tested in four low N environments of South Africa

Source	DF	Potchefstroom		Cedara		Tshiombo		Towoomba	
		MS	%Variation	MS	%Variation	MS	%Variation	MS	%Variation
Replication	2	0.006	0.27	0.017	0.37	0.134	4.32	0.152	5.13
Genotype	11	0.347**	84.76	0.692**	85.04	0.418**	73.86	0.325**	60.20
Residual	22	0.031	14.97	0.059	14.58	0.062	21.82	0.093	34.67
CV (%)		3.30		4.79		5.30		5.72	

**P≤0.01; MS=Mean squares; CV=Coefficient of variation

Table 4.5 Mean values and rankings of oil concentration for 12 QPM inbred lines in four low N environments

Genotype	Potchefstroom		Cedara		Tshiombo		Towoomba	
	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank
SO503W	**5.77**	**1**	5.47	2	4.93	4	5.67	2
Hans Male	5.23	7	**6.00**	**1**	4.93	5	**5.73**	**1**
CML144	4.57	12	4.13	12	3.83	12	4.47	12
RO424W	5.40	6	5.03	8	4.63	8	5.17	11
FO215W	5.76	2	5.00	9	4.77	6	5.57	3
RO559W	5.17	9	4.37	11	5.00	3	5.33	7
SO713W	4.93	11	5.30	3	5.07	2	5.53	4
BO163W	5.50	4	5.00	10	4.33	11	5.23	10
CML176-2	5.43	5	5.10	7	4.53	9	5.50	5
KO54W	5.53	3	5.23	5	**5.13**	**1**	5.27	9
RO450W	5.23	8	5.13	6	4.67	7	5.30	8
CZL01005	5.17	10	5.30	4	4.40	10	5.40	6
Average	**5.31**		**4.79**		**4.69**		**5.34**	
LSD $_{(0.05)}$	**0.36**		**0.49**		**0.51**		**0.63**	

LSD=least significant difference

4.4.1.3 Protein concentration

In the ANOVA for protein concentration (Table 4.6), mean squares for genotype were highly significant (P≤0.01) at all four locations and 71.4-95.5% of the total variance was accounted for by genotypes. Genotypes at Potchefstroom had the highest percentage total sum of squares (95.5%). Variation was observed in the rankings of genotypes across four locations (Table 4.7). Hans Male ranked first in three locations namely Cedara (11.9%), Tshiombo (11.4%) and Towoomba (11.4%) and second in Potchefstroom with CML144 ranking first. SO503W ranked last in all the locations except at Cedara where it ranked 8th which is still below the average mean, with FO215W ranking last. The average mean was highest at Tshiombo and lowest at Potchefstroom. Protein concentrations ranged between 8.0-11.9%, 8.5-11.4%, 8.1-11.4%, and 6.7-10.3% at Cedara, Tshiombo, Towoomba and Potchefstroom respectively.

4.4.1.4 Quality index

ANOVA for QI (Table 4.8) indicated significant mean squares (P≤0.05) for replication at Potchefstroom. Genotype mean squares were highly significant (P≤0.01) for Cedara and Tshiombo, significant (P≤0.05) for Towoomba and non-significant for Potchefstroom. As a percentage of total sum of squares, the genotype accounted for 40.9, 56.0, 56.4 and 74.0 of the variation in Potchefstroom, Tshiombo, Towoomba, and Cedara respectively. Variations were observed in rankings of genotypes across the four locations, due to changes in performance of genotypes in response to the different environments (Table 4.9). SO503W ranked first in Potchefstroom and Tshiombo; while KO54W ranked first in Cedara and Towoomba. Hans Male and BO163W ranked last in Potchefstroom, Cedara, Towoomba and Tshiombo respectively. The mean values across the locations ranged between 0.60-1.30% in Towoomba, 0.60-1.33% in Cedara, 0.70-1.20% in Tshiombo and 0.73-1.13% in Potchefstroom. Generally Cedara had the highest average mean value (1.08%) and Towoomba had the lowest mean value for QI.

Table 4.6 Mean squares from analysis of variance and proportion of variance components for protein concentration of 12 QPM inbred lines tested in four low N environments of South Africa

Source	DF	Potchefstroom		Cedara		Tshiombo		Towoomba	
		MS	%Variation	MS	%Variation	MS	%Variation	MS	%Variation
Replication	2	0.443	1.80	0.084	0.32	0.087	0.66	2.130	8.80
Genotype	11	3.592**	80.07	4.525**	95.47	2.064**	87.26	3.145**	71.44
Residual	22	0.497	18.13	0.100	4.21	0.143	12.08	0.435	19.77
CV (%)		7.56		3.45		3.92		7.18	

**P≤0.01; MS=Mean squares; CV=Coefficient of variation

Table 4.7 Mean values and rankings of protein concentration for 12 QPM inbred lines in four low N environments

Genotype	Potchefstroom		Cedara		Tshiombo		Towoomba	
	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank
SO503W	6.73	12	8.43	8	8.47	12	8.07	12
Hans Male	10.00	2	**11.87**	**1**	**11.43**	**1**	**11.40**	**1**
CML144	**10.27**	**1**	8.30	9	10.00	4	9.13	5
RO424W	7.87	8	9.50	4	9.00	9	8.80	7
FO215W	9.00	4	7.97	12	9.40	8	8.87	6
RO559W	8.77	5	10.33	3	9.60	7	10.37	2
SO713W	7.80	9	9.20	4	9.80	6	9.75	4
BO163W	9.63	3	10.67	2	10.00	3	10.17	3
CML176-2	7.47	11	8.03	11	9.80	5	8.40	9
KO54W	7.90	7	8.13	10	8.62	11	8.17	11
RO450W	7.73	10	8.67	6	9.00	10	8.77	8
CZL01005	8.03	6	8.67	7	10.50	2	8.40	10
Average	**8.43**		**9.15**		**9.63**		**9.19**	
LSD $_{(0.05)}$	**1.31**		**0.65**		**0.77**		**1.35**	

LSD=least significant difference

Table 4.8 Mean squares from analysis of variance and proportion of variance components for quality index of 12 QPM inbred lines tested in four low N environments of South Africa

Source	DF	Potchefstroom		Cedara		Tshiombo		Towoomba	
		MS	%Variation	MS	%Variation	MS	%Variation	MS	%Variation
Replication	2	0.084*	16.63	0.030	4.26	0.069	8.61	0.004	0.61
Genotype	11	0.038ns	40.94	0.095**	73.97	0.081**	56.03	0.075*	56.43
Residual	22	0.020	42.42	0.014	21.77	0.026	35.36	0.029	42.95
CV (%)		13.58		10.90		16.45		17.93	

*P≤0.05; **P≤0.01; MS=Mean squares; CV=Coefficient of variation

Table 4.9 Mean values and rankings for quality index of QPM inbred lines in four low N environments

Genotype	Potchefstroom		Cedara		Tshiombo		Towoomba	
	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank
SO503W	**1.13**	**1**	1.03	9	**1.20**	**1**	0.93	8
Hans Male	0.73	12	0.60	12	0.77	11	0.60	12
CML144	0.93	11	1.00	11	0.83	10	0.83	11
RO424W	0.97	10	1.03	10	1.10	4	0.93	6
FO215W	1.03	8	1.23	2	1.17	2	1.03	2
RO559W	1.13	2	1.10	8	0.97	7	0.90	9
SO713W	1.10	4	1.10	7	1.00	6	0.97	5
BO163W	1.03	9	1.10	6	0.70	12	1.00	3
CML176-2	1.03	7	1.17	3	1.00	5	1.00	4
KO54W	1.10	5	**1.33**	**1**	1.17	3	**1.30**	**1**
RO450W	1.07	6	1.13	5	0.90	8	0.90	10
CZL01005	1.10	3	1.17	4	0.87	9	0.93	7
Average	**1.03**		**1.08**		**0.97**		**0.94**	
LSD $_{(0.05)}$	**0.29**		**0.24**		**0.33**		**0.35**	

LSD=least significant difference

4.4.1.5 Tryptophan concentration

Replication mean squares were significant (P≤0.05) at Potchefstroom. Only Cedara showed significant genotype mean squares (P≤0.01) and about 66.7% of the total variance was contributed by genotypes at Cedara (Table 4.10). Variation was observed in rankings of genotypes across four locations (Table 4.11). BO163W, FO215W, KO54W and ranked first in Cedara (0.12%), Tshiombo (0.11%), Towoomba (0.11%), respectively, and RO559W and B0163W ranked first and second with a mean of 0.10% at Potchefstroom (0.10%); while Hans Male ranked last at Potchefstroom, Cedara and Towomba and BO163W ranking last at Tshiombo. The tryptophan concentration ranged between 0.07-0.12%, 0.07-0.11%, 0.07-0.11% and 0.07-0.10% at Cedara, Tshiombo, Towoomba and Potchefstroom respectively. Generally, genotypes had the lowest tryptophan concentration in Potchefstroom and Towoomba and the highest tryptophan concentration at Cedara and Tshiombo. In terms of average means Cedara gave the highest average tryptophan concentration (0.10%), while the other three locations had similar average lowest tryptophan concentration (0.09%).

4.4.1.6 Starch concentration

Mean squares for genotype were highly significant (P≤0.01) at all the locations except for Towoomba which had significant (P≤0.05) mean square values (Table 4.12). About 46.6-86.6% of the total variance was accounted for by genotype across all four locations. Variation was observed in the rankings of genotypes across all four locations (Table 4.13). Hans Male ranked last at Cedara and Towoomba, and BO163W at Tshiombo and Potchefstroom. CML144 ranked first at Cedara and Tshiombo, CML176-2 at Potchefstroom and BO163W at Towoomba. The average mean for starch concentration was highest at Potchefstroom and lowest at Tshiombo. Starch concentration ranged between 65.3-72.6%, 68.7-73.2%, 69.7-72.3 and 70.1-72.6% at Tshiombo, Cedara, Towoomba and Potchefstroom respectively.

Table 4.10 Mean squares from analysis of variance and proportion of variance components for tryptophan concentration of 12 QPM inbred lines tested in four low N environments of South Africa

Source	DF	Potchefstroom		Cedara		Tshiombo		Towoomba	
		MS	%Variation	MS	%Variation	MS	%Variation	MS	%Variation
Replication	2	0.001*	14.29	0.000	0.00	0.001	11.11	0.000	0.00
Genotype	11	0.000ns	42.86	0.001**	66.67	0.000ns	44.44	0.000ns	44.44
Residual	22	0.000	57.14	0.000	33.33	0.000	44.44	0.000	55.55
CV (%)		15.04		11.15		15.12		16.89	

*P≤0.05; **P≤0.01; MS=Mean squares; CV=Coefficient of variation

Table 4.11 Mean values and rankings of tryptophan concentration for 12 QPM inbred lines in four low N environments

Genotype	Potchefstroom		Cedara		Tshiombo		Towoomba	
	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank
SO503W	0.08	10	0.09	10	0.10	4	0.07	11
Hans Male	0.07	12	0.07	12	0.09	9	0.07	12
CML144	0.09	4	0.08	11	0.08	10	0.08	10
RO424W	0.08	11	0.10	5	0.10	6	0.08	7
FO215W	0.09	3	0.10	8	**0.11**	**1**	0.09	5
RO559W	**0.10**	**1**	0.11	2	0.09	7	0.09	4
SO713W	0.09	5	0.10	7	0.10	2	0.09	3
BO163W	0.10	2	**0.12**	**1**	0.07	12	0.10	2
CML176-2	0.08	9	0.09	9	0.10	5	0.08	6
KO54W	0.09	6	0.11	3	0.10	3	**0.11**	**1**
RO450W	0.08	8	0.10	6	0.08	11	0.08	8
CZL01005	0.09	7	0.10	4	0.09	8	0.08	9
Average	**0.09**		**0.10**		**0.09**		**0.09**	
LSD (0.05)	**0.03**		**0.02**		**0.03**		**0.03**	

LSD=least significant difference

Table 4.12 Mean squares from analysis of variance and proportion of variance components for starch concentration of 12 QPM inbred lines tested in four low N environments of South Africa

Source	DF	Potchefstroom		Cedara		Tshiombo		Towoomba	
		MS	%Variation	MS	%Variation	MS	%Variation	MS	%Variation
Replication	2	0.972	8.16	0.697	2.33	1.306	2.11	2.238	12.79
Genotype	11	1.255**	57.95	4.678**	85.99	9.962**	88.62	1.482*	46.61
Residual	22	0.367	33.88	0.318	11.68	0.521	9.26	0.646	40.59
CV (%)		0.85		0.79		1.02		1.13	

*P≤0.05; **P≤0.01; MS=Mean squares; CV=Coefficient of variation

Table 4.13 Mean values and rankings of starch concentration for 12 QPM inbred lines in four low N environments

Genotype	Potchefstroom		Cedara		Tshiombo		Towoomba	
	Mean	Rank	mean	Rank	mean	Rank	mean	Rank
SO503W	72.03	3	71.03	7	71.47	3	71.30	6
Hans Male	71.07	10	68.70	12	70.07	10	69.73	12
CML144	71.57	6	**73.17**	**1**	**72.60**	**1**	71.60	4
RO424W	71.73	5	70.47	10	71.23	6	71.27	7
FO215W	71.07	11	72.80	2	71.25	5	71.40	5
RO559W	71.17	8	71.87	4	70.00	11	70.80	10
SO713W	72.27	2	70.53	9	70.80	7	70.35	11
BO163W	70.17	12	70.63	8	65.33	12	**72.33**	**1**
CML176-2	**72.57**	**1**	72.50	3	71.97	2	71.80	3
KO54W	71.13	9	71.30	5	70.80	8	71.93	2
RO450W	71.87	4	71.17	6	71.30	4	71.20	8
CZL01005	71.50	7	70.20	11	70.23	9	71.07	9
Average	**71.51**		**71.20**		**70.59**		**71.23**	
LSD (0.05)	**1.24**		**1.15**		**1.48**		**1.65**	

LSD=least significant difference

4.4.2 Separate analysis of variance for grain quality traits in optimum N environments

4.4.2.1 Endosperm hardness

Genotype mean squares were highly significant (P≤0.01) for Potchefstroom, significant (P≤0.05) for Tshiombo and non-significant for Towoomba and Cedara (Table 4.14). Replication mean squares were significant (P≤0.05) for the Potchefstroom trial. As a percentage of total sum of squares, the genotypes accounted for 75.2% and 56.4% of variation in Potchefstroom and Tshiombo respectively. Variations were observed in rankings of genotypes across the four locations (Table 4.15). The mean values across the locations ranged between a score of 1.11-2.83 in Cedara, 1.25-3.16 in Potchefstroom, 1.75-3.26 in Towoomba and 2.34-3.69 in Tshiombo. On average Tshiombo produced the softest kernels with a mean score of 3.06 with Cedara and Towoomba mostly had hard kernels with mean scores of 2.37 and 2.41, respectively. Hans Male performed better than the other inbred lines and ranked first in three locations. SO503Wand KO54W ranked last at Tshiombo and Potchefstroom, respectively.

4.4.2.2 Oil concentration

The ANOVA and mean values for four optimum N trials are presented in Table 4.16 and Table 4.17 respectively. Highly significant genotype mean squares (P≤0.01) were observed at all the locations except for Towoomba. About 74.7%, 85.5% and 87.2% of the variance was contributed by genotypes at Tshiombo, Potchefstroom and Cedara respectively. Hans Male ranked first at Cedara (6.2%), KO54W at Potchefstroom (5.8%) and Tshiombo (5.2%), and CZL01005 (5.2%) at Towoomba. The oil concentration ranged between 1.95-6.2% in Cedara, 4.2-5.2% in Tshiombo, 4.3-5.2% in Towoomba and 4.3-5.8% in Potchefstroom. Generally, genotypes had lower oil concentrations in Tshiombo and higher oil concentrations at Potchefstroom. In terms of average means Tshiombo yielded the lowest oil concentration (4.7%), and Potchefstroom the highest (5.4%).

Table 4.14 Mean squares from analysis of variance and proportion of variance components for endosperm hardness of 12 QPM inbred lines tested in four optimum N environments of South Africa

Source	Potchefstroom		Cedara		Tshiombo		Towoomba	
	MS	%Variation	MS	%Variation	MS	%Variation	MS	%Variation
Replication	0.489*	6.74	0.218	2.51	0.082	1.57	1.815	19.13
Genotype	0.993**	75.19	0.789	49.85ns	0.535*	56.42	0.697ns	40.42
Residual	0.119	18.06	0.377	47.64	0.199	42.76	0.349	40.45
CV (%)	14.26		29.87		14.58		24.48	

*P≤0.05; **P≤0.01; MS=Mean squares; CV=Coefficient of variation

Table 4.15 Mean values and rankings of endosperm hardness of 12 QPM inbred lines in four optimum N environments

Genotype	Potchefstroom		Cedara		Tshiombo		Towoomba	
	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank
SO503W	1.97	3	2.02	7	3.69	12	2.90	10
Hans Male	**1.25**	**1**	**1.11**	**1**	**2.34**	**1**	1.80	2
CML144	1.62	2	1.71	3	2.65	3	**1.75**	**1**
RO424W	2.31	4	1.51	2	3.10	8	2.03	3
FO215W	2.76	8	2.57	10	3.02	6	2.27	6
RO559W	2.84	10	2.83	12	3.53	11	2.58	9
SO713W	2.49	7	1.79	5	2.61	2	2.22	5
BO163W	3.08	11	2.30	8	3.09	7	2.40	7
CML176-2	2.36	5	2.01	6	3.52	10	3.26	12
KO54W	3.16	12	2.72	11	2.74	4	3.10	11
RO450W	2.42	6	1.79	4	3.01	5	2.13	4
CZL01005	2.80	9	2.32	9	3.42	9	2.51	8
Average	**2.42**		**2.37**		**3.06**		**2.41**	
LSD $_{(0.05)}$	**0.71**		**1.22**		**0.91**		**1.21**	

LSD=least significant difference

Table 4.16 Mean squares from analysis of variance and proportion of variance components for oil concentration of 12 QPM inbred lines tested in four optimum N environments of South Africa

Source	Potchefstroom		Cedara		Tshiombo		Towoomba	
	MS	%Variation	MS	%Variation	MS	%Variation	MS	%Variation
Replication	0.076	2.20	0.043	0.82	0.012	0.41	0.083	3.31
Genotype	0.536**	85.51	0.838**	87.19	0.397**	74.71	0.223ns	48.49
Residual	0.039	12.29	0.058	11.99	0.066	24.88	0.111	48.20
CV (%)			4.65		5.51		6.68	

*P≤0.05; **P≤0.01; MS=Mean squares; CV=Coefficient of variation

Table 4.17 Mean values and rankings of oil concentration of 12 QPM inbred lines in four optimum N environments

Genotype	Potchefstroom		Cedara		Tshiombo		Towoomba	
	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank
SO503W	5.67	3	5.43	4	5.20	2	4.97	8
Hans Male	5.60	4	**6.23**	**1**	4.90	4	4.97	9
CML144	4.30	12	1.95	12	4.30	10	4.33	12
RO424W	5.53	6	5.03	8	4.50	8	5.13	4
FO215W	5.70	2	5.03	9	4.87	5	5.10	6
RO559W	4.83	11	4.60	11	4.30	11	4.77	10
SO713W	5.40	8	5.33	5	4.73	6	5.23	2
BO163W	5.60	5	5.57	2	4.17	12	5.20	3
CML176-2	5.50	7	5.13	6	4.63	7	4.63	11
KO54W	**5.77**	**1**	5.50	3	**5.23**	**1**	5.03	7
RO450W	5.23	10	4.80	10	4.30	9	5.10	5
CZL01005	5.27	9	5.10	7	4.93	3	**5.23**	**1**
Average	**5.36**		**5.16**		**4.67**		**5.00**	
LSD $_{(0.05)}$	**0.40**		**0.49**		**0.53**		**0.68**	

LSD=least significant difference

4.4.2.3 Protein concentration

ANOVA and mean values for protein concentration are presented in Table 4.18 and Table 4.19. Genotype mean squares for protein concentration were highly significant (P≤0.01) at all four locations and 78.7%, 83.9%, 93.2% and 94.4% of the total variance was accounted for by genotypes at Towoomba, Tshiombo, Cedara and Potchefstroom respectively. Genotypes performed differently at different locations. Hans Male ranked first in three locations namely Potchefstroom (11.6), Cedara (11.3%), and Towoomba (13.4%); while it ranked second in Tshiombo (11.0%) with BO163W (11.7%) ranking first there. SO503W ranked last in all the locations and the mean values were below the average mean. The average mean for protein concentration was high at Towoomba (10.6) and lowest at Cedara (8.8). Protein concentration ranged between 7.0-11.6%, 7.2-11.7%, 7.5-11.3% and 8.5-13.4 at Potchefstroom, Tshiombo, Cedara and Towoomba respectively.

4.4.2.4 Quality Index

Mean squares were significant (P≤0.05) for replication at Towoomba (Table 4.20). Genotype mean squares were highly significant (P≤0.01) for Potchefstroom, Tshiombo and Towoomba and non-significant for Cedara. As a percentage of total sum of squares, the genotype accounted for 60.1%, 68.0% and 85.1 of variation in Towoomba, Potchefstroom and Tshiombo respectively. Variations were observed in rankings of genotypes across the four locations (Table 4.21). SO503W (1.93%) ranked first in Towoomba, KO54W (1.60%) at Potchefstroom and at Cedara (1.80%) and S0713W (1.87%) at Tshiombo. Hans Male ranked last with QI values of 0.70%, 0.83% and 1.00% at Tshiombo, Potchefstroom and Towoomba respectively. Generally Tshiombo had the highest average mean value (1.43%) and Potchefstroom the lowest mean value (1.25) for QI.

Table 4.18 Mean squares from analysis of variance and proportion of variance components for protein concentration of 12 QPM inbred lines tested in four optimum N environments of South Africa

Source	Potchefstroom		Cedara		Tshiombo		Towoomba	
	MS	%Variation	MS	%Variation	MS	%Variation	MS	%Variation
Replication	0.021	0.070	0.050	0.18	1.712*	5.55	0.784	2.00
Genotype	5.138**	94.36	4.816**	93.23	4.706**	83.87	5.637**	78.70
Residual	0.152	5.57	0.170	6.59	0.297	10.58	0.691	19.31
CV (%)	4.25		4.70		6.04		7.83	

*P≤0.05; **P≤0.01; MS=Mean squares; CV=Coefficient of variation

Table 4.19 Mean values and rankings for protein concentration of 12 QPM inbred lines in four optimum N environments

Genotype	Potchefstroom		Cedara		Tshiombo		Towoomba	
	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank
SO503W	7.00	12	7.50	12	7.20	12	8.47	12
Hans Male	**11.63**	**1**	**11.33**	**1**	10.97	2	**13.37**	**1**
CML144	10.70	2	8.17	8	8.97	6	11.43	3
RO424W	8.20	10	9.03	4	8.53	8	10.87	5
FO215W	9.30	5	7.87	9	9.30	4	11.20	4
RO559W	9.73	4	9.97	3	9.30	3	9.63	10
SO713W	9.27	6	8.59	5	8.63	7	10.70	7
BO163W	10.50	3	10.77	2	**11.67**	**1**	12.20	2
CML176-2	8.57	8	7.50	11	7.97	11	10.77	6
KO54W	8.63	7	7.83	10	8.47	9	10.13	8
RO450W	7.90	11	8.43	6	7.97	10	9.83	9
CZL01005	8.53	9	8.37	7	9.30	5	8.87	11
Average	**9.16**		**8.78**		**9.02**		**10.62**	
LSD (0.05)	**0.80**		**0.85**		**1.12**		**1.70**	

LSD=least significant difference

Table 4.20 Mean squares from analysis of variance and proportion of variance components for quality index of 12 QPM inbred lines tested in four optimum N environments of South Africa

Source	Potchefstroom		Cedara		Tshiombo		Towoomba	
	MS	%Variation	MS	%Variation	MS	%Variation	MS	%Variation
Replication	0.004	0.31	0.008	0.50	0.001	0.04	0.218*	11.71
Genotype	0.140**	67.97	0.146	50.63	0.400**	85.05	0.203**	60.08
Residual	0.033	31.67	0.071	48.89	0.035	14.91	0.048	28.24
CV (%)	14.50		19.35		13.11		15.86	

*P≤0.05; **P≤0.01; MS=Mean squares; CV=Coefficient of variation

Table 4.21 Mean values and rankings for quality index of QPM inbred lines in four optimum N environments

Genotype	Potchefstroom		Cedara		Tshiombo		Towoomba	
	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank
SO503W	1.23	7	2.64	5	1.63	4	**1.93**	**1**
Hans Male	0.83	12	1.00	12	0.70	12	1.00	12
CML144	1.00	11	1.47	4	1.57	6	1.33	7
RO424W	1.13	9	1.10	11	1.43	7	1.30	8
FO215W	1.30	5	1.30	8	1.60	5	1.10	11
RO559W	1.23	8	1.40	5	1.40	8	1.67	2
SO713W	1.30	6	1.63	2	**1.87**	**1**	1.40	5
BO163W	1.43	3	1.27	9	0.83	11	1.13	10
CML176-2	1.07	10	1.37	6	1.77	2	1.27	9
KO54W	**1.60**	**1**	**1.80**	**1**	1.77	3	1.50	4
RO450W	1.33	4	1.23	10	1.40	9	1.33	6
CZL01005	1.50	2	1.53	3	1.17	10	1.57	3
Average	**1.25**		**1.37**		**1.43**		**1.38**	
LSD $_{(0.05)}$	**0.37**		**0.54**		**0.38**		**0.45**	

LSD=least significant difference

4.4.2.5 Tryptophan concentration

Replication showed significant mean squares (P≤0.05) at Towoomba (Table 4.22). Highly significant genotype mean squares (P≤0.01) were observed at Potchefstroom and Tshiombo with the other two locations non-significant. About 21.7-78.3% of the total variance was contributed by genotypes across the locations. Variation was observed in rankings of genotypes between the locations (Table 4.23). SO713W was ranked first at Tshiombo (0.16%) and Cedara (0.14%), while BO163W and SO503W were ranked first at Potchefstroom (0.15%) and Towoomba (0.16%), respectively. The tryptophan concentration ranged between 0.10-0.14%, 0.08-0.16%, 0.12-0.16% and 0.09-0.15% at Cedara, Tshiombo, Towoomba and Potchefstroom respectively. Generally, genotypes had the lowest tryptophan concentration in Tshiombo (0.08%) and Potchefstroom (0.09%); and the highest tryptophan concentration at Towoomba and Tshiombo respectively. The means are all above the acceptable value of 0.08%. In terms of average means Tshiombo (0.13) and Towoomba (0.14%) gave the highest average tryptophan concentration, while Potchefstroom (0.11%) gave the average lowest concentration.

4.4.2.6 Starch concentration

Mean squares for genotype were highly significant (P≤0.01) at all the locations except for Towoomba which had significant (P≤0.05) mean squares (Table 4.24). About 52.7%, 62.0%, 75.3% and 82.5% of the total variance was accounted for by genotypes at Towoomba, Tshiombo, Potchefstroom and Cedara respectively. Variation was observed in the rankings of genotypes across the four locations (Table 4.25). Hans Male ranked last in three locations namely Cedara (69.1%), Tshiombo (70.6%) and Potchefstroom (69.3%) and second last in Towoomba (69.2%) with RO450W ranking last there with 69.0%. CML144 ranked first in all the locations (72.7-74.2%) except at Towoomba where it ranked fourth, with CML176-2 ranking first (71.5%) there. The average mean of starch concentration was high at Tshiombo (71.8%) and lowest at Towoomba (70.4%). Starch concentration ranged between 69.0-71.5%, 69.1-74.2% in, 69.3-73.0%, and 70.6-72.7% at Towoomba, Cedara, Potchefstroom, and Tshiombo, respectively.

Table 4.22 Mean squares from analysis of variance and proportion of variance components for tryptophan concentration of 12 QPM inbred lines tested in four optimum N environments of South Africa

Source	Potchefstroom		Cedara		Tshiombo		Towoomba	
	MS	%Variation	MS	%Variation	MS	%Variation	MS	%Variation
Replication	0.000	0.00	0.000	0.00	0.000	0.00	0.003*	21.74
Genotype	0.001	65.00**	0.001ns	45.45	0.002**	78.26	0.000ns	21.74
Residual	0.000	35.00	0.001	54.56	0.000	17.39	0.001	56.52
CV (%)	16.06		19.40		11.17		17.32	

*P≤0.05; **P≤0.01; MS=Mean squares; CV=Coefficient of variation

Table 4.23 Mean values and rankings for tryptophan concentration of 12 QPM inbred lines in four optimum N environments

Genotype	Potchefstroom		Cedara		Tshiombo		Towoomba	
	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank
SO503W	0.09	12	0.10	10	0.12	7	**0.16**	**1**
Hans Male	0.10	9	0.11	7	0.08	12	0.13	10
CML144	0.11	7	0.12	6	0.14	4	0.15	4
RO424W	0.09	10	0.10	11	0.12	8	0.14	7
FO215W	0.12	6	0.10	12	0.15	2	0.12	12
RO559W	0.12	5	0.14	3	0.13	6	0.16	2
SO713W	0.12	4	**0.14**	**1**	**0.16**	**1**	0.15	5
BO163W	**0.15**	**1**	0.13	4	0.10	11	0.13	9
CML176-2	0.09	11	0.10	8	0.14	5	0.13	8
KO54W	0.14	2	0.14	2	0.15	3	0.15	3
RO450W	0.10	8	0.10	9	0.11	9	0.13	11
CZL01005	0.13	3	0.13	5	0.11	10	0.14	6
Average	**0.11**		**0.12**		**0.13**		**0.14**	
LSD $_{(0.05)}$	**0.04**		**0.05**		**0.05**		**0.05**	

LSD=least significant difference

Table 4.24 Mean squares from analysis of variance and proportion of variance components for starch concentration of 12 QPM inbred lines tested in four optimum N environments of South Africa

Source	Potchefstroom		Cedara		Tshiombo		Towoomba	
	MS	%Variation	MS	%Variation	MS	%Variation	MS	%Variation
Replication	0.324	1.41	0.709	2.00	0.902	7.84	0.881	4.11
Genotype	3.154**	75.26	5.306**	82.46	1.297**	62.01	2.050*	52.66
Residual	0.489	23.33	0.500	15.54	0.316	30.16	0.841	43.22
CV (%)	0.99		0.99		0.78		1.30	

*P≤0.05; **P≤0.01; MS=Mean squares; CV=Coefficient of variation

Table 4.25 Mean values and rankings for starch concentration of QPM inbred lines in four optimum N environments

Genotype	Potchefstroom		Cedara		Tshiombo		Towoomba	
	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank
SO503W	71.83	2	71.33	9	71.63	9	71.20	2
Hans Male	69.33	12	69.10	12	70.63	12	69.20	11
CML144	**73.00**	**1**	**74.17**	**1**	**72.67**	**1**	71.00	4
RO424W	70.63	8	71.57	6	72.27	4	69.80	9
FO215W	70.87	6	73.03	2	71.67	8	70.60	6
RO559W	70.90	5	71.53	8	71.80	7	70.73	5
SO713W	70.10	10	71.13	10	72.00	6	70.13	8
BO163W	69.47	11	70.17	11	72.00	5	69.50	10
CML176-2	71.60	3	72.87	3	72.60	2	**71.53**	**1**
KO54W	70.40	9	71.53	7	70.93	11	70.53	7
RO450W	71.47	4	72.47	4	72.45	3	69.00	12
CZL01005	70.77	7	71.63	5	71.10	10	71.07	3
Average	**70.86**		**71.71**		**71.81**		**70.36**	
LSD $_{(0.05)}$	**1.43**		**1.45**		**1.15**		**1.88**	

LSD=least significant difference

4.4.3. Combined analyses of variance within and across N environments

Results of combined ANOVA for within environments and across environments are presented in Tables 4.26-4.28. In low and optimum N the environments and genotype mean squares were highly significant (P≤0.01) for all traits. GEI was highly significant (P≤0.01) for protein, oil and starch concentration, significant (P≤0.05) for tryptophan concentration and non-significant for endosperm hardness and QI across the low N environments; while for the optimum N environments GEI was highly significant for all traits except endosperm hardness. For tryptophan concentration KO54W, RO599W and BO163W were the best three genotypes in low N and KO54W, SO713W and RO559W were best for optimum N environments (Table 4.27). The highest performing genotypes for tryptophan concentration had values of 0.10% and 0.15%, while the worst performing genotypes had values of 0.08% and 0.11%, with average means of 0.09% and 0.13% in low and optimum N environments, respectively. Protein concentration ranged between 7.9-11.2% and 7.5-11.8% with average means of 9.1% and 9.4% for low and optimum N environments respectively. The highest yielding genotype for oil concentration had values of 5.48% and 5.38%, with average means of 5.11% and 5.04% for both N environments respectively. Starch concentration was higher in optimum than low N environments.

The combined ANOVA across all environments indicated highly significant (P≤0.01) environment, genotype and GEI mean squares for all traits except endosperm hardness (Table 4.28). It showed that tryptophan and QI were highly affected by environment, which explained 46.1% and 33.1% of the total (G + E + GEI) variation, whilst protein, starch, endosperm and oil were highly affected by genotype which explained 51.7%, 32.6%, 31.7% and 35.9% of variation, respectively. GEI explained 16.7%, 16.7%, 20.4%, 20.6% and 32.4% of variation for tryptophan, protein, QI, oil and starch respectively. The mean values for all the traits are presented in Table 4.29. Tryptophan concentration varied from 0.09% to 0.12% across the eight N environments with average concentration of 0.11%. The highest and lowest ranking genotypes for tryptophan and QI were KO54W and Hans Male respectively. For protein and oil concentration Hans Male was the best genotype while SO503W and CML144 were the worst genotypes respectively. CML144 and Hans Male were the best and worst performing genotypes for starch concentration respectively.

Table 4.26 Mean squares of combined analysis of variance for endosperm hardness, quality index, tryptophan, protein, oil and starch concentration of 12 QPM inbred lines within low and optimum N environments of South Africa

Source	EH		Tryptophan		Protein		Quality index		Oil		Starch	
	LowN	OptN	LowN	OptN	LowN	OptN	LowN	OptN	LowN	OptN	LowN	OptN
Environ	2.168**	6.274**	0.001**	0.005**	8.889**	24.953**	0.138**	0.213**	3.309**	3.122**	5.453**	17.484**
Rep in E	0.245	0.651*	0.000	0.001	0.686*	0.642	0.047*	0.058	0.077ns	0.054ns	1.303**	0.704
Gen	2.414**	2.4027**	0.001**	0.002**	9.885**	16.620**	0.209**	0.497**	1.199**	1.313**	7.470**	7.838**
GEI	0.301ns	0.329ns	0.000*	0.001**	1.147**	1.226**	0.027ns	0.131**	0.194**	0.227**	3.303**	1.323**
Residual	0.284	0.261	0.000	0.000	0.271	0.328	0.022	0.047	0.061	0.068	0.463	0.536
CV (%)	20.60	20.54	14.53	16.32	5.72	6.09	14.70	15.90	4.58	5.18	0.96	1.03

*P≤0.05; **P≤0.01; ns=non-significant; EH=Endosperm hardness; LowN=Low nitrogen; OptN=Optimum nitrogen; Env=Environment; Rep in E=Replication in environment; Gen-Genotype; GEI=Genotype by environment interaction; CV=Coefficient of variation

Table 4.27 Mean values for endosperm hardness, quality index, tryptophan, protein, oil and starch concentration of 12 QPM inbred lines within low and optimum N environments of South Africa

Genotype	EH		Tryptophan		Quality Index		Protein		Oil		Starch	
	LowN	OptN	LowN	OptN	LowN	OptN	LowN	OptN	LowN	OptN	LowN	OptN
SO503W	2.68	2.65	0.08	0.12	1.08	1.54	7.93	7.54	5.46	5.32	71.46	71.50
Hans Male	**1.63**	**1.62**	0.08	0.11	0.68	0.88	**11.18**	**11.83**	**5.48**	**5.38**	69.89	69.57
CML144	2.09	1.93	0.08	0.13	0.90	1.34	9.43	9.82	4.25	4.27	72.23	72.71
RO424W	2.24	2.24	0.09	0.11	1.01	1.24	8.79	9.16	5.06	5.05	71.18	71.07
FO215W	2.90	2.65	0.10	0.12	1.12	1.33	8.81	9.42	5.28	5.18	71.63	71.54
RO559W	2.89	2.94	0.10	0.14	1.03	1.43	9.77	9.66	4.97	4.63	70.96	71.24
SO713W	2.27	2.28	0.10	0.14	1.04	1.55	9.14	9.29	5.21	5.18	70.99	70.84
BO163W	3.04	2.72	0.10	0.13	0.96	1.17	10.12	11.28	5.02	5.13	69.62	70.28
CML176-2	2.85	2.79	0.09	0.12	1.05	1.37	8.43	8.70	5.14	4.98	**72.21**	**72.15**
KO54W	2.95	2.93	**0.10**	**0.15**	**1.23**	**1.67**	8.20	8.77	5.29	5.38	71.29	70.85
RO450W	2.44	2.34	0.09	0.11	1.00	1.33	8.54	8.53	5.08	4.86	71.38	71.35
CZL01005	3.05	2.76	0.09	0.13	1.02	1.44	8.90	8.77	5.07	5.13	70.75	71.14
Average	**2.59**	**2.49**	**0.09**	**0.13**	**1.01**	**1.36**	**9.10**	**9.40**	**5.11**	**5.04**	**71.13**	**71.19**
LSD $_{(0.05)}$	**0.361**	**0.347**	**0.009**	**0.014**	**0.101**	**0.146**	**0.353**	**0.388**	**0.168**	**0.177**	**0.462**	**0.497**

EH=Endosperm hardness; LowN=Low nitrogen; OptN=Optimum nitrogen; LSD= Least significance difference

Table 4.28 Combined analysis of variance for endosperm hardness, quality index, tryptophan, protein, oil and starch concentration of 12 QPM inbred lines across eight N environments of South Africa

Source	Endosperm hardness		Tryptophan		Protein		Quality Index		Oil		Starch	
	MS	%Var	MS	%Var	MS	%Var	MS	%Var	MS	%Var	MS	%Var
Environment	3.719**	17.26	0.015**	46.05	15.400**	19.93	1.400**	33.09	2.799**	26.63	9.861**	13.97
Rep in Env	0.448ns	4.76	0.001*	3.51	0.664**	1.96	0.052ns	2.82	0.065ns	1.42	1.004*	3.25
Genotype	4.349**	31.73	0.002**	10.53	25.420**	51.69	0.630**	23.39	2.401**	35.89	14.645**	32.61
GEI	0.283ns	14.45	0.000**	16.67	1.172**	16.68	0.078**	20.37	0.196**	20.56	2.077**	32.37
Residual	0.284	31.80	0.000	22.81	0.299	9.74	0.034	20.33	0.065	15.49	0.500	17.80
CV (%)			15.93		5.92		15.65		5.01		0.99	

*P≤0.05; **P≤0.01; ns=non-significant; MS=Mean squares; Var=Variation; Rep in Env=Replication in Environment; GEI=Genotype by environment interaction; CV=Coefficient of variation

Table 4.29 Mean values and rankings for endosperm hardness, quality index, tryptophan, protein, oil and starch concentration of 12 QPM inbred lines across eight N environments of South Africa

Genotype	EH		Tryptophan		Quality Index		Protein		Oil		Starch	
	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank
SO503W	2.66	6	0.10	10	1.31	2	7.73	12	5.39	2	71.48	4
Hans Male	**1.63**	**1**	0.09	12	0.78	12	**11.50**	**1**	**5.45**	**1**	69.73	12
CML144	2.01	2	0.11	7	1.12	10	9.62	4	4.26	12	**72.47**	**1**
RO424W	2.24	3	0.10	9	1.13	9	8.98	7	5.05	9	71.12	6
FO215W	2.78	7	0.11	5	1.22	6	9.11	6	5.23	4	71.59	3
RO559W	2.92	11	0.12	3	1.23	5	9.71	3	4.80	11	71.10	7
SO713W	2.28	4	0.12	2	1.30	3	9.21	5	5.19	5	70.91	10
BO163W	2.88	9	0.11	4	1.06	11	10.70	2	5.08	7	69.95	11
CML176-2	2.82	8	0.10	8	1.21	7	8.56	9	5.06	8	72.18	2
KO54W	2.94	12	**0.12**	**1**	**1.45**	**1**	8.49	11	5.34	3	71.07	8
RO450W	2.39	5	0.10	11	1.16	8	8.54	10	4.97	10	71.36	5
CZL01005	2.90	10	0.11	6	1.23	4	8.83	8	5.10	6	70.95	9
Average	**2.54**		**0.11**		**1.18**		**9.25**		**5.07**		**71.16**	
LSD $_{(0.05)}$	**0.249**		**0.008**		**0.088**		**0.261**		**0.122**		**0.337**	

EH=Endosperm hardness; LowN=Low nitrogen; OptN=Optimum nitrogen; LSD= Least significance difference

4.4.4 Additive main effects and multiplicative interaction analysis across N environments

4.4.4.1 Tryptophan concentration

The AMMI analysis showed that IPCA axis 1 and IPCA axis 2 were highly significant (P≤0.01) and significant (P≤0.05) for tryptophan concentration (Table 4.30), explaining 56.5% and 24.9% of the total variation respectively, and when combined they explained 81.4% of the genotype main effect and GEI (Table 4.31). Few genotypes were clustered around the zero point (Figure 4.1). Genotypes with IPCA 1 scores close to zero are more stable than those furthest away from zero. KO54W and RO559W were the best performing genotypes with regards to tryptophan concentration and stability. However CML144 and RO450W were relatively stable with tryptophan values that were average and below average respectively. Genotypes with tryptophan concentration that was above average were KO54W, RO559W, SO713W, FO215W, BO163W and CZL01005. Of all those genotypes, BO163W, FO215W and CZL01005 were considered to be unstable. Hans Male had unstable and lower tryptophan concentration. Environments TWH, TSH, CH and PH were higher yielding; whilst environments PL, TWL, TSL and CL were lower yielding for tryptophan concentration.

Table 4.30 Additive main effects and multiplicative interaction model mean squares for tryptophan, protein, oil and starch concentration across eight N environments in South Africa

Source	Mean squares				
	Trytophan	**Protein**	**QI**	**Oil**	**Starch**
Environments	0.015**	15.400**	1.441**	2.799**	9.861**
Genotype	0.002**	25.420**	0.651**	2.401**	14.645**
GEI	0.001**	1.172**	0.082**	0.196**	2.077**
IPCA1	0.001**	2.571**	0.173**	0.347**	5.036**
IPCA2	0.001*	1.242**	0.117**	0.210**	2.725**
IPCA3	0.000	1.070**	0.071*	0.190**	1.320**
IPCA4	---	0.852**	0.032	0.167**	0.609
Residual	0.000	0.299	0.034	0.065	0.500

*P ≤0.05, **P≤0.01; GEI= Genotype by environment interaction; IPCA= Interactive principle component axes; QI=Quality index

Table 4.31 Contribution of IPCA scores to the total variation for GEI of tryptophan, protein, oil and starch concentration across eight N environments

IPCA Axis	Protein		Tryptophan		Quality Index		Oil		Starch	
	%GEI Exp	Cum %	%GEI Exp	Cum %	%GEI Expl	Cum %	%GEI Expl	Cum %	%GEI Expl	Cum %
1	50.52	50.52	56.52	56.52	49.23	49.23	44.21	44.21	56.94	56.94
2	22.00	72.52	24.94	81.47	29.38	78.61	23.61	67.82	27.19	84.13
3	16.47	88.93	18.53	100	15.56	94.17	18.45	86.27	11.42	95.55
4	11.07	100	---	---	5.83	100	13.73	100	4.45	100

IPCA: Interactive principle component axes, GEI Expl: Genotype by environment interaction explained; Cum=Cumulative

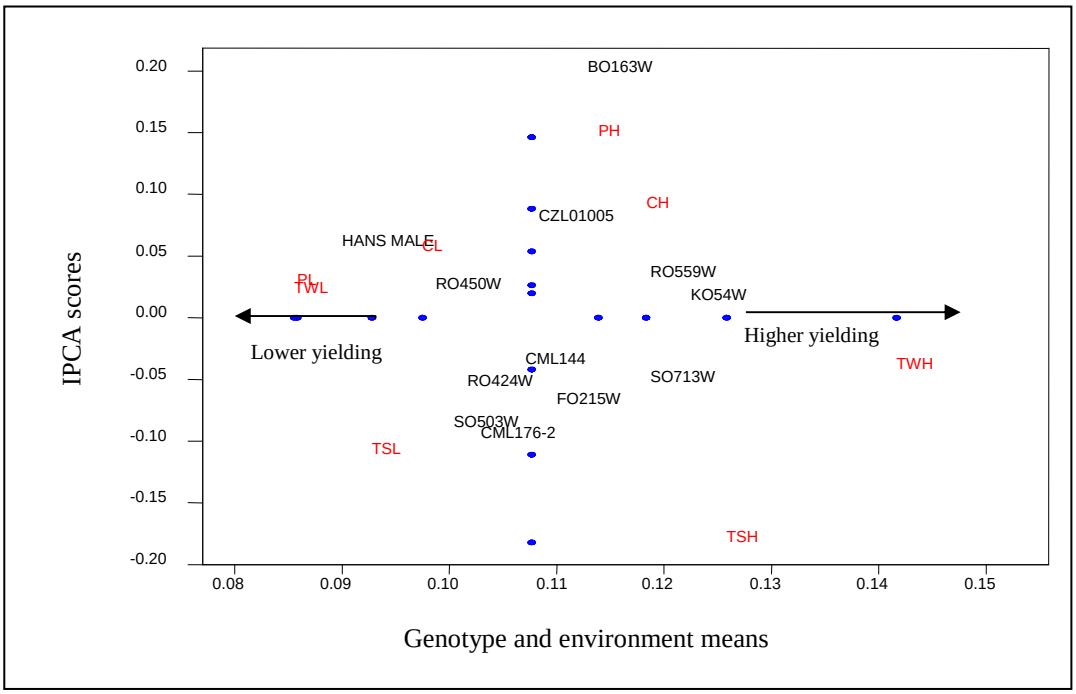

Figure 4.1 Additive main effects and multiplicative interaction biplot for genotype tryptophan concentration in eight environments.

PH =Potchefstroom optimum N; PL =Potchefstroom low N; CH=Cedara optimum N; CL=Cedara low N; TSH =Tshiombo optimum N; TSL =Tshiombo low N; TWH =Towoomba optimum N; TWL =Towoomba low N

4.4.4.2 Protein concentration

The AMMI analysis revealed that PC1 and PC2 were highly significant (P≤0.001) for protein concentration (Table 4.30) and explained 50.5% and 22.0% of the total variation. Together they explained 72.5%, of the genotype main effect and GEI (Table 4.31). SO713W was the most stable genotype with above average protein concentration (Figure 4.2). BO163W and Hans Male were relatively stable with the highest protein concentration. The most unstable genotypes were CML144 and RO559W. Genotype CZL01005 and KO54W were relatively stable but with lower protein concentration. Higher yielding environments were TWH and TSL; whilst lower yielding environments were PL, CH and TSH. PH, CL and TWL were average yielding environments.

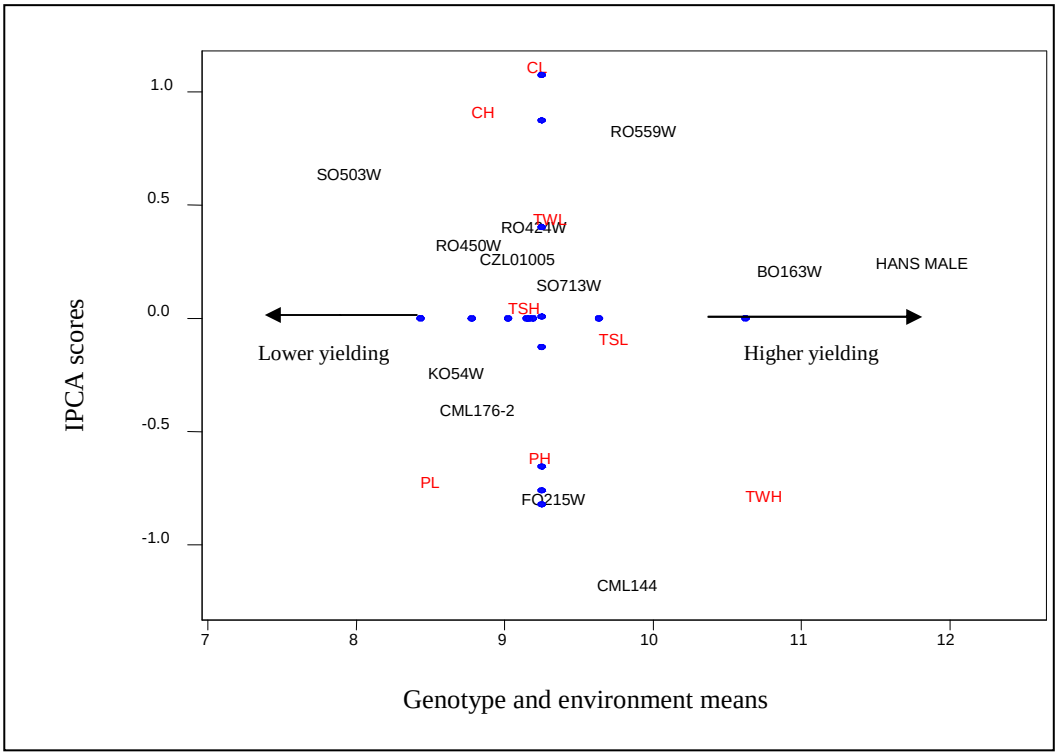

Figure 4.2 Additive main effects and multiplicative interaction biplot for genotype protein concentration in eight environments.

PH =Potchefstroom optimum N; PL =Potchefstroom low N; CH=Cedara optimum N; CL=Cedara low N; TSH =Tshiombo optimum N; TSL =Tshiombo low N; TWH =Towoomba optimum N; TWL =Towoomba low N

4.4.4.3 Quality Index

The partitioning of GGE through AMMI analysis revealed that IPCA axis 1 and IPCA axis 2 were highly significant (P≤0.001) for QI (Table 4.30), explaining 49.2% and 29.4% of the total variation respectively. When combined, they explained 78.6% of the genotype

main effect and GEI (Table 4.31). KO54W was the best genotype with the highest stable QI of all the genotypes (Figure 4.3). Other relatively stable genotypes were RO559W, RO424W and RO450W with high QI values. B0163W and Hans Male were the most unstable genotypes with below average QI, while SO713W, CML176-2 and CZL01005 had above average QI values. The genotypes with the highest QI were KO54W, SO503W and SO713W, whilst the ones with the lowest QI were Hans Male, BO163W and CML144. TSH, TWH, CH and PH were the environments with the highest QI; whilst the environments with the lowest QI were TWL, TSL, PL and CL.

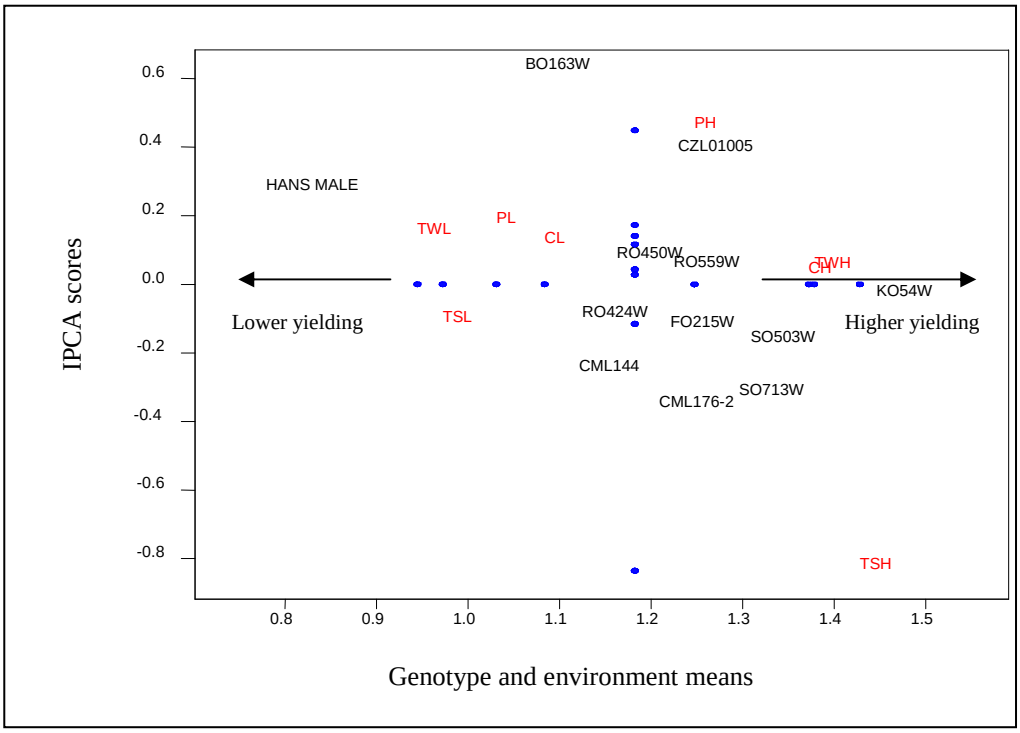

Figure 4.3 Additive main effects and multiplicative interaction biplot for genotype quality index in eight environments.

PH =Potchefstroom optimum N; PL =Potchefstroom low N; CH=Cedara optimum N; CL=Cedara low N; TSH =Tshiombo optimum N; TSL =Tshiombo low N; TWH =Towoomba optimum N; TWL =Towoomba low N

4.4.4.4 Oil concentration

The AMMI analysis showed that IPCA axis 1 and IPCA axis 2 were highly significant ($P \leq 0.001$) for oil concentration (Table 4.30), explaining 44.2% and 23.6% of the total variation respectively. When combined, they explained 67.8% of the genotype main effect and GEI (Table 4.31). SO503W, CML176-2, KO54W and CZL01005 were the most stable genotypes, with SO503W, KO54W and CZL01005 having the highest oil concentration and CML176-2 having the lowest (Figure 4.4). The most unstable

genotypes with the highest oil concentration were Hans Male and FO215W, while the lowest yielding and unstable genotypes were RO559W and CML144. Environments with the highest oil concentration were PH, TWL, PL, CH and CL; whilst those with the lowest oil concentration were TSH, TSL and TWH.

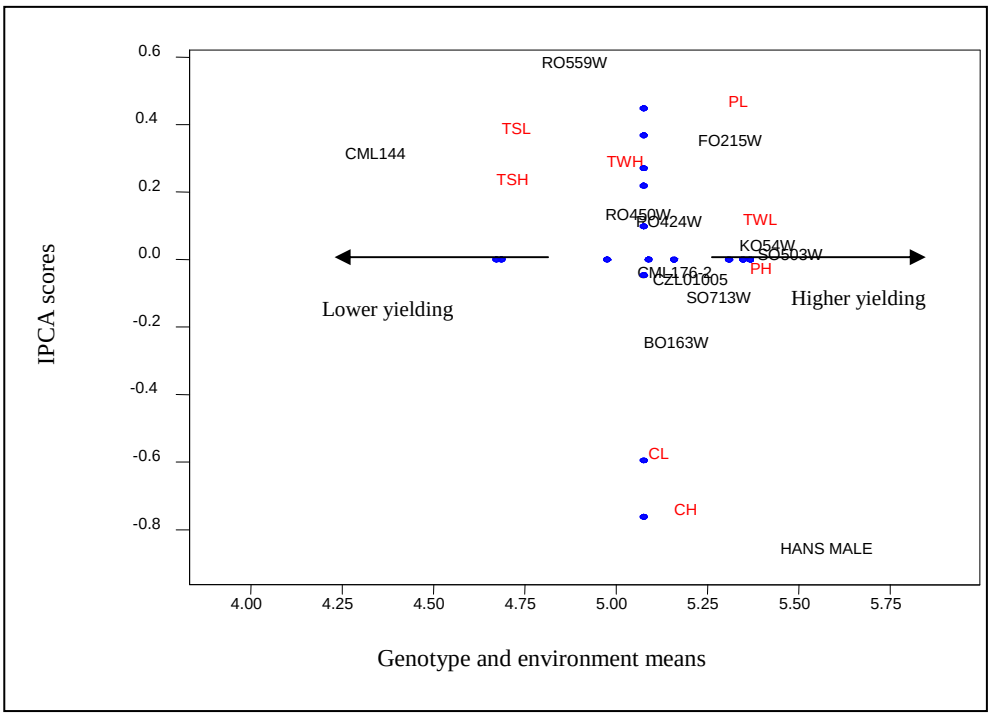

Figure 4.4 Additive main effects and multiplicative interaction biplot for genotype oil concentration in eight environments.

PH =Potchefstroom optimum N; PL =Potchefstroom low N; CH=Cedara optimum N; CL=Cedara low N; TSH =Tshiombo optimum N; TSL =Tshiombo low N; TWH =Towoomba optimum N; TWL =Towoomba low N

4.4.4.5 Starch concentration

The partitioning of GGE through AMMI analysis showed that IPCA axis 1 and IPCA axis 2 were highly significant (P≤0.001) for starch concentration (Table 4.30), explaining 56.9 and 27.2% of the total variation respectively. Together they explained 84.1% of the genotype main effect and GEI (Table 4.31). In Figure 4.5 most of the genotypes were centred in the region of the biplot origin. The most stable genotypes were KO54W and CZL01005. CML144 was associated with the highest yielding environments with regards to starch concentration; however it was considered the most unstable of them all. The genotype with the lowest and unstable starch concentration was Hans Male. Higher yielding environments for starch concentration were CH, TSH and PL; with the lowest yielding environments being TWH, TSL and PH.

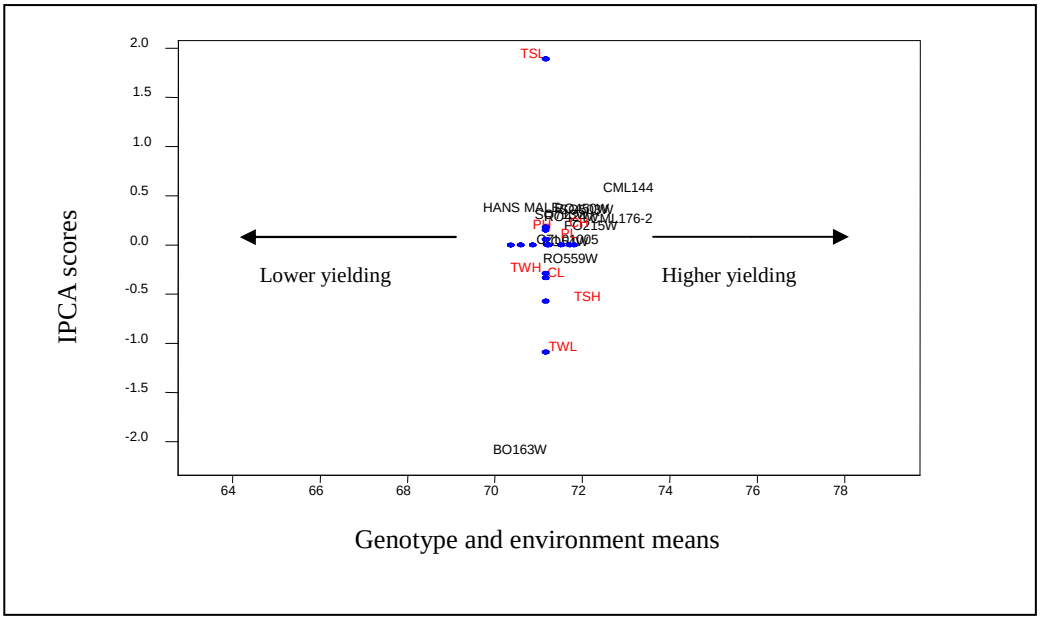

Figure 4.5 Additive main effects and multiplicative interaction biplot for genotype starch concentration in eight environments.

PH =Potchefstroom optimum N; PL =Potchefstroom low N; CH=Cedara optimum N; CL=Cedara low N; TSH =Tshiombo optimum N; TSL =Tshiombo low N; TWH =Towoomba optimum N; TWL =Towoomba low N

4.4.5 Genotype and genotype by environment interaction biplots for five traits

The GGE biplot analysis with visual positioning of the genotypes is presented in Figures 4.6a to 4.6e. It explained a total of 78.9%, 89.3%, 78.7%, 79.5% and 86.5% of GGE variation for tryptophan, protein, QI, oil and starch respectively. The high variation explained by the GGE biplot indicates its appropriateness in further carrying out other biplot types (such as discriminating environments, genotype evaluation and ideal environments). The two principal components for tryptophan, protein, QI, oil and starch had scores of 48.5% and 30.4%, 77.8% and 11.6%, 61.2% and 17.5%, 69.8% and 9.77% and, 65.6% and 20.9% for the GGE variation respectively. In the case of tryptophan RO559W was associated with TWL, PL, CL and CH environments, while FO215W and CZL01005 corresponded to TSL and PH environment respectively. KO54W had the highest tryptophan concentration. For protein concentration CML144 and FO215W were mostly associated with PL, PH and TWH, while BO163W and Hans Male were associated with TSH and TWL, and RO559W with CL and CH. It was found that Hans Male and BO163W performed better, with high protein concentration. With regards to QI genotype

SO713W, SO503W and FO215W were suitable for the TSL environment, while RO559W, KO54W and RO450W corresponded to PL, TWL, TWH, CH and CL environments and CML176-2 and CZL01005 corresponded to TSH and PH respectively. KO54W had the highest QI. For oil concentration genotypes KO54W and SO503W were suitable for PH, TSH, TWL and TWH environments, while FO215W was associated with the PL and TSL environments, and Hans Male corresponded with CL and CH environments. The genotype with the highest oil concentration was Hans Male. In terms of starch concentration SO503W was mostly suitable for the PL environment, while FO215W was associated with TSH and TWH environments. PH and CH environments were mostly associated with genotype CML144, while environments PH and TWH were associated with genotype CML176-2. CML144 was the genotype with the highest starch content.

Graphical presentation of GGE biplot analysis for mean vs stability of genotypes for tryptophan, protein, QI, oil and starch is shown in Figures 4.7a to 4.7e.The PC1 is on the X axis and PC2 on the Y axis respectively and they represent the mean performance (genotypes with high PC1 are more productive) and stability (genotypes with PC2=0 are more stable) of genotypes. The mean performance and stability were determined by the average environment co-ordinates (AEC) method (Yan, 2001; Yan and Hunt, 2001), with the average environment defined by PC1 and PC2 average values for all the environments indicated with a circle (Kaya *et al.,* 2006; Choukan, 2011). The line passing through the biplot origin and the average environment is known as the average environment axis (AEA). The line passing through the biplot origin and which is perpendicular to the AEA is called AOE and divides genotypes into those that perform above average and those with lower values than average. The genotypes are ranked by mean performance on AEA, increasing in the position of the arrow. Genotype stability is based on their distance from the AE abscissa i.e. the closer they are to the abscissa the more stable they are and *vice versa* (Kaya *et al.,* 2006; Yan and Tinker, 2006).

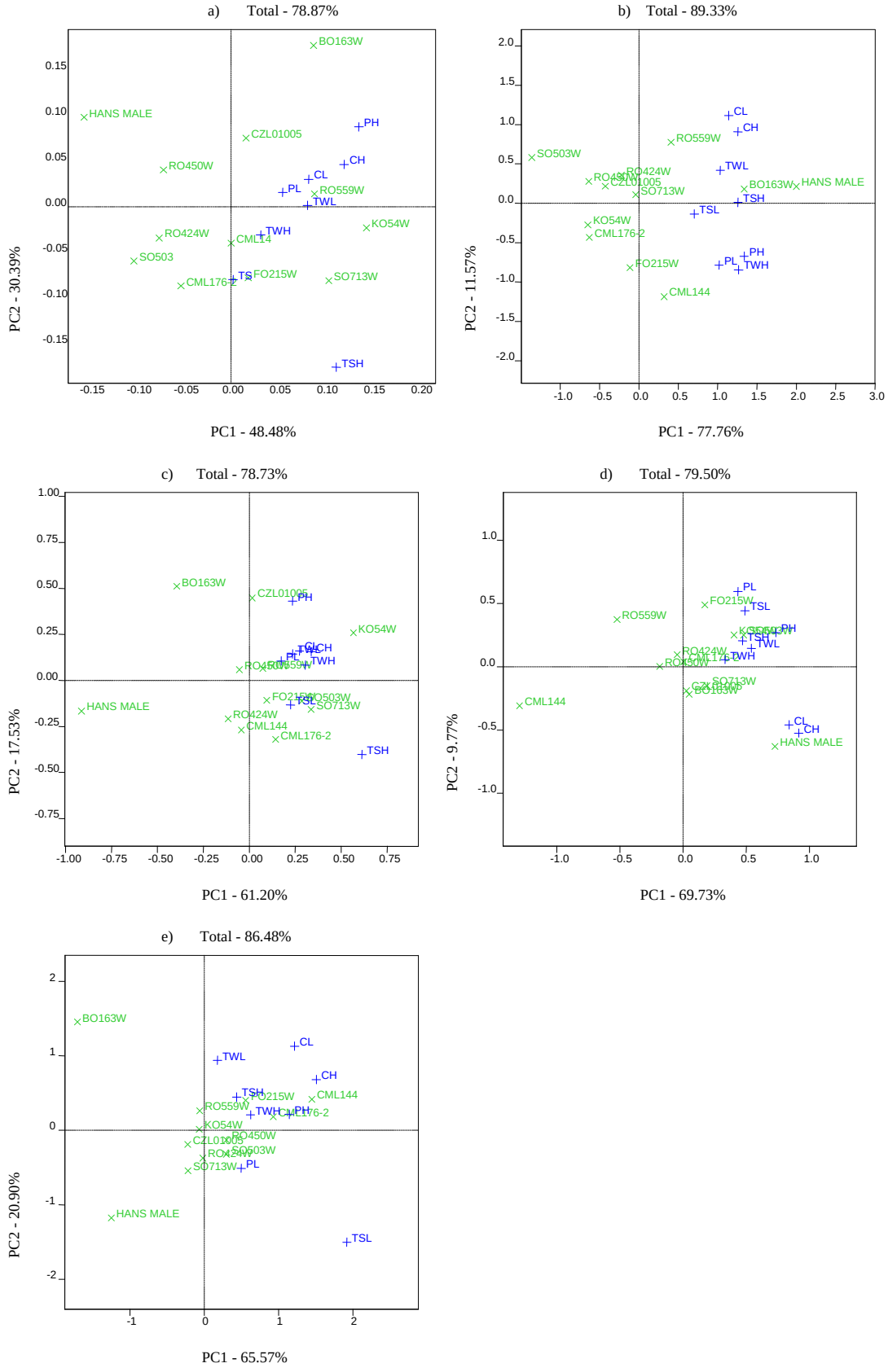

Figure 4.6 Genotype and genotype by environment interaction biplot of tryptophan (a), protein (b), QI (c), oil (d) and starch (e). PH =Potchefstroom optimum N; PL =Potchefstroom low N; CH=Cedara optimum N; CL=Cedara low N; TSH =Tshiombo optimum N; TSL =Tshiombo low N; TWH =Towoomba optimum N; TWL =Towoomba low N

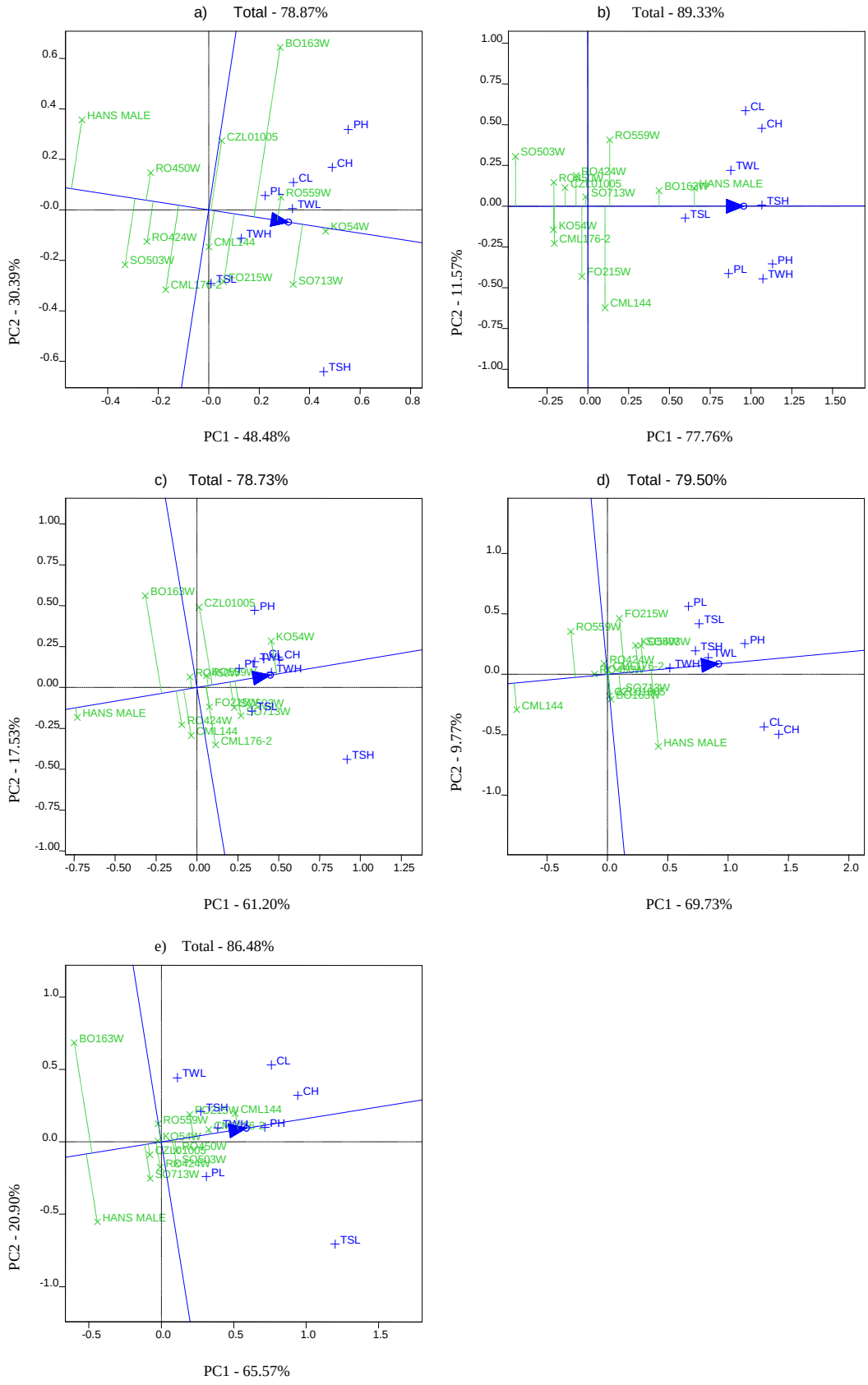

Figure 4.7 **Genotype and genotype by environment interaction biplot based on average environment co-ordinate view for tryptophan (a), protein (b), QI (c), oil (d) and starch (e).** PH =Potchefstroom optimum N; PL =Potchefstroom low N; CH=Cedara optimum N; CL=Cedara low N; TSH =Tshiombo optimum N; TSL =Tshiombo low N; TWH =Towoomba optimum N; TWL =Towoomba low N

In the case of tryptophan concentration the highest mean performers were KO54W, SO713W and RO559W and the lowest were Hans Male, SO503W and RO450W. KO54W was the most stable of all the genotypes, followed by RO559W. Genotypes with the highest protein concentration were Hans Male, BO163W and RO559W, while SO503W and KO54W had the lowest protein concentration. SO713W, BO163W and Hans Male were the most stable genotypes. The genotypes which had the highest QI were KO54W, SO713W and FO215W, while Hans Male, BO163W and RO450W had the lowest. Hans Male was the most stable of all the genotypes, followed by RO559W and RO450W. For oil concentration Hans Male, SO503W and KO54W were the best performers, while CML144, RO559W and RO450W were the worst. The most stable genotypes were RO450W and CML176-2. In the case of starch concentration the best performers were CML144, CML176-2 and FO215W, while the worst performers were Hans Male, BO163W and SO713W. The stable genotypes were KO54W and CML176-2.

Graphical presentation of the "ideal" genotype using GGE biplot analysis is shown by Figure 4.8. The arrow on the line is an indication of the "ideal" genotype. An ideal genotype has the highest and absolute stable mean across all test environments (Kaya *et al.,* 2006). The closer the genotype is located closest to the "ideal" genotype the more desirable it becomes (Kaya *et al.*, 2006; Choukan, 2011). In this study the genotype that was closest to the "ideal" genotype for tryptophan, protein, QI, oil and starch concentration were KO54W, Hans Male, KO54W, SO503W and CML144 respectively.

Graphical presentation of the "ideal" environment is presented in Figure 4.9. The "ideal" environment is the one that is both discriminating of genotypes and representative of the average tester (Yan *et al.,* 2007; Badu-Apraku *et al.,* 2011). An average tester is shown with the circle, while the arrow points to the "ideal" environment and the vertical line that passes through the biplot origin and the circle is called the average environment axis. The testing environment which is closely located to the "ideal" environment is the one which is more desirable in terms of discrimination ability of genotypes and representativeness of the environments.

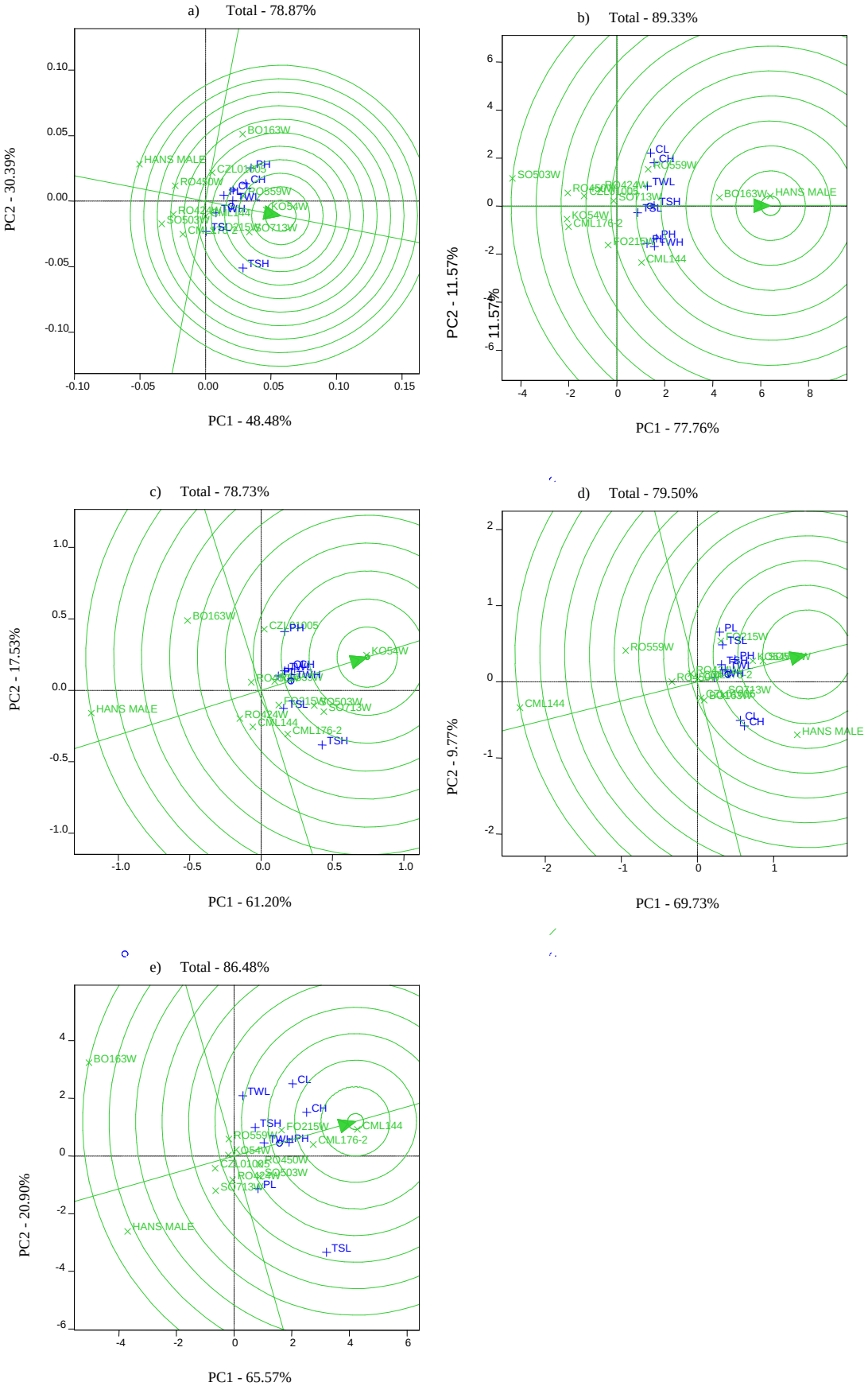

Figure 4.8 GGE biplots for "ideal" genotype for tryptophan (a), protein (b), QI (c), oil (d) and starch (e).

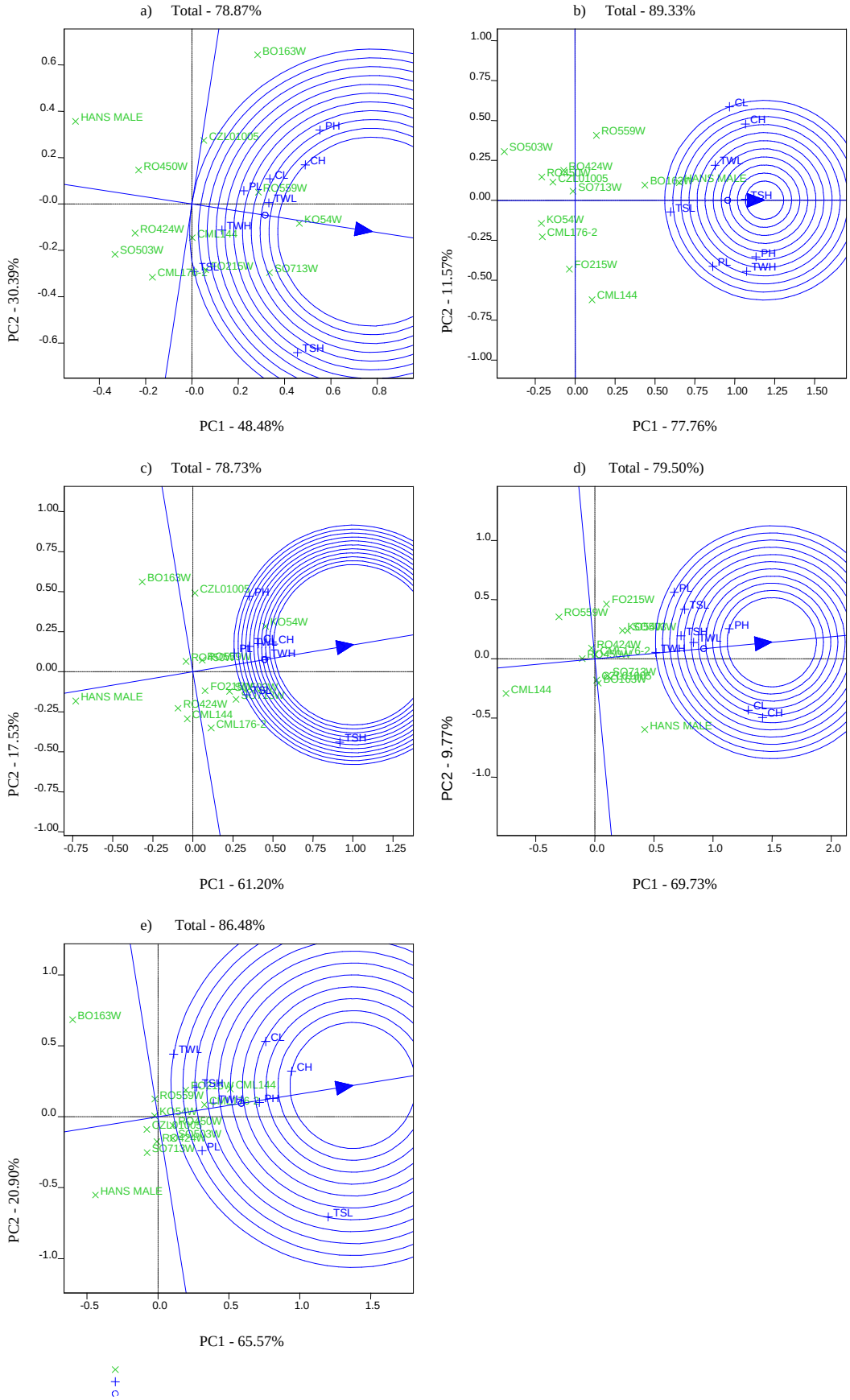

Figure 4.9 **Ranking of environments based on both discriminating power and representativeness for tryptophan(a), protein(b), QI(c), oil(d) and starch(e).**
PH =Potchefstroom optimum N; PL =Potchefstroom low N; CH=Cedara optimum N; CL=Cedara low N; TSH =Tshiombo optimum N; TSL =Tshiombo low N; TWH =Towoomba optimum N; TWL =Towoomba low N.

The ranking of tester environments for tryptophan concentration is as follows: CH > TWL > PH > CL > PL > TSH > TWH > TSL, with CH being the “ideal” environment. In case of protein the “ideal” environment was TSH, with ranking as TSH > PH = TWL > TWH > CH > PL > TSL > CL. The ranking of tester environments for QI is as follows: CH > TWH > CL > TSH > TWL > PH > TSL > PL, with CH as the “ideal” environment. For oil the “ideal” environment was PH and for starch it was CH, with rankings as follows: PH > CL > CH > TWL > TSH > TSL > PL > TWH and CH > PH > CL > TSL > TWH > TSH > PL > TWL respectively.

The GGE biplot is drawn with a polygon view of “which-won-where” or “which is best for what” in order to assess which genotypes performed well in which environments (Figure 4.10). The genotypes located away from the biplot origin are connected with a straight line that forms a polygon, resulting in other genotypes being contained within the polygon. Then a set of perpendicular lines are drawn from the origin of the plots and extends beyond the polygon to divide the polygon into several sectors. Each sector represents environments where certain genotypes ranked the highest. For tryptophan concentration the polygon biplot was drawn from BO163W, KO54W, SO713W, CML176-2, SO503W and Hans Male with perpendicular lines dividing the biplot into six sectors (Figure 4.10a). Genotypes that performed similarly for PL environments were KO54W and BO163W, while for the TSL environment they were SO713W and CML176-2. Genotype BO163W wins for PH, CH, CL and environments, while KO54W wins for TWL and PL, and SO713W wins for TWH, TSL and TSH. Most of the other genotypes were less responsive than the vertex genotypes since they fell within the polygon. The polygon was drawn on RO559W, CML44, SO503W and Hans Male in the case of protein concentration (Figure 4.10b). The perpendicular lines divided the polygon in six sectors with all the eight environments falling into one sector and the vertex genotypes were Hans Male and RO559W. The remaining genotypes were less responsive to the environments.

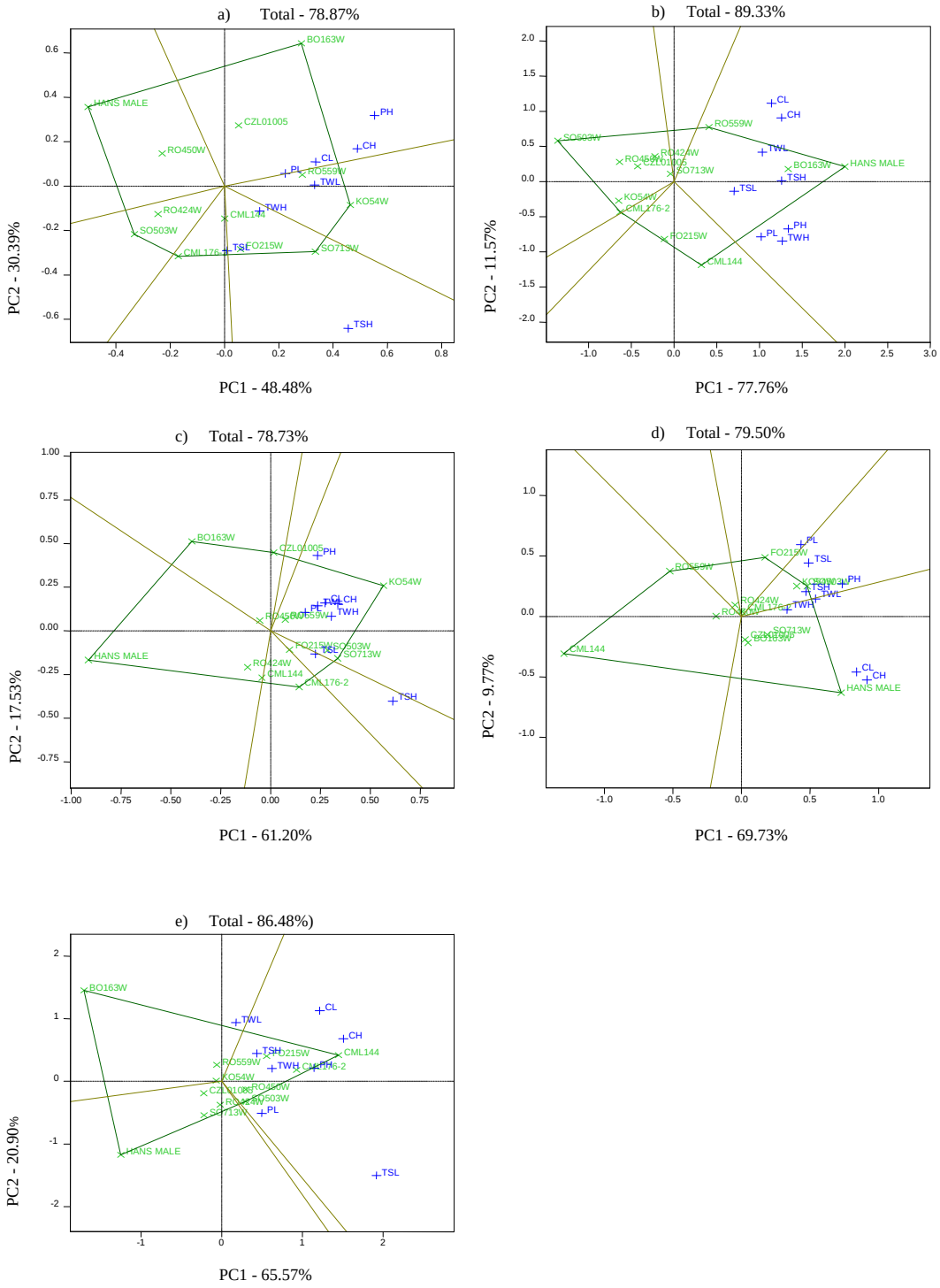

Figure 4.10 Polygon views of GGE biplots showing "which won where" or "what is best for what" for tryptophan(a), protein(b), QI(c), oil(d) and starch(e). PH =Potchefstroom optimum N; PL =Potchefstroom low N; CH=Cedara optimum N; CL=Cedara low N; TSH =Tshiombo optimum N; TSL =Tshiombo low N; TWH =Towoomba optimum N; TWL =Towoomba low N.

In QI the polygon had six sectors drawn on KO54W, SO713W, CML176-2, Hans Male, BO163W and CZL01005 (Figure 4.10c). CH, PH, TWH, CL, TWL, PL and TSL were best for KO54W and SO713W. The remaining genotypes fell within the polygon and were less responsive compared to the vertex genotypes. The polygon was drawn on Hans Male, CML144, RO559W, FO215W and SO503W and it divides by perpendicular line into five sectors with regards to oil concentration (Figure 4.10d). Hans Male won at CH, TWH, CL and TWL, while SO503W won at TWL, TSH, PH and TSL, with FO215W winning at PL. Hans Male and SO503W performed similarly with regards to the TWL environment. For starch concentration the polygon was drawn on CML144, Hans Male and BO163W with the perpendicular line dividing it into four sectors (Figure 4.10e). Environment CH, PH, TWH, TSH, CL, PL and TSL were best for CML144, while TWL was best for BO163W. The remaining genotypes fell within the polygon and thus were less responsive of target environments for both traits.

4.5 Discussion

Evaluation of QPM inbred lines for grain quality characteristics in multi-environment trials is crucial for understanding the effect of environment on those characteristics because understanding GEI will assist in identifying and selecting superior QPM inbred lines which will be useful in QPM breeding programmes. Highly significant genotypic differences observed among the QPM inbred lines across the grain quality characteristics indicated the existence of variation for endosperm hardness, QI, tryptophan, protein, oil and starch content. Worku *et al.* (2007) in a study with QPM and normal maize cultivars tested in nine environments for determination of kernel endosperm quality, observed significant genotypic differences for QI, protein and tryptophan concentrations, while Hohls *et al.* (1996) in a study with opaque-2 maize germplasm observed highly significant genotypic differences amongst crosses for kernel hardness. In the present study environments were significantly different, which showed that each environment used was different in N levels. The QI and tryptophan content values observed in this study were similar to those achieved by Ignjatovic-micic *et al.* (2009) and higher than those achieved by Okello *et al.* (2006). Various researchers also found genetic variation in endosperm hardness, QI, tryptophan and protein under low and optimum N

environments (Pixley and Bjarnason, 1993; Worku, 2005; Gissa, 2008; Ngaboyisonga, 2008).

The results also revealed significant GEI for all measured traits except for endosperm hardness. Non-significant GEI for endosperm hardness meant that the QPM inbred lines were generally adapted to low and optimum N environments, which is what most breeders would want to achieve. Significant variation of GEI indicated the differences in the response of genotypes to the various environments. The result of the study was in agreement with findings of Hohls *et al.* (1996) and Pixley and Bjarnason (2002). However, it was in disagreement with the findings by Machida (2008) for endosperm hardness, and with Worku *et al.* (2007) who observed non-significant GEI differences for QI and protein concentration. The significant GEI of oil and starch are consistent with findings of other researchers (Lambert *et al.*, 1998; Letchworth and Lambert, 1998; Guria, 2006). These findings indicated that the responses of QPM inbred lines to the environments were not similar for measured traits. Oikeh *et al.* (1998) found that varieties performed differently in different N environments, which makes it difficult to select superior genotypes for different end users. Kniep and Mason (1991), Tsai *et al.* (1992), Kaye *et al.* (2007), Ngaboyisonga (2008) and Surma *et al.* (2012) indicated that an increase in N fertiliser caused an increase in protein concentration. N application in most cases resulted in reduced grain hardness and this was shown by various researchers (Tsai *et al.*, 1992; Kettlewell, 1996); however other researchers (Duarte *et al.*, 2005; Kaye *et al.*, 2007; Surma *et al.*, 2012) observed an increase in grain hardness as N levels increased. Protein and tryptophan concentration of the grain endosperm increased markedly as available N in the soil increased, indicating that all protein fractions in the grain are reduced when N in the soil is limiting; while QI decreases (Worku, 2005; Worku *et al.*, 2007). The results were consistent with findings of this study except for QI, which increased as N was increased. Low N supply results in a lowered starch concentration (Griess *et al.*, 2010). This was not the case with the present study where average starch content was found to be higher under low N than optimum N conditions.

Ngaboyisonga (2008) in a study to determine the effects of water deficiency on protein quality traits, found that water deficiency increased the concentration of protein in grain and that of tryptophan. The findings of the current study disagreed with these findings by

indicating that the Cedara site, which had the lowest rainfall, produced genotypes with the lowest protein concentration. It did, however, agree with the finding that tryptophan content was increased in environments where rainfall was limited. Water deficiencies negatively affected endosperm hardness from hard kernels to soft kernels (Ngaboyisonga, 2008). However the findings of this study indicated that when rainfall was limited endosperm hardness was positively affected, while high rainfall (Tshiombo) resulted in soft kernels. High rainfall sites resulted in low oil content compared to low rainfall sites which resulted in increased oil content**.** According to Jurgens *et al.* (1978) severe drought results in reduced oil content.

Genotype and GEI variations measure the response of the genotypes across environments while the environment effects measure the differences of the cultivar means to the environments (Aremu *et al.,* 2008). In the present study, genotype had the largest effect on endosperm hardness, protein, oil and starch content. According to Duarte *et al.* (2005) genotype had a larger effect on grain quality parameters. Surma *et al.* (2012) observed that environment had a large effect on the performance of genotypes for protein content, except for endosperm hardness and starch content (Machida, 2008; Surma *et al.,* 2012). Similar to findings in this study, oil content was less influenced by the effects of environment and GEI (Berke and Rocheford, 1995). Environment had a larger effect than genotype on tryptophan and QI. For starch content GEI was larger than genotype effects as compared to results for other traits measured where in most instances genotype effect was greater than GEI. These results are not consistent with that of Aremu *et al.* (2008) and Badu-Apraku *et al.* (2011) who reported that GEI was greater than genotype effects. In the present study tryptophan and QI were highly affected by environment, which explained 46.1% and 33.1% of the total (G + E + GEI) variation, whilst protein, starch, endosperm and oil were highly affected by genotype which explained 51.7%, 35.9% 32.6% and 23.4% of variation. GEI explained 16.7%, 16.7%, 20.4%, 20.6% and 32.4% of variation for tryptophan, protein, QI, oil and starch respectively. For various traits, the high genotype contribution in this study indicated the variability in genotypes, while the high E effect meant that environments were very different, which influenced genotype performance. The high GEI on the other hand meant that it was difficult to recommend genotypes for various mega-environments (Machida, 2008). The findings in the present study agrees with the above studies on GEI significance; however the partitioning of

components deviates from the most commonly observed pattern of results, possibly because of large genotypic differences and smaller differences in environments (Table 4.1). Under low and optimum N environments the best genotype for endosperm hardness, tryptophan, protein, QI, starch and oil content were Hans Male, KO54W, Hans Male, KO54W, CML176-2 and Hans Male, respectively. The best performer across environments for endosperm hardness, protein and oil content was Hans Male, while for tryptophan and QI it was KO54W and CML144 for starch content.

The existence and magnitude of GEI in oil yield differ. Ghafoor *et al.* (2005) found that GEI was important in a study with sunflower genotypes by contributing more than 85% of the total variation in oil yield, which meant that with respect to oil yield the stability analysis of genotypes based on the location index was important. Mekonen and Mohammed (2010) found that GEI only contributed 4.6% of variation and genotypes had the largest effect on the oil yield. Findings in the present study were different from the reports by these researchers. Gutierrez-Rojas *et al.* (2008) in their study with a recombinant inbred population on endosperm texture modification and amino acid composition found highly significant genotype and environment effects for endosperm texture, opacity, and tryptophan and lysine content. They also found significant differences for endosperm texture in the field, however there were no significant GEI effects for tryptophan, lysine and vitreousness/hardness for the recombinant inbred population. The findings in the present study were different from the reports of these researchers. The need for extensive germplasm testing in multi-environment trials is confirmed by highly significant GEI effects (Badu-Apraku *et al.,* 2011). Because highly significant GEIs were observed for traits measured, further multivariate analysis were done in order to determine the stability and adaptability of genotypes across locations for grain quality characteristics. This was done using the AMMI model and GGE biplots.

IPCA1 of AMMI analysis accounted for 49.2%, 50.5%, 56.5%, 44.2% and 56.9% and IPCA2 for 29.4%, 22.0%, 24.9%, 23.6% and 27.2% of the GEI variation for QI, protein, tryptophan, oil and starch concentrations. Findings indicated that the first two IPCAs explained more than 60%-80% of the GEI. Similarly Choukan (2011) in a study on genotype, environments and GEI effects on performance of maize inbred lines observed the two IPCAs explained more than 80% of GEI variation. Other researchers who

obtained similar results are Yan *et al.* (2007) and Mitrovic *et al.* (2012). Inbred lines KO54W and RO559W were the best performing genotypes in terms of tryptophan content and stability. For protein content SO713W was the most stable genotype. KO54W was considered to be the best genotype with the highest and stable QI. SO503W and KO54W were the most stable genotypes, with the highest oil concentration. FO215W and CML176-2 were considered the best because they were stable with a good starch concentration. Environments TWH, TSH, CH and PH were higher yielding; whilst environments PL, TWL, TSL and CL were lower yielding for tryptophan concentration. Higher yielding environments for protein content were TWH and TSL; whilst lower yielding environments were PL, CH and TSH. PH, CH and TWL were average yielding environments. TSH, TWH, CH and PH were the environments with the highest QI; whilst the environments with the lowest QI were TWL, TSL, PL and CL. Environments with the highest oil concentration were PH, TWL, PL, CH and CL; whilst those with the lowest oil concentration were TSH, TSL and TWH. Higher yielding environments for starch concentration were CH, TSH and PL; with the lowest yielding environments being TWH, TSL and PH.

According to Yan and Kang (2003) and Yan *et al.* (2007) the most important three aspects of genotype and environment data analysis for GGE biplots are genotype evaluation, test environment evaluation and mega-environment identification. In this study the GGE biplot results were used to show the positioning of the genotypes in different environments and total variation due to G and GEI, the relative mean performance vs stability of genotypes across environments, the ideal genotype, ideal environment, and which was best for what/which wins where pattern analysis. The GGE biplot average environment co-ordinate view allows test environments to be evaluated by discriminating power and representativeness and genotypes by mean performance and stability (Yan, 2001; Yan and Hunt, 2001; Yan *et al.*, 2007). Yan (2001) also defined the ideal environments and genotypes. An ideal genotype is represented by a high mean performance and high stability across environments, with an ideal environment being the one that is most discriminating and representative of the environments (Yan, 2001; Yan and Hunt, 2001; Kaya *et al*., 2006; Yan and Tinker, 2006; Yan *et al.*, 2007; Badu-Apraku *et al.*, 2011; Choukan, 2011). The graphical visualisation of "which is best for what" pattern is essential for reviewing the potential existence of different mega environments

in the target region of the multi-environment data set (Gauch and Zobel, 1997; Yan *et al*., 2000; Yan *et al*., 2001).

The GGE biplot analysis of tryptophan, protein, QI, oil and starch content for 12 QPM inbred lines explained, respectively, 78.9%, 89.3%, 78.7%, 79.5% and 86.5% of G + GE variation. The GGE variation explained suggests some strong and complex GEI in the multi-environment data; and the high proportion of total variation explained justifies the use of GGE biplots (Aremu *et al.,* 2008). Various researchers reported similar findings for total G + GE variation with GGE biplots (Yan *et al.,* 2007; Aremu *et al.,* 2008; Badu-Apraku *et al.,* 2011; Choukan, 2011). RO559W was associated with TWL, PL, CL and CH environments and KO54W had the highest tryptophan concentration. CML144 and FO215W were mostly associated with PL, PH and TWH, while BO163W and Hans Male with TSH and TWL with Hans Male and BO163W having high protein concentration. With regards to QI RO559W, KO54W and RO450W corresponded to PL, TWL, TWH, CH and CL environment with KO54W having the highest QI. KO54W and SO503W were suitable for four environments, while Hans Male had the highest oil content and corresponded to CL and CH environments. CML144 had the highest starch content and was associated with PH and CH environments. All the environments associated with the genotypes were in the above average zone. The GGE biplot is able to do a lot of graphical visualisations in analysis of multi-environment data and in interpretation of GEI; and explains more of total variance compared to AMMI analysis (Yan *et al.,* 2007). The findings of this study agree with the above reports except for tryptophan content where AMMI (81.47%) was better than GGE (78.90%) analysis in partitioning of the total G + GEI variation.

According to Yan **(**2002) in the GGE biplot of mean vs stability PC1 approximates G which is the mean performance of the various genotypes and PC2 measures the stability and approximates the GEI effects. The highest mean performers for tryptophan concentration were KO54W, SO713W and RO559W and lowest were Hans Male, SO503W and RO450W, with KO54W and RO559W being the most stable. Hans Male, BO163W and RO559W had the highest protein content, while SO503W and KO54W had the lowest. SO713W, BO163W and Hans Male were most stable. The genotypes which had the highest QI were KO54W, SO713W and FO215W, while Hans Male, BO163W

and RO450W had the lowest. Hans Male, RO559W and RO450W were the most stable of all the genotypes. Hans Male, SO503W and KO54W were the best performers, while CML144, RO559W and RO450W were the worst for oil content. RO450W and CML176 were stable for oil content. For starch concentration the best performers were CML144, CML176-2 and FO215W, while the worst were Hans Male, BO163W and SO713W. KO54W and CML176-2 were stable. The study was able to identify both high mean performers and stable inbred lines for all the traits measured. Choukan (2011) in a GGE biplot for mean vs stability study used the same type of germplasm as used in the current study and was able to identify the highest yielding and very stable line which could be further used in a breeding programme.

A genotype that is selected as an ideal genotype can be used as a reference during cultivar evaluation trials (Kaya *et al.,* 2006; Choukan, 2011). In most cases an ideal genotype is one that is closest to the visual "ideal" genotype because in most cases an ideal genotype does not exist (Kaya *et al.,* 2006). This study was able to identify ideal genotypes for various traits measured. The ideal genotypes were KO54W, Hans Male and KO54W for QI, protein and tryptophan concentration. In the case of oil and starch concentration the ideal genotypes were SO503W and CML144 respectively. Consistent with the current results, Choukan (2011) was able to identify an ideal genotype which can be used as a reference in cultivar evaluation.

In most studies the discriminating ability vs representativeness are visualised with vectors and angles on the GGE biplot graph (Yan and Tinker, 2006; Yan *et al.,* 2007; Aremu *et al.,* 2008; Badu-Apraku *et al.,* 2011; Choukan, 2011). However, in this study the GGE biplots plotted were for discriminating power and representativeness. According to Badu-Apraku *et al.* (2011) both biplots are able to identify suitable locations to select superior genotypes. The biplot indicates an ideal environment as an environment that is both discriminating and representative of the test environments through the rankings of environments (Badu-Apraku *et al.,* 2011). The ranking of environments for tryptophan concentration were as follows: CH > TWL > PH > CL > PL > TSH > TWH > TSL, with CH being the "ideal" environment. In case of protein the "ideal" environment was TSH, with ranking as TSH > PH = TWL > TWH > CH > PL > TSL > CL. For QI the ranking of the environments were as follows: CH > TWH > CL > TSH > TWL > PH > TSL > PL,

with CH as the “ideal” environment. For oil the “ideal” environment was PH while for starch it was CH, with rankings as follows: PH > CL > CH > TWL > TSH > TSL > PL > TWH and CH > PH > CL > TSL > TWH > TSH > PL > TWL respectively. Similar to Badu-Apraku *et al.* (2011) the findings of this study were able to identify the most discriminating and representative of the test environments and regarded those environments as ideal environments for selecting superior genotypes.

The GGE biplot of “which was best for what” is used to identify superior genotypes in each environment or mega-environment (Yan and Hunt, 2002). The most responsive genotypes are best performers in those environments; while the less responsive genotypes are those that fall within the areas closer to the biplot origin of the polygon, whereas genotypes where environments did not feature in their polygon sectors were the lowest yielding in all or some of those environments (Badu-Apraku *et al.*, 2011; Choukan, 2011). In the GGE biplots for “which was best for what” the best genotype in the test locations was BO163W which was superior for PH, CH and CL environments, KO54W for TWL and PL environments, and SO713W for TWH, TSL and TSH environments for tryptophan content. For protein content the vertex genotypes were Hans Male and RO559W in all eight environments. Environments CH, PH, TWH, CL, TWL, PL and TSL were best for KO54W and SO713W for QI. In terms of oil content Hans Male won at CH, TWH, CL and TWL, while SO503W won at TWL, TSH, PH and TSL, with FO215W winning at PL. Hans Male and SO503W performed similarly with regards to the TWL environment. For starch content, environment CH, PH, TWH, TSH, CL, PL and TSL were best for CML144, while TWL was best for BO163W. The GGE biplots for “which was best for what” was a clear summary of the GE pattern of a multi-environment trial data set by simply identifying the best performers in various environments. Similar to what was reported by Badu-Apraku *et al.* (2011) it was easier to visualise the “which was best for what” pattern with the GGE biplot than the AMMI biplot.

4.6 Conclusions

Significant genotypic differences were observed for all traits measured in different environments indicating the existence of genetic variability amongst the QPM inbred lines, which will allow for selection for improvement in the breeding programme.

Genotype effects in the QPM inbred lines for all traits measured were greater than environment effects except in the case of tryptophan content and QI and greater than GEI effects except for the case of starch content. The combined ANOVA within environments revealed that in some instances both low and optimum N environments discriminated the inbred lines similarly, which gave an indication of a good possibility of genetic improvement and selection of genotypes that will perform well in both the low N environments used by small-scale farmers who apply no or small amounts of fertiliser during planting and for optimal conditions. The large genetic variation encountered under low N environments creates an opportunity to exploit the QPM inbred lines even more effectively in order to develop hybrids that are adaptable or tolerant to these environments. The low and optimum N environments sometimes discriminated the genotypes differently, whereas in some cases environments sharing the same location but with different stress levels discriminated the genotypes similarly. This showed the possibility of developing genotypes under both stress and optimal environments. The environment effects were found to be significant even though the environmental effects were lower than the other effects. An increase in N fertiliser caused an increase in protein content, grain hardness, tryptophan content, QI and oil content while low N conditions resulted in higher starch content. Endosperm hardness and tryptophan and oil content were improved while protein was reduced in low N sites. The inbred lines used in this study performed well for the majority of the traits under both optimum and stress environments, which indicated the tolerance of the germplasm available in the country for low N environments. These materials will be beneficial to farmers since they usually plant maize under low N conditions. In a combined ANOVA across the eight environments GEI was significant for all traits except for endosperm hardness, indicating the differential response of the QPM inbred lines to contrasting N levels which also indicated the possibility of developing hybrids or the formation of specific populations for each environment in the maize breeding programme. ANOVA was able to detect the existence of GEI effects, and AMMI and GGE analyses were used to further analyse this.

The AMMI and GGE analysis were efficient in analysing and interpreting GEI effects and this was shown by the large GEI extracted by both analyses. However, overall GGE biplots were superior for partitioning of the total G + GEI variation except for the case of tryptophan content. The GGE biplots were easy to interpret because of their ability to visualise the results, and due to the availability of graphical tools for visualization of G +

GEI interpretation as compared to the AMMI graphs. AMMI and GGE biplots were successful in the identification of high performance and stability and the overall ideal genotypes. Both methods were similar in the identification of genotype performance for QI, tryptophan and protein content; and similar for determining stability of inbred lines for tryptophan, protein and starch content but different for QI and oil content. The ideal genotypes were KO54W, Hans Male and KO54W, SO503W and CML144 for QI, protein tryptophan oil and starch concentration, respectively.

The AMMI identified the highest yielding environments as TWH, TWH, TSH, PH and TSH for tryptophan, protein, QI, oil and starch content, respectively. The test environment evaluations of GGE biplots were able to identify the ideal environment which is the most discriminating and representative of the environments. The ideal environments for tryptophan, protein, QI, oil and starch concentration were CH, TSH, CH, PH and CH, respectively. All of these ideal environments were optimum N environments. BO163W won for PH, CH and CL environments, KO54W for TWL and PL environments, and SO713W for TWH, TSL and TSH environments in tryptophan content. Hans Male and RO559W were superior in eight environments for protein content. KO54W and SO713W won in CH, PH, TWH, CL, TWL, PL and TSL environments for QI. Hans Male was superior at CH, TWH, CL and TWL, SO503W at TWL, TSH, PH and TSL, and FO215W at PL for oil content. CML144 won at CH, PH, TWH, TSH, CL, PL and TSL environments, and BO163W at TWL in starch content. As for further using GGE biplots for identification of mega-environments future studies need to be done which include year interactions in order to identify and validate mega-environment results.

4.7 References

Agronomix Software Inc (2005) AGROBASE Generation II User's Manual. Version II, Revised Edition. www.agronomix.com. Agronomix Software, Winnipeg, M.B., Canada.

Akpalu W, Hassan RM and Ringler C (2008) Climate variability and maize yield in South Africa. International Food Policy Research Institute (IFPRI) research brief pp.15-10. Available on the internet from: http://www.ifpri.org/sites/default/files/publicat

ions/rb15_10.pdf [Date Accessed: 01 June 2012].

Alake CO and Ariyo OJ (2012) Comparative analysis of genotype x environment interaction techniques in West African Okra, (*Abelmoschus caillei*, A. Chev Stevels). Journal of Agricultural Science 4: 135-150.

Alberts MJA (2004) A comparison of statistical methods to describe genotype × environment interaction and yield stability in multi-location maize trials. MSc Thesis, Department of Plant Sciences, University of the Free State, Bloemfontein, South Africa.

Aremu CO, Adebayo MA and Adeniji OT (2008) Seasonal performance of cowpea (Vigna unguiculata) in humid tropics using GGE biplot analysis. World Journal of Biological Research 1: 8-13.

Badu-Apraku B, Akinwale RO, Menkir A, Obeng-Antwi K, Osuman AS, Coulibaly N, Onyibe JE, Yallou GC, Abdullai MS and Didjera A (2011) Use of GGE biplot for targeting early maturing maize cultivars to mega-environments in West Africa. African Crop Science Journal 19: 79-96.

Becker HC and Leon J (1988) Stability analysis in plant breeding. Plant Breeding 101: 1-23.

Benhin JKA (2006) Climate change and South African agriculture: Impact and adaptation options. Centre for Environmental Economics and Policy in Africa, Discussion Paper no.21. University of Pretoria, South Africa. Available on the internet from: http://www.ceepa.co.za/docs/POLICY%20NOTE%2028.pdf [Date Accessed: 01 November 2012].

Berke TG and Rocheford TR (1995) Quantitative trait loci for flowering, plant and ear height and kernel traits in maize. Crop Science 35: 1542-1549

Bjarnason M and Vasal SK (1992) Breeding of Quality Protein Maize (QPM). In: J Jarick (Eds.). Plant breeding Reviews. Vol 9. John Wiley and Sons, Inc., New York, USA, pp. 181-216.

Boyer CD and Hannah LC (1994) Kernel mutants of corn. In: AR Hallauer (Eds.). Speciality Corn. CRC Press, Boca Raton, FL, pp. 1-28.

Choukan (2011) Genotype, environment and genotype x environment interaction effects on the performance of maize inbred lines (*Zea mays* L.). Crop Breeding Journal 1: 97-103.

Commuri PD and Jones RJ (2001) High temperatures during endosperm cell division in maize: A genotypic comparison under in vitro and field conditions. Crop Science 41: 1122-1130.

Crossa J (1990) Statistical analysis of multilocational trials. Advances in Agronomy 44: 55-85.

Downsell CR, Paliwali RC and Contrell RP (1996) Maize in the third world. West View Press, pp.1-37.

Duarte AP, Mason SC, Jackson DS and Kiehl JC (2005) Grain quality of Brazilian maize genotypes as influenced by nitrogen level. Crop Science 45: 1958-1964.

Durand W (2006) Assessing the impact of climate change on crop water use in South Africa. Centre for Environmental Economics and Policy in Africa, Discussion Paper no.28. University of Pretoria, South Africa. Available on the internet from: http://www.ceepa.co.za/docs/POLICY%20NOTE%2028.pdf [Date Accessed: 01 November 2012].

Falconer DS (1981) Introduction to Quantitative Genetics. 2nd ed. Longman Group Limited, New York, 340 pp.

FAO (2005) Fertiliser use by crop South Africa. Available on the internet from: http://www.ftp://ftp.fao.org/agl/agll/docs/fertusesouthafrica.pdf [Date Accessed: 01 November 2012].

Folch J, Lees M and Stanley GHS (1957) A simple method for the isolation and purification of total lipids from animal tissues. Journal of Biological Chemistry 226: 497-509.

Gauch HG (1992) Statistical analysis of regional yield trials: AMMI analysis of factorial designs. Elsevier, Amsterdam, Netherlands, 278 pp.

Gauch HG and Zobel RW (1988) Predictive and postdictive success of statistical analyses of yield trial. Theoretical and Applied Genetics 76: 1-10.

Gauch HG and Zobel RW (1996) AMMI analysis of yield trials. In: Kang MS and Gauch HG (Eds), Genotype by environment interaction. CRC Press, Boca Raton, FL, pp. 1-40.

Gauch HG and Zobel RW (1997) Identifying mega environments and targeting genotypes. Crop Science 37: 311-26.

GenStat (2012) Introduction to GenStat for Windows. 16th ed. VSN International, Hemel Hemstead, Hertfordshire HPI. IES, UK.

Ghafoor A, Arshad IA and Muhammad F (2005) Stability and adaptability analysis in sunflower specifically from eight locations in Pakistan. Journal of Applied Science 5: 118-121.

Gissa DW (2008) Genotypic variability and combining ability of quality protein maize inbred lines under stress and optimal conditions. PhD Thesis, Department of Plant Sciences, University of the Free State, South Africa.

Griess JK, Mason SC, Jackson DS, Galusha TD, Yaseen M and Pedersen JF (2010) Environment and hybrid influences on food-grade sorghum grain yield and hardness. Crop Science 50: 1480-1489.

Guria P (2006) Physico-chemical properties, nutritional quality and value addition to quality protein maize (*Zea mays* L.). MSc Thesis, Department of Food Science and Nutrition, University of Agricultural Sciences, Dharwad.

Gutierrez-Rojas A, Scott MP, Leyva OR, Menz M and Betrán J (2008) Phenotypic characterization of quality protein maize endosperm modification and amino acid contents in a segregating recombinant inbred population. Crop Science 48: 1714-1722.

Hadi G (2004) Effect of the length of the kernel filling period and the kernel filling rate on the grain yield of maize under different water supply conditions. Cereal Research Communication 32: 465-470.

Hohls T, Shanahan PE, Clarke GP and Gevers HO (1995) Genotype x environment interactions in a 10 x 10 diallel cross of quality protein maize (*Zea mays* L.). Euphytica 84: 209-218.

Hohls T, Shanahan PE, Clarke GP and Gevers HO (1996) Genetic control of kernel modification found in South African quality protein maize inbred lines. Euphytica 87: 103-109.

Ignjatovic-micic D, Markovic K, Ristic D, Mladenovic-drinic S, Stankovic S, Lazic-jancic V and Denic M (2009) Variability analysis of normal and opaque2 maize inbred lines. Genetika 41: 81-93.

Jurgens SK, Johnson RR and Boyer JS (1978) Dry matter production and translocation in maize subjected to drought during grain fill. Agronomy Journal 70: 678-682.

Kang, MS (2004) Breeding: Genotype-by-environment interaction. In: RM Goodman (Eds.). Encyclopedia of plant and crop science. Marcel-Dekker, New York, pp. 218-221.

Kaya Y, Akcura M and Taner S (2006) GGE biplot analysis of multi-environment yield trials in bread wheat. Turkish Journal of Agriculture 30: 325-337.

Kaye NM, Mason SC, Jackson DS and Galusha TD (2007) Crop rotation and soil amendment alters sorghum grain quality. Crop Science 47: 722-729.

Kettlewell PS (1996) Agronomy and cereal quality. In: RJ Henry, and PS Kettlewell, (Eds.). Cereal grain quality. Chapman and Hall, London, Cambridge, pp. 407-437.

Kniep KR and Mason SC (1991) Lysine and protein content of normal and opaque-2 maize grain as influenced by irrigation and nitrogen. Crop Science 31: 177-181.

Lambert RJ, Alexander DE and Han ZJ (1998) A high oil pollinator of kernel oil and effects on grain yields of maize hybrids. Agronomy Journal 90: 211-215.

Laubscher MC, Roux CZ and Geerthesen JMP (2000) An explanation of genotype by environment interaction for maize in South Africa. South African Journal of Plant and Soil 17: 147-150.

Laurie SM (2010) Agronomic performance, consumer acceptability and nutrient content of new sweet potato varieties in South Africa. PhD Thesis, Department of Plant Sciences, University of the Free State, Bloemfontein, South Africa.

Letchworth MB and Lambert RJ (1998) Pollen parent effects on oil, protein, and starch concentration in maize kernels. Crop Science 38: 363-367.

Machida (2008) 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. PhD Thesis, African Centre for Crop Improvement, University of KwaZulu-Natal, Pietermaritzburg, South Africa.

Mekonen Z and Mohammed H (2010) Study on genotype x environment interaction of oil content in sesame (*Sesamum indicum* L.). World Journal of Fungal and Plant Biology 1: 15-20.

Mitrovic B, Stanisavljevi D, Treski S, Stojakovic M, Ivanovic M, Bekavac G and Rajkovic M (2012) Evaluation of experimental maize hybrids tested in multi-location trials using AMMI and GGE biplot analyses. Turkish Journal of Field Crops 17: 35-40.

Monjardino P, Smith AG and Jones RJ (2005) Heat stress effects on protein accumulation of maize endosperm. Crop Science 45: 1203-1210.

Mukharib DS (2006) Studies on drought tolerance and molecular characterization of maize genotypes. MSc Thesis, Department of Agronomy, University of Agricultural Sciences, Dharwad.

Ngaboyisonga C (2008) Quality protein maize under stress environments: Gene action and genotype × environment effects. PhD Thesis, Department of Plant Science and Crop Protection, University of Nairobi, Kenya.

Ngaboyisonga C, Njoroge K, Kirubi D and Githiri SM (2006) Effects of low nitrogen and drought on grain yield and endosperm hardness of quality protein maize single cross hybrids. International Plant Breeding Symposium, Mexico City, Mexico, 137pp.

Nurit E, Tiessen A, Pixley KV and Palacios-Rojas N (2009) Reliable and inexpensive colorimetric method for determining protein-bound tryptophan in maize kernels. Journal of Agricultural and Food Chemistry 57: 7233-7238.

Oikeh SO, Kling JG and Okoruwa AE (1998) Nitrogen fertiliser management effects on maize grain quality in the West African moist savanna. Crop Science 38: 1056-1061.

Okello DK, Manna R, Imanywoha J, Pixley K and Edema R (2006) Agronomic performance of selected inbred lines for improvement of protein quality of adapted Ugandan maize germplasm. African Crop Science Journal 14: 37-47.

Paterniani E (1990) Maize breeding in the tropics. Critical Review in Plant Science 9: 125-154.

Pixley KV and Bjarnason MS (1993) Combining ability for yield and protein quality among modified endosperm opaque-2 tropical maize inbreds. Crop Science 33: 1229-1234.

Pixley KV and Bjarnason MS (2002) Stability of grain yield, endosperm modification, and protein quality of hybrid and open-pollinated quality protein maize cultivars. Crop Science 42: 1882-1890.

Preciado-Ortiz R, Guerrero R, Ortega A, Terrón A, Crossa F, Cordova H, Reyes C, Aguilar G, Tut C, Gómez N and Cervantes E (2006) Identification of superior quality protein maize hybrids for different mega-environments using the biplot methodology. Maydica 51: 451-460.

Purchase JL, Hatting H and Van Deventer CS (2000) Genotype x environment interaction of winter wheat (*Triticum aestivum* L.) in South Africa: I. AMMI analysis of yield performance. South African Journal of Plant and Soil 17: 95-100.

Ramburan S, Zhou M and Labuschagne M (2011) Interpretation of genotype x environment interactions of sugar cane: Identifying significant environmental factors. Field Crops Research 124: 392-399.

SAGL (2011) South African maize crop quality 2010/2011 season. Available on the internet from: http://www. sagl.co. za/ [Date Accessed: 01 November 2012].

Schoeman LJ (2003) Genotype × environment interaction in sunflower (*Helianthus annuus*) in South Africa. MSc Thesis, Department of Plant Sciences, University of the Free State, Bloemfontein, South Africa.

Shava JG (2007) Genotype x environment interaction effects on the developmental time, kernel modification and yield of experimental quality protein maize (*Zea mays* L.) hybrid varieties. MSc Thesis, Department of Crop Science, University of Zimbabwe, Harare, Zimbabwe.

Solomon KF, Smit HA, Malan E and Du Toit WJ (2008) Parametric model based assessment of genotype×environment interactions for grain yield in durum wheat under irrigation. International Journal of Plant Production 2: 23-36.

Surma M, Adamski T, Banaszak Z, Kaczmarek Z, Kuczyn´ska1 A, Majcher M, Ługowska B, Obuchowski W, Salmanowicz B and Krystkowiak K (2012) Effect of genotype, environment and their interaction on quality parameters of wheat breeding lines of diverse grain hardness. Plant Production Science 15: 192-203.

Terasawa Jr. F, Vencovsky R and Koehler H (2008) Environment and genotype-environment interaction in maize breeding in Paraná, Brazil. Crop Breeding and Applied Biotechnology 8: 17-22.

Tsai CY, Dweikat I, Huber DM and Warren HL (1992) Interrelationship of nitrogen nutrition with maize (*Zea mays*) grain yield, nitrogen use efficiency and grain quality. Journal of the Science of Food and Agriculture 58: 1-8.

Van der Merwe R (2010) Genotype by environment interaction for oil quality in high oleic acid sunflower lines. PhD Thesis, Department of Plant Sciences, University of the Free State, Bloemfontein, South Africa.

Vivek BS, Krivanek AF, Palacios-Rojas N, Twumasi-Afriyie S and Diallo AO (2008) Breeding Quality Protein Maize (QPM): Protocols for Developing QPM Cultivars. Mexico, D.F.: CIMMYT, 50 pp.

Worku M (2005) Genetic and crop-physiological basis of nitrogen efficiency in tropical maize: Field studies. PhD Dissertation, Faculty of Natural Sciences, University of Hannover, Germany.

Worku M, Bänziger M, Schulte auf´m Erley G, Friesen D, Diallo AO and Horst WJ (2007) Nitrogen uptake and utilization in contrasting nitrogen efficient tropical maize hybrids. Crop Science 47: 519-528.

Yan W (2001) GGE biplot- a Windows application for graphical analysis of multi-environmental data and other types of two-way data. Agronomy Journal 93: 1111-1118.

Yan W (2002) Singular value partitioning in biplot analysis of multi-environment trial data. Agronomy Journal 94: 990-996.

Yan W and Hunt LA (2001) Genetic and environmental causes of genotype by environment interaction for winter yield in Ontario. Crop Science 41: 19-25.

Yan W and Hunt LA (2002) Biplot analysis of multi-environment trial data: In: M.S. Kang (Eds.). Quantitative Genetics, Genomics and Plant Breeding. CAB International, Willingford, pp. 289-303.

Yan W and Kang MS (2003) GGE Biplot Analysis: A Graphical Tool for Breeders, Geneticists and Agronomists. CRC Press, Boca Raton, FL, pp. 3-93.

Yan W and Tinker NA (2006) Biplot analysis of multi-environment trial data: Principles and applications. Canadian Journal of Plant Sciences 86: 623-645.

Yan W, Cornelius PL, Crossa J and Hunt HL (2001) Two types of GGE biplot for analysing multi-environment trial data. Crop Science 41: 656-63.

Yan W, Hunt LA, Sheng Q and Szlavnics Z (2000) Cultivar evaluation and mega-environment investigation based on the GGE biplot. Crop Science 40: 597-605.

Yan W, Kang MS, Ma B, Woods S and Cornelius PL (2007) GGE Biplot vs AMMI analysis of genotype by environment data. Crop Science 47: 643-53.

Zhou M, Joshi SV and Maritz T (2012) Trends and implications of genotype by environment interaction in South African sugarcane breeding. Journal of Crop Improvement 26: 163-176.

Zobel RW, Wright MJ and Gauch HG Jnr (1988) Statistical analysis of a yield trial. Agronomy Journal 80: 388-393.

Chapter 5

Evaluation of high quality protein maize (QPM) and non-QPM hybrids and open-pollinated varieties under two nitrogen levels for grain quality traits

5.1 Abstract

Low N in the soil affects grain quality traits of maize. Field experiments were conducted in two provinces of SA to evaluate grain quality traits of 20 QPM and non-QPM genotypes under low and optimum N conditions. Single and combined ANOVAs were conducted in low N, optimum N and across N environments for grain quality traits. Single and combined correlation matrix analyses were also conducted to determine relationships between the grain quality characters. There were non-significant genotype differences for all grain quality traits except for oil content at Cedara optimum N and tryptophan content and QI in all environments. The QPM had acceptable levels of hardness, which is an important trait for millers and QPM commercialisation. Environment effects were highly significant for all traits measured and played a major role in determining the expression of the traits. The GEI was significant for tryptophan content and QI, but was not larger than environment effects. Most of the traits improved with increased N level. Although many correlations were significant, values were relatively low except between tryptophan content and QI, and starch and oil content. Starch was significantly negatively correlated with oil content (r = -0.72, -0.65 and -0.67), whilst tryptophan content were highly significantly correlated with QI (r = 0.95, 0.98 and 0.97) in low, optimum and across N environments. Tryptophan content and QI values were lower in low N than optimum N environments. QPM varieties performed significantly better than non-QPM varieties for tryptophan and QI in all environments. Genetic variation observed for tryptophan content in the genotypes will be useful in the QPM breeding programmes, with better performing synthetics to be tested across more environments to determine their stability. Positively correlated traits will make it easier for simultaneous selection of grain quality traits in the ARC-GCI maize breeding programme.

5.2 Introduction

Several million people, particularly in the developing countries, derive their protein and calorie requirements from maize (Mbuya *et al.*, 2011). In SA, more than half of the maize consumed domestically is for human consumption and the rest is used for animal feed and industrial purposes (NDA, 2012). The maize kernel has poor nutritional quality and is mainly fortified in developed countries for human consumption, while for animal consumption the feed is supplemented with other protein sources. In most of the developing countries the reality is that the poorer communities are unable to acquire fortified products and other sources of protein to consume with maize. The majority of pregnant women and young children are at a higher risk of contracting protein deficiency diseases. Maize oil, due to a high degree of unsaturated fatty acids and low levels of saturated fatty acids (Zai and Gao, 2001), is widely used for human consumption and it is also an important energy source for livestock feed (Perry, 1988). Subsequently, in order to improve human and animal health, it is of great importance to improve the nutritional quality of maize. The discovery of the opaque-2 mutant in the 1960s brought a lot of hope for a breakthrough to improve the nutritional value of normal maize and bring relief to millions of people suffering from malnutrition and other diseases associated with the absence of essential amino acids. The improvement of maize using the opaque-2 mutant lead to the development of QPM varieties with about twice the levels of lysine and tryptophan and 10% higher grain yield than the most modern varieties of tropical maize. This created the possibility of significant improvement in human and animal nutrition (Akande and Lamidi, 2006; Olakojo *et al.*, 2007).

In developing countries most of the maize is grown under low N conditions (McCown *et al.*, 1992; Stoorvogel *et al.*, 1993) and this is mainly due to high fertiliser cost (Odhiambo and Magandini, 2008). In SA fertiliser use is higher compared to other African countries; however its application is still lower than the recommended optimum levels, especially in the case of small-scale farmers. Nitrogen significantly affects grain quality traits in normal maize (Tsai *et al.*, 1992; Oikeh *et al.*, 1998) and QPM (Wegary *et al.*, 2011; Ngaboyisonga *et al.*, 2012). Most of the research on maize at large focuses on grain yield and yield related traits, and the impact of low N on protein quality and quantity of QPM germplasm has not yet been sufficiently addressed (Gissa, 2008; Ngaboyisonga *et al.*, 2012). Determination of tryptophan content is a necessary step to develop QPM, and

every QPM breeding programme must have access to a laboratory equipped to do tryptophan analysis. Various researchers have conducted comparative studies between QPM and normal maize endosperm for yield, endosperm hardness, protein, tryptophan, fat, and starch concentration; and results show that QPM varieties perform better for tryptophan or lysine, equivalent or better for yield, endosperm hardness and protein, including fat, starch and carbohydrate concentration (Bjarnason and Vasal, 1992; Pixley and Bjarnason, 1993; Martinez *et al*., 1996; Pixley and Bjarnason 2002; Guria, 2006). Levels of tryptophan in opaque-2 maize range from 0.5% to 1.1% and in normal endosperm maize from 0.2% to 0.5% (Nurit *et al.,* 2009) while in QPM the levels have to be equal or greater than 0.8% (Krivanek *et al.,* 2007; Vivek *et al.,* 2008).

Negative and positive relationships amongst various maize grain quality characters have been reported (Song *et al.,* 1999; Uribelarrea *et al.,* 2004; Pradeepa, 2007; Ngaboyisonga *et al.,* 2012). It is important to target the most important grain quality characters in QPM breeding because they add value to the grain for animal feed, human health and industrial applications. But it is not easy to do that simultaneously since some traits are negatively correlated. There is no information with regards to correlation of starch, oil, protein and tryptophan in QPM. Most of the available QPM germplasm and commercial hybrids have been developed and tested under optimum conditions and there is limited information on their grain quality traits compared to those of non-QPM germplasm in SA. The aim of this study was to determine whether QPM (hybrids and OPVs) perform better or similar to normal maize and how the grain quality traits of the different genotypes are affected by low and optimum N environments, and to determine the relationship between grain quality traits.

5.3 Materials and methods

5.3.1 Germplasm, trial environment, design and management

Eight QPM and 12 non-QPM varieties were used in this study. The materials consisted of hybrids (four QPM hybrids and six non-QPM hybrids), and open-pollinated varieties (four QPM OPVs and six non-QPM OPVs). A list of varieties and their description is given in Table 5.1. The varieties were tested in Potchefstroom and Cedara under two N

levels (low and optimum N) resulting in four environments. The trial designs, N application rates and trial management were the same as those described in Chapter 4.

Table 5.1 List of maize hybrids and open-pollinated varieties evaluated in two locations

Entries	Types	Origin
PAN6479	Non-QPM hybrid	PANNAR
PAN6611	Non-QPM hybrid	PANNAR
PAN6616	Non-QPM hybrid	PANNAR
PhB30Y83	Non-QPM hybrid	PIONEER
CRN3505	Non-QPM hybrid	MONSANTO
DKC78-15 Bt	Bt maize hybrid	MONSANTO
QS7705	QPM hybrid	Quality Seed
QS7707	QPM hybrid	Quality Seed
QS7711	QPM hybrid	Quality Seed
QS7715	QPM hybrid	Quality Seed
Syn9QW	QPM Synthetic OPV	ARC-GCI
Syn15QW	QPM Synthetic OPV	ARC-GCI
Syn12 QW	QPM Synthetic OPV	ARC-GCI
Obatanpa-SR	QPM OPV	CIMMYT-Zim
SAM1109	Non-QPM OPV	ARC-GCI
ZM1421	Non-QPM OPV	CIMMYT-Zim
ZM1423	Non-QPM OPV	CIMMYT-Zim
ZM1523	Non-QPM OPV	CIMMYT-Zim
ZM1623	Non-QPM OPV	CIMMYT-Zim
ZM521	Non-QPM OPV	CIMMYT-Zim

5.3.2 Data collection and analyses

Data collection and analyses methods for grain quality traits were the same as described in Chapter 4. Single and combined ANOVAs were conducted in low N, optimum N and across N environments for grain quality traits. Single and combined correlation matrix analyses were also conducted for optimum and low N environments and across N environments to determine relationships between the grain quality characters.

5.4 Results

Single ANOVAs conducted for each environment showed significant differences amongst genotypes for oil content at Cedara optimum N and highly significant genotype variation for tryptophan content and QI in all environments. The single ANOVA's results are not shown in the thesis chapter as combined analyses of the two locations were deemed a better representation of trends than single location analysis. The environment effects were significant for QI, starch and oil content; and highly significant for the other traits except for tryptophan content in low N environment, and highly significant for all traits in the optimum N environment (Table 5.2). GEI was highly significant for only tryptophan and QI in all environments. Genotypes played a major role in the determination of grain quality traits when compared to environment and GEI effects in all environments.

Tryptophan content of the QPM varieties ranged between 0.07-0.09% across low N environments and 0.11-0.16% across optimum N environments; whilst it ranged between 0.03-0.07% and 0.05-0.07% for the non-QPM varieties across low N and optimum N environments respectively (Table 5.3). The QPM varieties had QI values which ranged from 0.70-1.05% under low N and 1.09-1.53% under optimum N; while for non-QPM varieties it ranged between 0.34-0.61% in low N and 0.47-0.72% in optimum N environments. Average means of the varieties for tryptophan content and QI in low N conditions were 0.06% and 0.68%, whilst those for optimum N environments were 0.10% and 0.94% respectively. The genotypes with best performance for tryptophan content and QI under both low and optimum N environments were QS7707 and Syn15QW respectively. The average means of tryptophan and QI for the QPM hybrids and non-QPM hybrids were 0.08 and 0.05% in low N sites respectively, whereas in optimum N sites it was 0.12 and 0.06% respectively. For QPM OPVs and non-QPM OPVs the average mean was 0.07 and 0.05% respectively in low N conditions, whereas it was 0.14 and 0.07% respectively in optimum N environments. Tryptophan content and QI value were lower in low N than optimum N environments, and higher in QPM than non-QPM. Therefore, QPM varieties performed much better than non-QPM varieties for tryptophan and QI.

Table 5.2 Mean squares for six grain quality traits of 20 QPM and non-QPM varieties grown in low and optimum N environments

Source	DF	Mean squares low N						Mean squares optimum N					
		Protein	Tryp	QI	EH	Starch	Oil	Protein	Tryp	QI	EH	Starch	Oil
Block	2	0.116	0.000	0.004	0.078	0.207	0.024	0.283	0.000	0.007	0.015	0.185	0.006
Genotype	19	0.506	0.002**	0.209**	0.233	0.655	0.140	0.528	0.007**	0.659**	0.197	0.990	0.264
Env	1	14.008**	0.000	0.043*	4.929**	5.002*	0.990*	8.112**	0.002**	0.046**	12.095**	25.669**	0.954**
GEI	19	0.426	0.000**	0.021**	0.184	0.928	0.158	0.378	0.001**	0.061**	0.278	0.726	0.127
Residual	76	0.620	0.000	0.007	0.195	0.808	0.187	0.709	0.000	0.007	0.324	0.654	0.138
Mean		9.22	0.06	0.64	2.51	70.3	4.87	10.05	0.09	0.88	2.26	69.91	4.91
$LSD_{(0.05)}$		1.08	0.01	0.12	0.61	1.23	0.59	1.16	0.0004	0.12	0.78	1.11	0.51
CV (%)		8.54	11.62	13.1	17.6	1.28	8.87	8.38	3.14	9.64	25.14	1.16	7.56

*P≤0.05; **P≤0.01; Env=Environments; GEI=Genotype by environment interaction; LSD=least significant difference; CV=Coefficient of variation; Tryp=Tryptophan; EH=Endosperm hardness; QI=Quality Index

Table 5.3 Mean values of 20 QPM and non-QPM varieties for grain quality traits grown in low and optimum N environments of South Africa

Entries	Low N						Optimum N					
	Protein	**Tryp**	**QI**	**EH**	**Oil**	**Starch**	**Protein**	**Tryp**	**QI**	**EH**	**Oil**	**Starch**
QS7705	9.18(11)	0.08(4)	0.83(4)	2.49(10)	4.83(11)	70.15(15)	9.80(16)	0.13(3)	1.36(3)	2.48(18)	4.95(11)	69.78(12)
QS7707	8.97(16)	0.09(1)	1.05(1)	2.57(13)	4.80(16)	70.37(11)	10.20(5)	0.11(8)	1.10(7)	2.20(8)	4.97(9)	69.60(18)
QS7711	8.73(20)	0.08(2)	0.94(2)	2.92(20)	4.85(12)	70.50(5)	9.53(20)	0.12(4)	1.21(4)	2.07(5)	4.47(20)	70.87(1)
QS7715	9.32(7)	0.07(8)	0.70(8)	2.53(12)	4.87(10)	70.42(8)	10.25(4)	0.11(7)	1.09(8)	2.03(3)	5.05(5)	69.10(20)
QPM hybrid mean	**9.05**	**0.08**	**0.88**	**2.63**	**4.84**	**70.36**	**9.95**	**0.12**	**1.19**	**2.20**	**4.86**	**69.84**
CRN3505	9.05(15)	0.06(9)	0.61(9)	2.45(8)	4.92(7)	70.28(12)	9.68(19)	0.07(10)	0.67(10)	2.55(20)	5.15(2)	69.87(9)
PAN6479	9.13(12)	0.05(18)	0.51(17)	2.34(5)	4.83(15)	70.48(6)	9.83(15)	0.06(19)	0.61(16)	2.38(14)	4.88(14)	69.77(13)
PAN6611	9.65(3)	0.05(13)	0.52(15)	2.30(4)	5.10(3)	69.68(20)	10.43(2)	0.06(17)	0.57(19)	2.04(4)	4.95(10)	60.73(14)
PAN6616	8.87(19)	0.05(10)	0.60(10)	2.58(14)	4.93(6)	70.75(2)	9.70(18)	0.07(12)	0.67(11)	2.48(17)	4.60(19)	70.55(2)
PHB30Y83	9.45(4)	0.05(12)	0.55(14)	2.28(3)	5.13(1)	70.00(17)	10.07(11)	0.07(15)	0.65(13)	2.39(15)	4.80(15)	70.07(6)
DKC78-15 Bt	9.37(5)	0.05(16)	0.52(16)	2.58(16)	5.00(4)	70.45(7)	10.80(1)	0.05(20)	0.47(20)	2.02(2)	5.03(6)	69.40(19)
Normal hybrid mean	**9.25**	**0.05**	**0.55**	**2.42**	**4.99**	**70.27**	**10.09**	**0.06**	**0.61**	**2.31**	**4.90**	**68.40**
Obatanpa-SR	9.88(1)	0.07(5)	0.73(7)	2.24(1)	5.10(2)	69.78(19)	10.07(10)	0.12(6)	1.14(6)	2.28(11)	5.27(1)	70.15(5)
Syn9QW	9.08(14)	0.08(3)	0.90(3)	2.51(11)	4.57(19)	70.75(3)	10.18(6)	0.14(2)	1.39(2)	2.47(16)	4.90(13)	70.02(7)
Syn12QW	8.95(17)	0.07(6)	0.77(5)	2.76(17)	4.75(17)	70.28(13)	9.88(13)	0.12(5)	1.17(5)	2.50(19)	4.70(16)	69.85(10)
Syn15QW	9.23(9)	0.07(7)	0.74(6)	2.80(18)	4.98(5)	70.40(9)	10.18(7)	0.16(1)	1.53(1)	2.37(13)	5.13(3)	69.68(15)
QPM OPV mean	**9.29**	**0.07**	**0.79**	**2.58**	**4.85**	**70.30**	**10.08**	**0.14**	**1.31**	**2.41**	**5.00**	**69.93**
ZM521	9.28(8)	0.03(20)	0.34(20)	2.58(15)	4.90(8)	70.25(14)	9.88(14)	0.06(16)	0.61(17)	2.20(9)	5.12(4)	69.78(11)
ZM1421	9.22(10)	0.03(19)	0.36(19)	2.25(2)	4.88(9)	70.38(10)	9.77(17)	0.07(9)	0.72(9)	2.29(12)	4.65(17)	70.37(3)
ZM1423	9.12(13)	0.05(15)	0.56(13)	2.38(7)	4.57(20)	70.63(4)	10.13(9)	0.07(14)	0.64(15)	2.16(7)	4.97(8)	69.67(16)
ZM1523	9.35(6)	0.05(11)	0.56(12)	2.36(6)	4.87(11)	70.03(16)	10.33(3)	0.06(18)	0.58(18)	2.26(10)	5.00(7)	69.62(17)
ZM1623	9.68(2)	0.07(17)	0.48(18)	2.48(9)	4.85(13)	69.83(18)	10.07(12)	0.07(11)	0.65(14)	2.12(6)	4.62(18)	70.37(4)
SAM1109	8.92(18)	0.05(14)	0.58(11)	2.81(19)	4.72(18)	70.92(1)	10.13(8)	0.07(13)	0.65(12)	2.00(1)	4.95(12)	69.92(8)
Normal OPV mean	**9.26**	**0.05**	**0.48**	**2.48**	**4.80**	**70.34**	**10.05**	**0.07**	**0.64**	**2.17**	**4.89**	**69.96**
Grand mean	**9.21**	**0.06**	**0.68**	**2.53**	**4.87**	**70.32**	**10.04**	**0.10**	**0.94**	**2.27**	**4.91**	**69.53**
LSD $_{(0.05)}$	**1.08**	**0.01**	**0.12**	**0.61**	**0.59**	**1.23**	**1.16**	**0.0004**	**0.12**	**0.78**	**0.51**	**1.11**

LSD=least significant difference; Tryp=Tryptophan; EH=Endosperm hardness; QI=Quality Index; OPV=Open pollinated variety

Combined ANOVA across N environments is presented by Table 5.4. Highly significant environmental effects were observed for all traits and genotypic effects for tryptophan and QI. Environmental effects played a major role in determination of the quality traits, followed by genotype effects and then GEI for all characters except for endosperm hardness where genotype effects were the least important. Averaged over all environments, tryptophan content for all varieties ranged from 0.05% to 0.11%, with QPM hybrids and OPVs having the highest tryptophan content compared to other types of varieties (Table 5.5). The average means of QPM and non-QPM hybrids for tryptophan was 0.10 and 0.06% whereas for OPVs they were 0.11% and 0.06% respectively. For QI the average means of QPM and non-QPM hybrids were 1.03% and 0.58% respectively, whereas for QPM and non-QPM OPVs they were 1.05% and 0.56% respectively. The average means of all the varieties for protein content, endosperm hardness, tryptophan, QI, oil and starch contents were 9.6%, 2.4%, 0.08%, 0.81%, 4.9% and 70.1% respectively. The top five performers for tryptophan and QI across N environments were Syn15QW, Syn9QW, QS7705, QS7707 and QS7711. Taking all measured traits into consideration Obatanpa-SR performed well overall for protein (9.98%), tryptophan content (0.09%), QI (0.93%), endosperm hardness (2.26%) and oil content (5.18%), followed by QS7715 and QS7707. QPM varieties performed much better than non-QPM varieties for tryptophan and QI.

Under low N environments tryptophan content was highly significantly (almost 100%) and positively correlated with QI ($r = 0.95$) and was non-significantly ($P>0.05$) correlated with all the other traits (Table 5.6). This was expected since tryptophan content values are used to calculate QI. Starch content showed a highly significant ($P<0.01$) negative relationship with oil content ($r = -0.72$). Protein content had a highly significant negative correlation with QI ($r = -0.32$), endosperm hardness ($r = -0.27$) and starch content ($r = -0.41$). Endosperm hardness was highly significantly positively correlated with QI ($r = 0.23$) and significantly positively correlated with starch content ($r = 0.18$).

Table 5.4 Mean squares of six grain quality traits in 20 QPM and non-QPM varieties grown across four N environments

Source	DF	Protein	Tryp	QI	EH	Starch	Oil
Block in loc	8	0.200	0.000	0.006	0.046	0.196	0.015
Genotype	19	0.759	0.007**	0.725**	0.184	0.847	0.237
Environment	3	20.986**	0.017**	1.114**	6.887**	13.586**	0.673**
GEI	57	0.360	0.001**	0.075**	0.236	0.817	0.151
Residual	152	0.664	0.000	0.007	0.260	0.731	0.162
Mean		9.63	0.073	0.76	2.39	70.11	4.89
$LSD_{(0.05)}$		0.78	0.01	0.08	0.49	0.82	0.37
CV (%)		8.46	7.12	11.10	21.34	1.22	8.24

**P≤0.01; Env=Environments; GEI=Genotype by environment interaction; LSD=least significant difference; CV=Coefficient of variation; Tryp=Tryptophan; EH=Endosperm hardness; QI=Quality Index

Table 5.5 Mean values and rankings of 20 QPM and non-QPM varieties for six grain quality traits across four N environments

Entries	Protein	Rank	Tryp	Rank	QI	Rank	EH	Rank	Oil	Rank	Starch	Rank
QS7705	9.49	14	0.10	3	1.09	3	2.49	15	4.89	10	69.97	16
QS7707	9.58	12	0.10	4	1.07	5	2.38	11	4.88	11	69.98	14
QS7711	9.13	20	0.10	5	1.07	4	2.50	16	4.66	20	70.68	1
QS7715	9.78	6	0.09	8	0.90	8	2.28	5	4.96	8	69.76	19
QPM hybrid mean	**9.50**		**0.10**		**1.03**		**2.41**		**4.85**		**70.10**	
CRN3505	9.37	18	0.06	9	0.64	9	2.50	17	5.03	3	70.08	9
PAN6479	9.48	16	0.05	17	0.56	16	2.36	10	4.86	12	70.13	7
PAN6611	10.04	2	0.06	16	0.55	17	2.17	1	5.03	4	69.71	20
PAN6616	9.28	19	0.06	10	0.64	10	2.53	18	4.77	16	70.65	2
PHB30Y83	9.76	7	0.06	11	0.60	13	2.33	9	4.97	7	70.03	12
DKC78-15 Bt	10.08	1	0.05	19	0.49	19	2.30	6	5.02	5	69.93	17
Normal hybrid mean	**9.67**		**0.06**		**0.58**		**2.37**		**4.95**		**70.09**	
Obatanpa-SR	9.98	3	0.09	6	0.93	7	2.26	2	5.18	1	69.97	15
Syn9QW	9.63	9	0.11	2	1.15	1	2.49	14	4.73	17	70.38	4
Syn12QW	9.42	17	0.09	7	0.97	6	2.63	20	4.73	19	70.07	10
Syn15QW	9.71	8	0.11	1	1.14	2	2.59	19	5.06	2	70.04	11
QPM OPV mean	**9.69**		**0.11**		**1.05**		**2.49**		**4.93**		**70.12**	
ZM521	9.58	11	0.05	20	0.48	20	2.39	12	5.01	6	70.02	13
ZM1421	9.49	15	0.05	18	0.54	18	2.27	3	4.77	14	70.38	5
ZM1423	9.63	10	0.06	12	0.60	12	2.27	4	4.77	15	70.15	6
ZM1523	9.84	5	0.06	14	0.57	14	2.31	8	4.93	9	69.83	18
ZM1623	9.88	4	0.06	15	0.56	15	2.30	7	4.73	18	70.10	8
SAM1109	9.53	13	0.06	13	0.62	11	2.41	13	4.83	13	70.42	3
Normal OPV mean	**9.66**		**0.06**		**0.56**		**2.33**		**4.84**		**70.15**	
Grand mean	**9.63**		**0.08**		**0.81**		**2.40**		**4.89**		**70.12**	
$LSD_{(0.05)}$	**0.78**		**0.01**		**0.08**		**0.49**		**0.39**		**0.82**	

LSD=least significant difference;Tryp=Tryptophan; EH=Endosperm hardness; QI=Quality Index; OPV=Open pollinated variety

Under optimum N environments protein content had a highly significant negative association with endosperm hardness (r = -0.65) and starch content (r = -0.66), a highly significant positive correlation with oil content (r = 0.39) and a significant negative correlation with QI (r = -0.20). Starch content, on the other hand, showed a highly significant positive relationship with endosperm hardness (r = 0.44) and a highly significant negative correlation with oil content (r = -0.65). Tryptophan content was highly significantly (almost 100%) and positively correlated with QI (r = 0.98).

Across all environments tryptophan content was highly significantly positively correlated with protein content (r = 0.20) and QI (r = 0.97); and significantly negative associated with starch content (r = -0.14) although these values were relatively low. Starch content had highly significant and negative correlation with oil content (r = -0.67) across all environments. Protein content on the other hand had a highly significant negative relationship with endosperm hardness (r = -0.52) and starch content (r = -0.57) and a highly significant positive correlation with oil content (r = 0.25). Endosperm hardness showed a highly significant positive association with starch content (r = 0.36).

Table 5.6 Correlation analysis of 20 QPM and non-QPM varieties for quality traits in low N, optimum N and across N environments

	Tryp	Protein	QI	EH	Starch
Low N environments					
Protein	-0.04 ^{ns}				
QI	0.95 **	-0.32 **			
EH	0.15 ^{ns}	-0.27 **	0.23 **		
Starch	0.004 ^{ns}	-0.41 **	0.11 ^{ns}	0.18 *	
Oil	-0.03 ^{ns}	0.13 ^{ns}	-0.07 ^{ns}	0.07 ^{ns}	-0.72 **
Optimum N environments					
Protein	-0.01 ^{ns}				
QI	0.98 **	-0.20 *			
EH	0.03 ^{ns}	-0.65 **	0.15 ^{ns}		
Starch	-0.07 ^{ns}	-0.66 **	0.06 ^{ns}	0.44 **	
Oil	0.09 ^{ns}	0.39 **	0.01 ^{ns}	-0.11 ^{ns}	-0.65 **
Across N environments					
Protein	0.20 **				
QI	0.97 **	-0.03 ^{ns}			
EH	-0.05 ^{ns}	-0.52 **	0.07 ^{ns}		
Starch	-0.14 *	-0.57 **	-0.01 ^{ns}	0.36 **	
Oil	0.06 ^{ns}	0.25 **	-0.004 ^{ns}	-0.04 ^{ns}	-0.67 **

*P≤0.05; **P≤0.01; Tryp= Tryptophan; Prot=Protein; QI=Quality index; EH=Endosperm hardness

5.5 Discussion

Grain quality traits such as endosperm hardness, protein, starch and oil content are extremely important for the maize industry. To make QPM more acceptable and appealing to the South African maize industry it is important to select QPM varieties performing similar or better than non-QPM varieties for the above traits as well as for tryptophan content and QI. Grain quality traits affect each other in different ways and are also affected by N levels in the soil. Therefore it is important in a breeding programme, when developing and testing new materials to take into consideration all these aspects.

The results of this study showed that genotypes performed similar for all traits except for tryptophan content and QI in all environments. The reason for non-significant protein, starch content and endosperm hardness differences between genotypes might be due to the fact that the commercial QPM hybrids available have been improved for those grain quality traits, especially protein content and endosperm hardness, so that they perform better or similar to non-QPM varieties which is extremely important for the maize industry. This shows the amount of dedication and effort given to the development of QPM varieties, and this is confirmed by the new developed QPM synthetics from the ARC-GCI which are also performing similar for all grain quality characters. It brings scientists a step closer to bringing better nutrition to millions of people who rely on maize. However, what is missing in SA currently is linking QPM to the maize industry because farmers are interested in producing the varieties but the produce is mixed with non-QPM at the millers and secondly there is no price incentive involved in producing this nutritious maize.

Bello *et al.* (2012) evaluated 22 QPM hybrids and OPVs with two local checks for nutritional quality and other agronomic traits. They indicated that genotypes were significantly different for carbohydrate content and grain crude protein and non-significant for oil content with the mean value of carbohydrates content ranging from 65 - 74% and protein content ranging between 7.50 - 10.67% and. This study disagreed with Bello *et al.* (2012), with regards to significant differences amongst genotypes for starch and protein content. However, agreed on mean values of starch and protein content ranging from 69.10 – 70.87% and 9.53 – 10.80%. The SAGL (2011) reported that South

African maize on average contains 3.9% oil, 8.7% protein and 72.1% starch. The value ranges between 2.8 - 5.8%, 6.1 - 12.7% and 58.3 - 77.0% for oil, protein and starch content, respectively. The levels of oil, protein and starch obtained in this study are similar to those of South African maize as reported by SAGL (2011).

Sentayehu (2008) tested 12 QPM varieties for protein, tryptophan and lysine and reported highly significant differences between treatments for all traits in all environments. Ngaboyisonga *et al.* (2012) evaluated 41 QPM varieties against one check variety under three low, three optimum N and two drought environments for endosperm modification, tryptophan and protein contents. They found highly significant differences amongst hybrids for all the traits, under low, optimum and across all N conditions. In the present study it was found that there were no genotypic differences for traits measured except for tryptophan content and QI. Ngaboyisonga *et al.* (2012) found highly significant environment effects for all traits under low, optimum and across N conditions. This was coupled with significant GEI effects for all traits in all N conditions except for endosperm modification under low and optimum N conditions. The present study observed significant environmental effects for all characters in all N environments except for tryptophan content in the low N environments and significant GEI effects for tryptophan content only, in all N environments. The environment played the most important role in defining most of the characters in both studies, followed by genotypes, except for EH in the present study where environment effect was followed by GEI. However, overall GEI was less important for all the characters measured in the present study in all N environments. This might be due to the few locations and genotypes used in this study.

CIMMYT (2003) in a study which focused on the effect of abiotic stresses on endosperm modification observed that low N conditions had a negative effect on endosperm modification and that prompted further investigation in the effects of abiotic stresses on tryptophan and protein content. The present study focused on the effect of low N conditions on a number of grain quality traits, where more severe effects were reported than in the above study. There was no significant genetic variation for endosperm hardness under low N and optimum N conditions, although some individual differences were observed. Endosperm modification was higher under low N conditions with a score of 2.53 compared to optimum N conditions with a score of 2.27, showing the presence of

softer kernels or poor modification under low N. This study agrees with CIMMYT (2003) indicating that low N has a negative effect on endosperm modification. Low N affects endosperm hardness negatively, resulting in development of softer kernels (Ngaboyisonga et al., 2012). The mean score of the present study was also within the acceptable array of below 3, similar to findings of Machida (2008). Even though the genotypes were not significantly different from one another for endosperm hardness, Obatanpa-SR was among the best performing genotypes with a score of 2.24 under low N and 2.26 across N environments, with the best performer being SAM1109 with a score of 2.00.

The present study is in agreement with the results obtained by various researchers that tryptophan content of QPM varieties under low and optimum N is higher as compared to normal maize endosperm, and also that tryptophan content is higher under optimum than low N conditions (Mosisa, 2005; Mosisa *et al.,* 2007; Gissa, 2008; Wegary *et al.,* 2011). Gissa (2008) reported a lower QI value under optimum N conditions when compared to low N conditions, and explained that it was due to a sharp reduction in protein content under low N conditions. The present study demonstrated an increase in protein QI value under optimum N together with an increase in protein and tryptophan content. Ngaboyisonga *et al.* (2012) concluded that low N conditions, especially when they occur together with drought, increase tryptophan content with poor grain quality and this raises concerns about its use for human and animal consumption.

Mbuya *et al.* (2011) in a study on six QPM and seven non-QPM varieties focused on profiling the nutritional quality of elite QPM varieties from the Democratic Republic of Congo breeding programme and selected the best QPM variety for local release. They found that tryptophan in the protein, which is also known as QI, was higher in QPM varieties than non-QPMs, and that the tryptophan content of QPM Longe 5 showed a 50% increase over non-QPMs. These results were in agreement with the present research findings, which illustrated that QI of QPM varieties were significantly better than those of the non-QPMs.

Grain quality traits that are negatively correlated make it difficult to select them simultaneously in the same plants. In most QPM studies it has been shown that various grain quality characters are negatively correlated, as a result making the breeding process

more complex. In the present study instances where traits were negatively correlated with one another, making it difficult to select them at the same time, was between starch and oil content in all environments; protein content with QI in low and optimum N enviromnents; protein content with endosperm hardness and protein content with starch in all environments. The levels of correlations were weak except between starch and oil content in all environments, protein content and endosperm hardness; protein and starch content in optimum N and across environments and tryptophan content and QI in all environments.

Pixley and Bjarnason (2002) reported that protein content was highly significantly and positively correlated with tryptophan in the grain, positively correlated with endosperm modification and highly significantly and negatively correlated with QI (r = -0.45); whilst tryptophan in the grain was positively correlated with QI. The correlation between protein and tryptophan content was very high (r = 0.84); however that of protein content and endosperm modification score, tryptophan content with QI and endosperm modification score, tryptophan in protein and endosperm modification score were weak and not significant. Olakojo *et al.* (2007) reported that tryptophan and protein were significantly and positively correlated at 0.56. This study disagrees with the findings by Pixley and Bjarnason (2002) and Olakojo *et al.* (2007); indicating that protein was highly significant and negatively correlated with endosperm hardness (r = -0.65), and non-significant with tryptophan content (r = -0.01) in optimum N environments. However, the study agrees with Pixley and Bjarnason (2002) indicating that protein content and QI are highly significantly and negatively correlated, and that the associations between tryptophan content with endosperm modification score and QI and endosperm modification score were weak and non-significant under optimum N environments.

Gissa (2008) reported that the negative correlation between QI and protein content confirms the probability that higher protein content will result in lower protein quality. An increase in total protein in maize kernels results in an increase in the zein fraction in endosperm protein (Glover, 1992), which has poor nutritional quality by lacking tryptophan and lysine. Gutierrez-Rojas *et al.* (2008) noted a negative correlation between tryptophan and kernel vitreousness (% hardness/total area) and a positive correlation between endosperm opacity and amino acids content in a cross between B73*o2* ×

CML161. They reported that as the recombinant inbred lines inclined from opaqueness towards hard modified kernels the amino acid content decreased. The negative association between QI and protein content is undesirable and very important for QPM breeders because they complicate simultaneous improvement of these traits (Pixley and Bjarnason, 2002). Conversely, lack of unfavourable correlations between endosperm modification score and other traits should facilitate QPM cultivar development efforts (Pixley and Bjarnason, 2002).

The significant positive correlation of protein content with oil content has been found in different populations, lines and cultivars (Mittelmann *et al.,* 2003; Song and Chen, 2004; Wassom *et al.,* 2008; Wang *et al.,* 2009; Li *et al.,* 2011; Cook *et al.,* 2012), however other researchers found non-significant correlations (Dudley and Lambert, 2004; Lambert *et al.,* 2004; Song and Chen, 2004). It has been mostly reported that oil content is significantly and negatively correlated with starch content (Doehlert and Lambert, 1991; Dudley and Lambert, 1992; 2004; Moose *et al.,* 2004; Song and Chen, 2004; Uribelarrea *et al.,* 2004; Pradeepa, 2007; Wang *et al.,* 2009). Mittelmann *et al.* (2003) observed that protein and oil content were positively correlated in all the experiments, but with low values, (0.21 to 0.41). Associations between protein and oil content varied from not significant (Dorsey-Redding *et al.,* 1991; Séne *et al.,* 2001) to highly significant positive values (Song *et al.,* 1999). Uribelarrea *et al.* (2004) reported a strong negative association between grain protein and starch concentration with maize hybrids. With regards to correlations amongst grain quality traits, oil content was reported to have a significant negative association with starch content (r = -0.624) (Pradeepa, 2007). Cook *et al.* (2012) studied genetic architecture of maize kernel composition in the nested association mapping (NAM) and inbred association panels (AP). They found a significant positive correlation between protein and oil (r = 0.32 and r = 0.29, NAM and AP, respectively) and a highly significant negative correlation between starch and protein (r = -0.66 and r = -0.56, NAM and AP, respectively) and starch and oil (r = -0.41 and r = -0.33, NAM and AP, respectively), in both the NAM and AP populations. The results of the present study are in line with most if not all of these findings are in line with the results of the present study.

According to Li *et al.* (2011) significant correlation among traits were generally consistent between low and high N levels. They found that the estimates were either positive or negative under both N levels, with all non-significant estimates except one observed under the high N level. Protein and oil content were positively correlated and showed a strong negative correlation with starch content. According to Alexander and Lambert (1968) the ability of the plant to produce carbohydrates and to synthesize oil are physiologically independent. The current study agreed with these findings. The inverse relationship between kernel protein and starch concentration is impacted not only by genotype, but also by N availability.

5.6 Conclusions

The results of this study showed that genotypes performed similar for all traits except for tryptophan content and QI in all environments. QPMs gave superior tryptophan content and QI value than non-QPMs under both high and low N. The best QPMs under low and optimum N environments were Syn15QW, Syn9QW, QS7705, QS7707 and QS7715. Genetic variation observed for tryptophan content in the genotypes can be useful in the QPM breeding programmes, with newly developed synthetics to be tested across more environments to determine their stability. The best five varieties for tryptophan and QI under low and optimum N environments were Syn15QW, Syn9QW, QS7705, QS7707 and QS7715. Genetic variation observed for tryptophan content in the genotypes can be useful in the QPM breeding programmes, with newly developed synthetics to be tested across more environments to determine their stability. The reason for non-significant protein, starch content and endosperm hardness differences between genotypes might be due to the fact that the commercial QPM hybrids available have been improved for those grain quality traits, especially protein content and endosperm hardness, so that they perform better or similar to non-QPM varieties. This shows the amount of dedication and effort given to the development of QPM varieties, and this is confirmed by the new developed QPM synthetics from the ARC-GCI which are also performing similar for all grain quality characters except for tryptophan content and QI value, for which they perform better than non-QPM.

Most of the traits measured in the present study were interdependent as evidenced by significant and negative association among them. Correlated traits will make it easier for simultaneous selection in the ARC-GCI maize breeding programme. Thus to come up with conclusive recommendations, further studies with more locations and a large number of QPM genotypes are required under low and optimum N environments to determine the stability of genotypes for grain quality traits, thus seeking a deeper understanding of the relationships and GEI, in the view of increasing the efficiency of breeding programmes to N stress conditions.

5.7 References

Akande SR and Lamidi GO (2006) Performance of quality protein maize varieties and disease reaction in the derived-savanna agro-ecology of South-West Nigeria. African Journal of Biotechnology 5: 1744-1748.

Alexander DE and Lambert RJ (1968) Relationship of kernel oil content to yield in maize. Crop Science 8: 273-274.

Bello OB, Oluleye F, Mahamood J, Afolabi MS, Azeez MA, Ige SA and Abdulmaliq SY (2012) Nutritional and agronomic evaluation of quality protein maize in the southern Guinea savanna of Nigeria. Scholarly Journal of Agricultural Science 2: 52-61.

Bjarnason M and Vasal SK (1992) Breeding of Quality Protein Maize (QPM). In: J Jarick (Eds.). Plant breeding Reviews. Vol 9. John Wiley and Sons, Inc., New York, USA, pp 181-216.

CIMMYT (2003a) The development and promotion of quality protein maize in sub-Saharan Africa. Progress Report Submitted to Nippon Foundation. Report on the effect of abiotic stresses on endosperm modification. CIMMYT, Mexico, D.F., Mexico.

Cook JP, McMullen MD, Holland JB, Tian F, Bradbury P, Ross-Ibarra J, Buckler ES and Flint-Garcia SA (2012) Genetic architecture of maize kernel composition in the nested association mapping and inbred association panels. Plant Physiology 158: 824-834.

Doehlert DC and Lambert RJ (1991) Metabolic characteristics associated with starch, protein, and oil deposition in developing maize kernels. Crop Science 31:151-157.

Dorsey-Redding CD, Hurburgh Jr. CR, Johnson LA and Fox SR (1991) Relationships among maize quality factors. Cereal Chemistry 68: 602-605.

Dudley JW and Lambert RJ (1992) Ninety generations of selection for oil and protein in maize. Maydica 37: 81-87.

Dudley JW and Lambert RJ (2004) 100 generations of selection for oil and protein in corn. Plant Breeding Review 24: 79-110.

Gissa DW (2008) Genotypic variability and combining ability of quality protein maize inbred lines under stress and optimal conditions. PhD thesis, Department of Plant Sciences, University of the Free State, Bloemfontein, South Africa.

Glover DV (1992) Corn protein - Genetics, breeding, and value in foods and feeds. In: ET Mertz (Eds.). Quality Protein Maize. American Association of Cereal Chemists, St.Paul, Minnesota, USA, pp. 9-26.

Gutierrez-Rojas A, Scott MP, Leyva OR, Menz M and Betrán J (2008) Phenotypic characterization of quality protein maize endosperm modification and amino acid contents in a segregating recombinant inbred population. Crop Science 48:1714-1722.

Guria P (2006) Physico-chemical properties, nutritional quality and value addition to quality protein maize (*Zea mays* L.). MSc Thesis, Department of Food Science and Nutrition, University of Agricultural Sciences, Dharwad.

Krivanek AF, De Groote H, Gunaratna N, Diallo A and Friesen D (2007) Breeding and disseminating quality protein maize (QPM) for Africa. African Journal of Biotechnology 6: 312-324.

Lambert RJ, Alexander DE and Mejaya IJ (2004) Single kernel selection for increased grain oil in maize synthetics and high oil hybrid development. Plant Breeding Reviews 24: 153-176.

Li L, Wegenast T, Li H, Dhillon BS, Longin CFH, Xu X, Melchinger AE and Chen S (2011) Estimation of quantitative genetic and stability parameters in maize under high and low N levels. Maydica 56: 25-34.

Machida L (2008) 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. PhD Thesis, University of KwaZulu-Natal, Pietermaritzburg, South Africa.

Martinez BF, Figueroa JDC and Larios SA (1996) High lysine extruded products of quality protein maize. Journal of the Science of Food and Agriculture 71: 151-155.

Mbuya K, Nkongolo KK and Kalonji-Mbuyi A (2011) Nutritional analysis of quality protein maize varieties selected for agronomic characteristics in a breeding program. International Journal of Plant Breeding and Genetics 5: 317-327.

McCown RL, Keating BA, Probert ME and Jones RK (1992) Strategies for sustainable crop production in semi-arid Africa. Outlook Agriculture 21: 21-31.

Mittelmann A, de Miranda Filho JB, de Lima GJMM, Hara-Klein C and Tanaka RT (2003) Potential of the ESA23B maize population for protein and oil content improvement. Scientia Agricola 60: 319-327.

Moose SP, Dudley JW and Rocheford TR (2004) Maize selection passes the century mark: A unique resource for 21st century genomics. Trends in Plant Science 9: 358-364.

Mosisa W (2005) Genetic and crop-physiological basis of nitrogen efficiency in tropical maize: Field Studies. PhD Dissertation, University of Hannover, Hannover, Germany.

Mosisa W, Banziger M, Erley GS, Friesen D, Diallo AO and Horst WJ (2007) Nitrogen uptake and utilization in contrasting nitrogen efficient tropical maize hybrids. Crop Science 47: 519-528.

NDA (2012) Maize market value chain profile 2011/2012, Department of Agriculture, Forestry and Fisheries. Available on the internet from: http://www.nda.agric.za/docs/AMCP/MaizeMVCP/2011.pdf [Date Accessed: 01 November 2012].

Ngaboyisonga C, Njoroge K, Kirubi D and Githiri SM (2012) Quality protein maize under low N and drought environments: Endosperm modification, protein and tryptophan concentration in grain. Agricultural Journal 7: 327-338.

Nurit E, Tiessen A, Pixley KV and Palacios-Rojas N (2009) Reliable and inexpensive colorimetric method for determining protein-bound tryptophan in maize kernels. Journal of Agricultural and Food Chemistry 57: 7233-7238.

Odhiambo JJO and Magandini VN (2008) An assessment of the use of mineral and organic fertilizers by smallholder farmers in Vhembe district, Limpopo province, South Africa. African Journal of Agricultural Research 3: 357-362.

Oikeh SO, Kling JG and Okoruwa AE (1998) Nitrogen management effects on maize grain quality in the West Africa moist savanna. Crop Science 38: 1056-1061.

Olakojo SA, Omueti O, Ajomale K and Ogunbodede BA (2007) Development of quality protein maize: Biochemical and agronomic evaluation. Tropical and Subtropical Agroecosystems 7: 97-104.

Perry TW (1988) Corn as a livestock feed. In: GF Sprague and JW Dudley (Eds.). Corn and Corn Improvement. American Society of Agronomy, Madison, WI, pp. 941-963.

Pixley KV and Bjarnason MS (1993) Combining ability for yield and protein quality among modified endosperm *opaque-2* tropical maize inbreds. Crop Science 33: 1229-1234.

Pixley KV and Bjarnason MS (2002) Stability of grain yield, endosperm modification and protein quality of hybrid and open-pollinated quality protein maize (QPM) cultivars. Crop Science 42: 1882-1890.

Pradeepa BP (2007) Genetic studies on quantitative and quality traits of three-way cross hybrids and evaluation of germplasm in maize (*Zea mays* L.). MSc Thesis, Department of Genetics and Plant Breeding. University of Agricultural Sciences, Dharwad.

SAGL (2011) South African Maize Crop Quality Report 2010/2011Season. Available on the internet from: http://www.sagl.co.za/Portals/0/FullMaizereport2.pdf [Date Accessed: 24 June 2013].

Séne M, Thévenot C, Hoffmann D, Bénétrix F, Causse M and Prioul JL (2001) QTLs for grain dry milling properties, composition and vitreousness in maize recombinant inbred lines. Theoretical and Applied Genetics 102: 591-599.

Sentayehu A (2008) Protein, tryptophan and lysine contents in quality protein maize, North India. Ethiopian Journal of Health Science 18: 9-15.

Song TM and Chen SJ (2004) Long term selection for oil concentration in five maize populations. Maydica 49: 9-14.

Song TM, Kong FL and Song GH (1999) Eleven cycles of single kernel phenotypic recurrent selection for oil in Zhongzong no. 2 maize synthetic. Journal of Genetics and Breeding 53: 31-35.

Stoorvogel JJ, Smaling EMA and Janssen BH (1993) Calculating soil nutrient balances in Africa at different scales. Fertiliser Research 35: 227-235.

Tsai CY, Dweikat I, Huber DM and Warren HL (1992) Interrelationship of nitrogen nutrition with maize (*Zea mays* L.) grain yield, nitrogen use efficiency and grain quality. Journal of Science Food and Agriculture 58: 1-8.

Uribelarrea M, Below FE and Moose SP (2004) Grain composition and productivity of maize hybrids derived from the illinois protein strains in response to variable nitrogen supply. Crop Science 44: 1593-1600

Vivek BS, Krivanek AF, Palacios-Rojas N, Twumasi-Afriyie S and Diallo AO (2008) Breeding Quality Protein Maize (QPM): Protocols for Developing QPM Cultivars. CIMMYT Mexico, D.F.

Wassom JJ, Wong JC, Martinez E and King JJ (2008) QTL associated with maize kernel oil, protein, and starch concentrations; kernel mass; and grain yield in Illinois high oil ×B73 backcross-derived lines. Crop Science 48: 243-252.

Wegary D, Labuschagne MT and Vivek BS (2011) Protein quality and endosperm modification of quality protein maize (*Zea mays* L.) under two contrasting soil nitrogen environments. Field Crops Research 121: 408-415.

Wang HW, Wu BY, Song TM and Chen SJ (2009) Effects of long-term selection for kernel oil concentration in KYHO, a high-oil maize population. Crop Science 49: 459-466.

Zai SW and Gao J (2001) Nutritional value and developing prospect of high-oil corn. Cereal and Feed Industry 6: 41-42.

Chapter 6

Combining ability of high quality protein maize (QPM) and non-QPM inbred lines in South Africa

6.1 Abstract

The combining ability of QPM inbred lines with normal maize germplasm for grain quality traits was determined using line x tester design, in order to identify suitable QPM donors for use in conversion programmes of normal maize lines to QPM and in the development of hybrids through QPM x QPM crosses. For the purpose of combining ability, seven normal maize inbred lines were used as females (lines) and crossed with six QPM inbreds (testers) in a line x tester design during the 2008/09 growing season, resulting in 42 crosses which were later evaluated during 2009/10 for use in analysis of GCA and SCA effects. Data for four traits viz., tryptophan, protein content, QI and endosperm modification, were collected and analysed. The GCA of lines was highly significant for protein content, QI and endosperm hardness, for testers it was significant for endosperm hardness and protein and tryptophan content, whilst the interaction of lines with testers was highly significant for all traits measured, showing that dominant gene effects were more important than additive gene action. SCA mean squares were highly significant for all traits except endosperm hardness. Contribution of SCA (lines x testers), was higher than that of GCA for lines and testers for all traits except for endosperm hardness where GCA of testers was higher. Larger GCA of lines than testers showed the importance of maternal effects in most of the traits. The lines J80W and T1162W were amongst the best general combiners for most traits. Among the testers SO607W, CML144 and SO503W were the best combiners for the majority of the traits. These testers can be used as donor parents for the development of new QPM cultivars. Four crosses viz., K64R x SO503W, T1162W x RO452W, K64R x RO452W and K64R x SO713W were promising for endosperm hardness, tryptophan content, QI and protein content, respectively. In general RO452W was the best specific combiner for most traits except for endosperm hardness of which SO503W was the best. Best lines, testers and crosses were identified for the ARC-GCI QPM breeding programme.

6.2 Introduction

Maize protein quality is poor due to low amounts of lysine and tryptophan. The primary reason for the poor protein quality of maize is the high proportion of zeins in the endosperm (Vasal, 2000). On average maize protein contains about 2% lysine, which according to the recommendation by the Food and Agriculture Organization of the United Nations, is less than one-half of the concentration recommended for human nutrition (Mbuya *et al.,* 2011). The structure and content of the maize endosperm influences the expression of traits targeted for genetic improvement (Mazur *et al.,* 1999). The improvement of protein in terms of tryptophan and lysine content is important in dealing with nutritional quality of malnourished communities. The introduction of *opaque-2* maize resulted in improvement of these two essential amino acids (Mertz *et al.,* 1964). However, there were problems associated with the *opaque-2* maize as shown by various researchers (Vasal *et al.,* 1980; Bjarnason and Vasal, 1992; Vasal, 1994) which prompted researchers to look for alternatives in better mutants, seed/endosperm ratio alterations, double mutant combinations, recurrent selection and backcross techniques.

The development of QPM was done through selection for genetic modifiers that restore the desirable hard endosperm texture in *opaque-2* whilst maintaining its high levels of lysine and tryptophan (Bjarnason and Vasal, 1992; Gevers and Lake 1992; Villegas *et al.,* 1992; Vasal, 1994; 2001). The main objective of QPM improvement programmes would be to obtain varieties with high nutritional quality to combat malnutrition, mainly among the rural poor children and women in many developing countries. Selecting inbred lines with hard endosperm, high levels of tryptophan and protein content and QI as well as yield potential will be useful in breeding programmes. The information on kernel modification and tryptophan content of inbred lines will be important for breeders to use in breeding programmes for development of superior QPM.

The line by tester mating design is important in maize breeding for selecting parental material and identifying their genetic potential for hybridisation; and by further providing information about the GCA and SCA of parents and estimates of other genetic parameters. The design is a modified form of the top-cross scheme proposed by Davis in 1927 for inbred evaluation and it was developed by Kempthorne (1957). The mating

design involves crossing lines to each of the testers resulting in hybrids, with the resultant crosses tested with or without parents in a replicated trial using a suitable field design (Kempthorne, 1957; Singh and Chaudhary 1985). Among the different biometrical methods employed to study combining ability, line x tester analysis is the most widely used; and it has been useful in identification of suitable parents and crosses displaying good performance for traits measured (Ahuja and Dhayal, 2007). The GCA and SCA effects are important tools used by breeders in selecting superior parents for developing crosses (Shukla and Pandey, 2008). The *per se* performance of parental lines does not always translate to GCA effects, therefore it is important for breeders to study GCA effects of parental lines of hybrids in order to determine their appropriateness for hybrid development.

According to Allard (1960) combining ability analysis provides an indication of the genetic behaviour of the parental material and assists in selecting parents to be used for hybridisation based on their combining ability, thus it is a highly useful technique for plant breeding. According to Shukla and Pandey (2008), GCA effects should be used for selection of particular parents, while SCA is used in combination with hybrid performance. Additive gene effects are indicated by high GCA values while non-additive effects are shown by high SCA values which are associated with dominance and epistasis (Rojas and Sprague, 1952). Combining ability studies rely on the availability of genetic diversity in genotypes involved in a breeding programme, in order to identify maize inbred lines with good GCA and SCA effects.

Studies on combining ability of South African QPM inbred lines have been previously reported for kernel modification and yield (Hohls *et al.*, 1996) and on CIMMYT lines for yield, endosperm modification, tryptophan and protein content, and QI (Gissa, 2008; Machida, 2008; Machida *et al.*, 2010). Pixley and Bjarnason (1993) studied 28 QPM crosses derived from five populations in a diallel and suggested that hybrids could be best obtained by crosses between the lines with high GCA because they found that the hybrids had a tryptophan and protein content improvement of 48% and 60%, respectively. Hohls *et al.* (1996) evaluated 12 inbred lines of *opaque-2* for endosperm modification and reported a positive GCA for grain hardness, with grain vitreousness positively correlated with the accumulation of key modifier genes of the grain. The researchers concluded that

inbred lines can be used in a programme to improve grain quality protein since they showed sufficient genetic potential. There is no available information on combining ability for tryptophan and protein content in South African QPM inbred lines. This study intends to fill the gap by examining combining ability of QPM and normal maize inbred lines for grain quality traits, in order to identify suitable QPM donor parents for use in the QPM breeding programme. In the light of the issues discussed above the objectives of the present study were to determine combining ability of South African QPM inbred lines and the identification of good donors for conversion of normal maize inbred lines to QPM.

6.3 Materials and methods

6.3.1 Germplasm, trial environment and management

QPM inbred lines were obtained from CIMMYT-Harare and ARC-GCI, with all normal maize inbred lines obtained from ARC-GCI. Seven normal maize inbred lines were used as females and crossed with six QPM inbred lines used as testers in a line x tester design, during the 2008/09 summer season. The resultant 42 F_1 hybrids were planted in a (0, 1) alpha lattice design with three replications, at ARC-GCI Potchefstroom during the 2009/10 summer season. All cultural practices were applied as described in Chapter 3. The F_1 plants were self-pollinated and then F_2 seeds (F_2 progeny) were harvested. For each plot, a sample of 1000 QPM seeds were randomly selected using a light table. The QPM seed samples (F_2 QPM progeny) were then used for determining endosperm hardness, tryptophan and protein content and QI as described in Chapters 4 and 5. However for endosperm evaluation class 1 data were not considered to avoid confusion with segregating normal endosperm kernels. QI was determined by taking the ratio of tryptophan concentration to protein content, and expressed as a percentage.

6.3.2 Data analyses

Agrobase Gen II software (Agronomix Software Inc., 2005) was used for computing ANOVA for all the studied traits. Agrobase Gen II software was further used for line × tester analysis, following the procedure presented by Kempthorne (1957), whereby

females were designated as lines and males as testers. The programme was also used to compute ANOVA for entries, GCA for lines and testers, SCA for line x tester of selected traits for individual sites, LSDs for entries, line and tester GCA and SCA and the proportional contribution of GCA and SCA to entry mean squares. Lines, testers and crosses were considered fixed effects whilst replications were considered random effects. Assessments of the inbred lines were made for the relative importance of GCA and SCA effects on tryptophan and protein content, QI and endosperm hardness.

Table 6.1 List of six QPM and seven non-QPM inbred lines used in the study

Entries	Endosperm types	Origin
SO607W	QPM	ARC-GCI
SO503W	QPM	ARC-GCI
SO713W	QPM	ARC-GCI
RO452W	QPM	ARC-GCI
CML144	QPM	CIMMYT
CZL01005	QPM	CIMMYT
R2565Y	Non-QPM	ARC-GCI
T1162W	Non-QPM	ARC-GCI
K64R	Non-QPM	ARC-GCI
J80W	Non-QPM	ARC-GCI
L116W	Non-QPM	ARC-GCI
P2579W	Non-QPM	ARC-GCI
M162W	Non-QPM	ARC-GCI

6.4 Results

6.4.1 Analysis of variance of lines, testers and line x tester for grain quality traits of F_2 QPM progeny

ANOVA for the line x tester analyses for four grain quality traits are presented in Table 6.2. The F_2 QPM progeny mean squares were highly significant (P≤0.01) for endosperm hardness, QI, protein and tryptophan content. The GCA mean squares for lines were highly significant (P≤0.01) for endosperm hardness, protein content and QI and were not significant (P>0.05) for tryptophan content. The GCA attributable to tester mean squares was highly significant (P≤0.01) for endosperm hardness, protein and tryptophan content but not for QI. The SCA mean squares were highly significant (P≤0.01) for QI, protein and tryptophan content and not significant for endosperm hardness. GCA mean squares of lines were higher than those of the testers for protein content and QI, while it was reversed for endosperm hardness. The GCA mean squares for lines and testers were larger than SCA mean squares for protein content and endosperm hardness.

Table 6.2 Mean squares of four grain quality traits in a line x tester analysis of F_2 QPM progeny of seven non-QPM lines and six QPM testers

Source	DF	Protein	Tryp	QI	EH
Block	2	0.216	0.000**	0.026	0.483
F_2 progeny	41	1.291**	0.000**	0.040**	0.385**
Lines (GCA)	6	2.690**	0.000	0.033**	0.563**
Testers (GCA)	5	1.872**	0.000**	0.014	1.572**
L x T (SCA)	30	0.915**	0.000**	0.046**	0.152
Residual	82	0.317	0.000	0.010	0.171
CV (%)		5.16	6.67	8.31	12.98

**P≤0.01; L x T=Lines x Testers; CV=Coefficient of variation; Tryp=Tryptophan; EH=Endosperm hardness; QI=Quality Index; GCA=General combining ability, SCA=Specific combining ability

6.4.2 Performance of F_2 progeny

Table 6.3 shows the mean performance and ranking of 42 F_2 QPM progeny of seven non-QPM lines crossed with six QPM testers. The mean values for endosperm hardness, protein and tryptophan content, and QI, were 3.19 (2-5 rating), 10.9, 0.13, and 1.20, respectively**.** The F_2 progeny means ranged from 0.10 to 0.14, 9.3 to 10.93, 0.91 to 1.45, and 2.65 to 4.06 for tryptophan and protein content, QI and endosperm hardness, respectively. P2579W/CML144 had the best F_2 progeny, followed by K64R/SO503W for endosperm hardness. For tryptophan content the best progenies had a tryptophan value of 0.14, with 21 of them achieving that. K64R/RO452W had the best F_2 progeny for QI, followed by the progeny of T1162W/CZL01005. For protein content the best progeny was that of M162W/SO607W, followed by that of M162W/CML144, J80W/SO713W, P2579W/S0503W and K64R/SO607W.

6.4.3 General combining ability effects of lines and testers

The GCA of lines and testers for QI, endosperm hardness, tryptophan and protein content are presented in Table 6.4. The lines with the best GCA effects for protein were M162W (0.46) and J80W (0.41) with T1162W having the most negative GCA effect for this trait (-0.69). K64R had the highest positive GCA effect for endosperm hardness (0.22), while T1162W had the highest negative GCA effect (-0.20) followed by R2565Y (-0.19). For endosperm hardness lines with negative GCA effects are considered the best; in this case these were T1162W (-0.204) and R2565Y (-0.195). The line with the best GCA effect value for QI was T1162W (0.073), while the line with the worst GCA effect was M162W (-0.056). The tester with the best GCA effect for protein was SO503W (0.33). SO607W, which was the second best tester for protein content (0.31), had the highest and best GCA tester effect for tryptophan (0.0073) and QI (0.034). RO452W was the worst tester with a negative GCA value for protein (-0.47) and tryptophan (-0.007) content. The tester with the best GCA value for endosperm hardness (-0.34) was CML144 followed by SO503W (-0.23), with CZL01005 having the poorest negative tester effect of 0.43.

Table 6.3 Means and rankings for four grain quality traits of 42 F_2 QPM progeny of seven line and six tester crosses

F_2 progeny	Endosperm hardness scores		Tryptophan		Quality Index		Protein	
	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank
J80W/CML144	2.95	12	0.140	5	1.2	22	11.73	7
J80W/CZL01005	3.28	28	0.130	24	1.19	24	10.95	21
J80W/RO452W	3.09	20	0.123	31	1.1	35	11.30	15
J80W/S0503W	3.05	17	0.140	6	1.3	9	10.80	25
J80W/SO607W	3.05	16	0.143	1	1.26	16	11.37	12
J80W/SO713W	3.12	22	0.120	35	1.02	40	11.83	3
K64R/CML144	2.86	7	0.127	27	1.18	26	10.73	26
K64R/CZL01005	4.06	42	0.123	32	1.12	33	11.03	19
K64R/RO452W	3.51	35	0.137	14	1.45	1	9.47	41
K64R/S0503W	2.66	2	0.127	28	1.14	31	11.10	17
K64R/SO607W	3.94	41	0.140	7	1.2	21	11.80	5
K64R/SO713W	3.45	34	0.137	15	1.18	27	11.57	9
L116W/CML144	3.16	23	0.120	36	1.16	28	10.37	34
L116W/CZL01005	3.70	37	0.137	16	1.21	19	11.33	14
L116W/RO452W	3.45	33	0.123	33	1.2	23	10.33	35
L116W/S0503W	3.30	29	0.127	29	1.12	34	11.30	16
L116W/SO607W	3.22	26	0.137	17	1.35	6	10.10	38
L116W/SO713W	3.31	30	0.140	8	1.29	10	10.87	23
M162W/CML144	2.84	6	0.143	2	1.21	18	11.83	2
M162W/CZL01005	3.77	38	0.133	22	1.14	29	11.73	6
M162W/RO452W	3.21	25	0.097	42	0.91	42	10.60	30
M162W/S0503W	2.99	15	0.143	3	1.26	15	11.35	13
M162W/SO607W	3.34	32	0.130	25	1.09	36	11.97	1
M162W/SO713W	3.84	40	0.137	18	1.27	13	10.80	24
P2579W/CZL01005	3.80	39	0.130	26	1.13	32	11.47	10
P2579W/RO452W	3.06	18	0.127	30	1.19	25	10.63	29
P2579W/S0503W	2.96	14	0.123	34	1.04	39	11.83	4
P2579W/O607W	3.23	27	0.140	9	1.27	14	11.05	18
P2579W/CML144	2.65	1	0.140	10	1.31	8	10.73	27
P2579W/SO713W	3.18	24	0.137	19	1.36	3	10.07	39
R2565Y/CZL01005	3.61	36	0.110	40	1.05	37	10.50	32
R2565Y/RO452W	2.87	9	0.120	37	1.14	30	10.55	31
R2565Y/S0503W	2.86	8	0.120	38	1.05	38	11.40	11
R2565Y/SO607W	2.78	5	0.140	11	1.2	20	11.67	8
R2565Y/SO713W	3.07	19	0.140	12	1.35	5	10.40	33
R2565Y/CML144	2.77	4	0.133	23	1.23	17	10.87	22
T1162W/CML144	2.70	3	0.120	39	1.29	11	9.37	42
T1162W/CZL01005	3.09	21	0.137	20	1.39	2	9.80	40
T1162W/RO452W	2.95	13	0.140	13	1.36	4	10.30	37
T1162W/S0503W	2.90	10	0.143	4	1.32	7	10.97	20
T1162W/SO607W	2.94	11	0.137	21	1.28	12	10.67	28
T1162W/SO713W	3.32	31	0.103	41	1.01	41	10.30	36
Mean	**3.19**		**0.130**		**1.2**		**10.93**	
Range	**2.65-4.06**		**0.10-0.14**		**0.91-1.45**		**9.3-11.97**	
LSD $_{(0.05)}$	**0.805**		**0.017**		**0.19**		**10.91**	

LSD= Least significance difference; Red colour=top ten rankings

Table 6.4 GCA effects of lines and testers for protein and tryptophan content, quality index and endosperm hardness

Inbred lines	Protein	Tryp	QI	EH
		Lines/females		
M162W	**0.4560**	-0.0002	**-0.0562**	0.1418
R2565Y	-0.0274	**-0.0036**	-0.0337	**-0.1948**
K64R	0.0254	0.0009	0.0099	**0.2246**
J80W	0.4060	**0.0020**	-0.0270	-0.0971
L116W	-0.2079	-0.0002	0.0203	0.1646
P2579W	0.0393	**0.0020**	0.0141	-0.0354
T1162W	**-0.6913**	-0.0008	**0.0726**	**-0.2037**
		Testers/males		
CML144	-0.1198	0.0011	0.0218	**-0.3427**
SO713W	-0.0913	-0.0003	0.0073	0.1397
CZL01005	0.0492	-0.0022	**-0.0278**	**0.4273**
SO503W	**0.3254**	0.0011	-0.0265	-0.2303
SO607W	0.3063	**0.0073**	**0.0341**	0.0311
RO452W	**-0.4698**	**-0.0070**	-0.0090	0.0251

Tryp=Tryptophan; EH=Endosperm hardness; QI=Quality Index; Black bolded colour=best value; Red colour=poorest value

6.4.4 Percentage contribution of lines, testers, and their interactions to the expression of four grain quality traits

SCA (lines x testers) contributed a larger percentage of variation to majority of the measured traits compared to lines and testers (Table 6.5). SCA contributed about 52%, 82%, 84% and 29%, respectively, to variation for protein content, tryptophan content, QI and endosperm hardness; while GCA of testers (QPM lines) contributed about 50% to endosperm hardness, 16% to tryptophan content, and 18% to protein, with the GCA of lines (normal endosperm) contributing about 31%, 2.7%, 12% and 21% to protein content, tryptophan content, QI and endosperm hardness, respectively. Overall the GCA

of lines and testers contributed less than 50% to all the traits except endosperm hardness (Table 6.5).

Table 6.5 Percentage contribution of GCA of lines and testers, and their SCA to the expression of four grain quality traits

Genetic components	Protein	Tryp	QI	EH
Lines	30.49	2.71	11.96	21.41
Testers	17.68	15.64	4.11	49.77
Lines x testers	51.83	81.66	83.93	28.82

Tryp=Tryptophan; EH=Endosperm hardness; QI=Quality Index

6.4.5 Specific combining ability effects

The SCA effects of the lines and testers for endosperm hardness, tryptophan content, QI and protein content are presented in Tables 6.6, 6.7, 6.8 and 6.9, respectively. For endosperm hardness the best cross was K64R/S0503W with a SCA effect of -0.52. The poorest SCA (0.50) was between K64R and SO607W. The best cross was T1162W/RO452W for tryptophan content (0.017), whilst the poorest crosses were M162W/RO452W and T1162W/SO713W with SCA values of -0.027 and -0.026, respectively. A cross between K64R and RO452W had the highest SCA effect (0.25), for QI and the lowest SCA effect (-0.28) was obtained for T1162W/SO713W. For protein content the cross with the best SCA (0.71) was K64R/ SO713W, and the worst (-1.013) was K64R/RO452W.

Table 6.6 Specific combining ability effects for endosperm hardness

	Line						
Tester	M162W	R2565Y	K64R	J80W	L116W	P2579W	T1162W
CML144	-0.149	0.118	-0.215	0.203	0.145	-0.162	0.060
SO713W	0.372	-0.061	-0.104	-0.109	-0.181	-0.144	0.198
CZL01005	0.014	0.184	0.222	-0.237	-0.085	0.222	-0.320
SO503W	-0.115	0.095	**-0.524**	0.191	0.173	0.033	0.148
SO607W	-0.026	-0.243	**0.498**	-0.074	-0.169	0.091	-0.077
RO452W	-0.097	-0.093	0.124	0.026	0.117	-0.069	-0.008

Black bolded colour=best value; Red colour=poorest value

Table 6.7 Specific combining ability effects for tryptophan content

	Line						
Tester	M162W	R2565Y	K64R	J80W	L116W	P2579W	T1162W
CML144	0.012	0.005	-0.006	0.006	-0.012	0.006	-0.011
SO713W	0.006	0.013	0.005	-0.012	0.010	0.004	**-0.026**
CZL01005	0.005	-0.015	-0.006	-0.001	0.008	-0.001	0.009
SO503W	0.012	-0.008	-0.006	0.006	-0.005	-0.011	0.012
SO607W	-0.008	0.005	0.001	0.003	-0.001	0.000	-0.001
RO452W	**-0.027**	0.000	0.012	-0.002	0.000	0.001	**0.017**

Black bolded colour=best value; Red colour=poorest value

Table 6.8 Specific combining ability effects for quality index

	Line						
Tester	M162W	R2565Y	K64R	J80W	L116W	P2579W	T1162W
CML144	0.044	0.037	-0.056	-0.002	-0.083	0.069	-0.008
SO713W	0.113	0.174	-0.037	-0.167	0.059	0.134	**-0.276**
CZL01005	0.017	-0.092	-0.066	0.039	0.011	-0.056	0.147
SO503W	0.144	-0.093	-0.045	0.148	-0.074	-0.148	0.067
SO607W	-0.092	-0.002	-0.044	0.051	0.096	0.018	-0.027
RO452W	-0.225	-0.024	**0.248**	-0.070	-0.009	-0.017	0.097

Black bolded colour=best value; Red colour=poorest value

Table 6.9 Specific combining ability effects for protein content

	Line						
Tester	M162W	R2565Y	K64R	J80W	L116W	P2579W	T1162W
CML144	0.573	0.089	-0.097	0.523	-0.230	-0.111	-0.747
SO713W	-0.489	-0.406	**0.708**	0.594	0.241	-0.806	0.158
CZL01005	0.304	-0.446	0.034	-0.430	0.567	0.454	-0.483
SO503W	-0.356	0.177	-0.175	-0.856	0.258	0.544	0.408
SO607W	0.280	0.463	0.544	-0.270	-0.923	-0.220	0.127
RO452W	-0.311	0.123	**-1.013**	**0.439**	0.087	0.139	**0.537**

Black bolded colour=best value; Red colour=poorest value

6.5 Discussion

According to Bernado (1991) and Utz *et al.* (1994) the basis for index selection for characteristics should be based on the genetic variation, heritability, and economic value of each characteristic. In the present study the possibility of selection for improved protein quality and hard endosperm phenotype was indicated by highly significant variation among the F_2 QPM progeny for all four traits measured. Several researchers have reported similar results for endosperm modification, tryptophan and protein content

(Gissa, 2008; Machida *et al.,* 2010; Mutimaamba *et al.,* 2010) and QI (Gissa, 2008; Machida *et al.,* 2010). Majority of the traits in QPM are based on endosperm hardness and tryptophan content. The three best crosses for endosperm hardness had F_2 QPM progeny with mean values of 2.65 (P2579W/CML144), 2.66 (K64R/SO503W) and 2.7 (T1162W/CML144). For tryptophan content half of the F_2 QPM progeny had a value of 0.14. M162W/CML144 had the best F_2 QPM progeny for both tryptophan and protein content, whilst for QI K64R/RO452W had the best. Therefore the top 10 crosses selected based on endosperm hardness of their F_2 QPM progeny, were P2579W/CML144, K64R/SO503W, T1162W/CML144, R2565Y/CML144, R2565Y/SO607W, M162W/CML144, K64R/CML144, R2565Y/SO503W, R2565Y/RO452W and T1162W/SO503W. The best crosses in terms of endosperm hardness, tryptophan content and QI of their F_2 QPM progeny were P2579W/CML144 and T1162W/SO503W.

Plant breeders have extensively used components of variance and heritability estimates in selection of promising genotypes and prediction of desirable traits (Morakinyo, 1996). Highly significant GCA mean squares for lines and testers and SCA mean squares for line x tester combination were obtained for the majority of the traits in the present study. These results indicated the importance of both additive and non-additive gene effects for most of the traits. Gissa (2008) conducted combining ability studies on QPM x QPM under optimum and stressed environments and reported that GCA mean squares for lines and testers were highly significant for endosperm hardness, tryptophan and protein content and QI; while SCA effects were only significant for endosperm hardness and protein concentration under optimum conditions. SCA was not significant for these traits under stressed environments. He further highlighted the importance of additive gene action for most traits**.** If the GCA mean squares are not significant, as is the case for tryptophan content for lines and QI for testers in the current study, Narro *et al*. (2003) indicated that it is advisable to select lines based only on SCA. However if SCA is not significant as in the case of endosperm hardness, performance of single-cross hybrids can be predicted adequately on the basis of GCA (Baker, 1978). Kooner *et al.* (2005) reported highly significant mean squares due to lines for tryptophan and protein content, testers for protein content and line x tester for both traits. Their results were different from the present study in the sense that GCA of lines was not significant for tryptophan content while for the tester it was highly significant. Kooner *et al.* (2005) indicated the

predominance of non-additive effects for protein and tryptophan content which is similar to the present study.

GCA effects means additive gene action is associated with alleles at the same locus; while SCA indicates dominant gene action plus epistasis (interaction of genes at different loci) and other gene interactions (Holland, 2001). SCA effects were highly significant and contributed more than 80% to F2 QPM progeny variation for tryptophan content and QI, and more than 50% for protein content, while for endosperm hardness it was not significant and its contribution was less than that of the testers (50%). The results of this study were consistent with the known genetic basis of tryptophan content and endosperm hardness. In QPM, tryptophan content is controlled by the recessive opaque-2 gene and its interaction with modifier genes and hence the importance of both dominance and epistasis as indicated by the high SCA. However, additive gene action seems to be more important than dominance and epistasis as indicated by higher GCA effects. These results are not in agreement with those of Mutimaamba *et al.* (2010) who reported that additive gene action was predominant in the control of protein and tryptophan content in a combining ability analysis study of QPM and non-QPM inbred lines. The results for endosperm hardness were however in agreement, that additive gene effects played a major role in the control of the trait.

Machida *et al.* (2010) reported highly significant GCA and SCA effects. According to their report GCA effect was the largest contributor to hybrid variation with more than 70% contribution of the hybrid sum of squares for tryptophan and protein content, endosperm hardness and QI, thus confirming that additive gene action was more important than non-additive effects for all the traits. Their findings were similar to findings of the current study on the significance of GCA effects for the majority of the traits and differed for SCA effects since SCA effects were highly significant for protein, tryptophan and QI and non-significant for endosperm hardness. Furthermore it differed in terms of gene action and components of F_2 QPM progeny variation, because they emphasised that additive gene effects were more important than dominant gene effects and that GCA effects were the major contributor to hybrid sum of squares; while in the present study it is the opposite since non-additive gene effects were more important and SCA was the biggest contributor to F_2 progeny variation for all traits except for

endosperm hardness, where GCA of the testers was the largest contributor with 50%. Hohls *et al.* (1996) studied gene action of QPM inbreds for endosperm hardness and indicated highly significant GCA and SCA effects. They further indicated that GCA effects were more important and additive gene action made a major contribution to sum of squares of the crosses and that selection should be based on GCA.

Good GCA for a normal endosperm line means that such a line can easily be converted by any QPM donor line whereas a normal endosperm line with poor GCA cannot be easily converted. The normal lines with the best GCA values were M162W for protein content and T1162W for QI and endosperm hardness. Although GCA effects of lines for tryptophan were not significant, J80W and P2579W were the best general combiners. The lines with the most desirable and significant GCA effects for endosperm hardness were T1162W and R2565Y. J80W and T1162W were identified as the best general combiners for QI, whilst for protein M162W and J80W were good general combiners.

A QPM tester with good GCA means it is a good donor because it converts many lines to desirable levels of tryptophan content, endosperm hardness, QI and protein content. Also the best donor converts well for all important QPM traits namely high tryptophan content, endosperm hardness and good quality protein. The donor with the best GCA for protein content and endosperm hardness was SO503W, whilst the best donor for tryptophan content, QI and protein content was SO607W. CML144 was also the second best donor for QI although the GCA tester effect for QI was not significant. These donors can be used as parents for the development of new QPM cultivars either through the conversion of normal maize to QPM germplasm or QPM x QPM crosses. Gissa (2008) and Machida *et al.* (2010) also reported that CML144 was identified as the best general combiner for endosperm hardness. In this study RO452W had a positive GCA effect for endosperm modification. These results are similar to the findings of Hohls *et al.* (1996), who also reported a positive GCA effect for the inbred line.

The best SCA refers to or identifies the best specific combination(s) for the trait(s) of interest. For example which specific combination of normal line x QPM donor provides the highest tryptophan content, best hardness, best QI, best protein or a combination of essential QPM traits. For endosperm hardness the best cross was K64R/S0503W with a

SCA effect of -0.52, whilst the poorest SCA (0.50) was between line K64R and SO607W. The best cross was T1162W/RO452W for tryptophan content (0.017), whilst the poorest crosses were M162W/RO452W and T1162W/SO713W with SCA values of -0.027 and -0.026, respectively. A cross between K64R and RO452W had the highest SCA effect (0.25), for QI and the lowest SCA effect (-0.28) was obtained for T1162W/SO713W. For protein content the cross with the best SCA (0.71) was K64R/SO713W, while the worst (-1.013) was K64R/RO452W. SO503W was a good specific combiner as indicated by the significant negative SCA effects for endosperm hardness; whilst SO607W was a poor combiner for the trait. The GCA effects for RO452W and CZL01005 were significantly positive, while that of SO503W was significantly negative. For tryptophan content RO452W and SO503W had a positive significant SCA effect. The GCA effect for RO452W was significantly negative and SO503W non-significantly positive. RO452W had the highest SCA effect, with SO713 and SO503W being the best specific combiners for QI. GCA effects for these testers were non-significant for this trait. As for protein content most of the testers had significantly positive SCA effects, with SO713W and CZL01005 considered the best specific combiners. These testers had GCA effects that are non-significantly negative and positive, respectively. According to Tyagi and Lal (2005) parents with poor GCA might produce better hybrids while parents with good GCA effects sometimes produce better hybrids. Some of the findings for the current study agree with the above statement.

Maternal effects were important in the expression of protein content and QI, and this was shown by higher GCA mean squares of lines than that of testers. GCA mean squares of testers were higher than those of lines for endosperm hardness and tryptophan content, suggesting that paternal effects were more important for endosperm hardness and tryptophan content. This means that these traits were dependent on the direction of the cross. The implication of the results in a QPM conversion/breeding programme means that QPM donors need to be used as females in an initial cross for protein content and QI, while for tryptophan content and endosperm hardness QPM donors are to be used as males in order to confer the traits. Machida *et al.* (2010) reported that maternal and paternal effect exists, and that the presence of reciprocal crosses accounted for about 7 to 13% of the hybrid sum of squares. Results of the current study were in agreement with Machida *et al.* (2010), that maternal and paternal effects were present in F_2 progeny.

6.6 Conclusions

Highly significant mean squares of lines, testers and lines x testers were observed for all traits except for tryptophan content (lines), QI (testers) and endosperm hardness (lines x testers), indicating that both additive and non-additive gene action are important for controlling the majority of the traits. Contribution of SCA (lines x testers), was higher than that of GCA for lines and testers for all traits except endosperm hardness. GCA of females was larger than GCA of males, indicating that maternal effects are important for the majority of the traits.

The present study helped in identification of lines, testers and crosses for grain quality traits which can be exploited for further improvements in a breeding programme. In terms of GCA effects the best inbred lines which can easily be converted to QPM for the majority of the traits were J80Wand T1162W. The best donors based on GCA for all or specific QPM traits were CML144, SO503W and SO607W. RO452W was identified as a donor line with good SCA effect for the majority of the traits except endosperm hardness. Identified donors can be utilised in future breeding programmes for development of QPM with enhanced grain quality traits and for the conversion of well-adapted normal maize genotypes into QPM counterparts. The following normal x QPM inbred combinations were good and therefore recommended for further advancement to homozygous/permanent QPM inbred lines for the ARC breeding programme; K64R x SO503W, T1162W x RO452W, K64R x RO452W and K64R x SO713W. SO503W was a good specific combiner for endosperm hardness; whilst SO607W was a poor combiner for the trait. RO452W and SO503W were good specific combiners for tryptophan content. RO452W, SO713W and SO503W being the best specific combiners for QI. As for protein content SO713W and CZL01005 were the best specific combiners. However the performance of those testers also needs to be confirmed, by crossing them to more inbred lines.

6.7 References

Agronomix Software, Inc., (2005) AGROBASE Generation II User's Manual. Version II, Revised Edition. www.agronomix.com. Agronomix Software, Winnipeg, M.B., Canada.

Ahuja SL and Dhayal LS (2007) Combining ability estimates for yield and fibre quality traits in 4x13 line x tester crosses of *Gossypium hirsutum*. Euphytica 153: 87-98.

Allard RW (1960) Hybrid varieties: Principles of Plant Breeding. John Wiley and Sons, Inc. USA.

Baker RJ (1978) Issues in diallel analysis. Crop Science 18: 533-536.

Bernardo R (1991) Retrospective index weights used in multiple trait selection in a maize breeding program. Crop Science: 31: 1174-1179.

Bjarnason M and Vasal SK (1992) Breeding of Quality Protein Maize (QPM). In: J Jarick (Eds.). Plant breeding Reviews. Vol 9. John Wiley and Sons, Inc., New York, USA, pp. 181-216.

Davis RL (1927) Report of the plant breeder. Uerto Rico Agricultural Experimental Station. Annual Report, pp. 14-15.

Gevers HO and Lake JK (1992) Development of modified opaque-2 maize in South Africa. In: ET Mertz (Eds.), Quality Protein Maize. The American Association of Cereal Chemists, St. Paul, Minnesota, pp. 49-78.

Gissa DW (2008) Genotypic variability and combining ability of quality protein maize inbred lines under stress and optimal conditions. PhD Thesis, University of the Free State, Bloemfontein, South Africa.

Hohls T, Shanahan PE, Clarke GP and Gevers O (1996) Genetic control of kernel modification found in South African quality protein maize inbred lines. Euphytica 87: 103-109.

Holland JB (2001) Epistasis and Plant Breeding. In: J Jarick (Eds.). Plant breeding Reviews. Vol 2. John Willey and Sons, Inc., New York, pp. 27-112.

Kempthorne O (1957) An Introduction to Genetic Statistics. John Willey and Sons, Inc., New York, pp. 458-471.

Kooner R, Malhi NS, Pal SS and Singh H (2005) Identification of promising parental inbred lines for development of quality protein maize hybrids. Crop Improvement 32: 44-48.

Machida L (2008) 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. PhD Thesis, University of KwaZulu-Natal, Pietermaritzburg, South Africa.

Machida L, Derera J, Tongoona P and MacRobert J (2010) Combining ability and reciprocal cross effects of elite quality protein maize inbred lines in Subtropical environments. Crop Science 50: 1708-1717.

Mazur B, Krebbers E and Tingey S (1999) Gene discovery and product development for grain quality traits. Science 285: 372.

Mbuya K, Nkongolo KK and Kalonji-Mbuyi A (2011) Nutritional Analysis of Quality Protein Maize Varieties Selected for Agronomic Characteristics in a Breeding Program. International Journal of Plant Breeding and Genetics 5: 317-327.

Mertz ET, Bates LS and Nelson OE (1964) Mutant gene that changes protein composition and increases lysine content of maize endosperm. Science 145: 279-280.

Morakinyo JA (1996) Heritability, correlation and expected responses to selection of some yield components in grain sorghum, Sorghum bicolor *L.Moench.* Nigerian Journal of Genetics 11: 48-54.

Mutimaamba C, Lungu, D and MacRobert J (2010) Combining ability analysis of quality protein maize (QPM) and non-PM inbred lines for kernel quality and some agronomic characteristics. Second RUFORUM Biennial Meeting 20 - 24 September 2010, Entebbe, Uganda, pp. 437-440.

Narro L, Pandey S, Crossa J, De Leon C and Salazar F (2003) Using line x tester interaction for the formation of yellow maize synthetics tolerant to acid soils. Crop Science 43: 1718-1728.

Pixley KV and Bjarnason MS (1993) Combining ability for yield and protein quality among modified endosperm opaque-2 tropical maize inbreds. Crop Science 33: 1229-1234.

Rojas BA and Sprague GF (1952) A comparison of variance components in corn yield trials: III. General and specific combining ability and their interaction with locations and years. Agronomy Journal 44: 462-466.

Shukla SK and Pandey MP (2008) Combining ability and heterosis over environments for yield and yield components in two-line hybrids involving thermo-sensitive genic male sterile lines in rice (*Oryza sativa* L.) Plant Breeding 127: 28-32.

Singh RK and Chaudhary BD (1985) Biometrical methods in quantitative genetics. Kalyani Publishers, New Delhi, pp. 102-127.

Tyagi AP and Lal P (2005) Line x tester analysis in sugar cane (*Saccharum officinarum*). South Pacific Journal of Natural Science 23: 30-36.

Utz H F, Melchinger A E, Seitz G, Mistele M and Zeddies J (1994) Economic aspects of breeding for yield and quality traits in forage maize. 11. Derivation and evaluation of selection indices. Plant Breeding 112: 110-119.

Vasal SK (1994) High quality protein corn. In: AR Hallauer (Eds.), Specialty Corns. CRC Press, Boca Raton, Fl, pp. 79-121.

Vasal S K (2000) Quality Protein Maize Story. Food and Nutritional Bulletin 21: 445-450.

Vasal SK 2001. High quality protein corn. In: AR Hallauer (Eds.). Speciality Corns. 2nd ed. CRC Press, Washington, D.C., USA, pp. 85-129.

Vasal SK, Villegas E, Bjarnason M, Gelaw B and Goertz P (1980) Genetic modifiers and breeding strategies in developing hard endosperm opaque-2 materials. In: WG Pollmer and RH Phipps, (Eds.). Improvement of Quality Traits of Maize for Grain and Silage Use. Nighoff, The Hague, pp. 37.

Villegas E, Vasal SK and Bjarnason M (1992) Quality protein maize – What is it and how was it developed. In: ET Mertz (Eds.). Quality Protein Maize. American Association of Cereal Chemist. St. Paul, Minnesota, USA, pp. 27-48.

Chapter 7

General conclusions and recommendations

In SA maize is the staple food to the majority of rural communities and it is grown by both commercial and small-scale farmers. The nutritional quality of maize is, however, extremely poor in two essential amino acids, lysine and tryptophan. Malnutrition mainly affects rural communities and is considered a national priority in SA. QPM can be useful since it has been used in other African countries to address malnutrition problems, because its protein has twice the amount of lysine and tryptophan as compared to normal maize. The levels of these limiting amino acids in normal maize can be genetically improved. Index selection for each trait should be based on genetic variation, heritability, and economic value. The objectives of this study were to (1) investigate the effect of pollen parents on tryptophan content in QPM inbred lines; (2) analyse GEI and do stability analysis of QPM inbred lines for kernel hardness, protein, tryptophan, oil, and starch content under optimum and low N environments; (3) compare the performance of QPM genotypes to normal maize genotypes for grain quality traits and investigate the relationship between grain traits under low and optimum N environments; and (4) estimate general combining ability and specific combining ability of South African QPM inbred lines and the identification of good donors for grain quality traits.

It was important to study pollination effects on tryptophan content, in order to determine whether it is necessary to self-pollinate inbred lines when planting them for evaluation in field trials. Tryptophan concentration of the self-pollinated inbred lines did not differ significantly from the cross-pollinated material. This indicated that cross-pollination effect does not play a major role in the control of tryptophan content in QPM. The findings of this study meant that pollen from Hans Male, which was the male parent, did not have a major effect on the tryptophan content of the 12 inbred lines. These results indicate that it is not necessary to self-pollinate QPM inbred lines when evaluating them in small research plots, since the potential of the inbreds can be accurately determined in open pollinated fields. However, in order to confirm these results additional research should be done with more than one male parent for the same number of females. Future research on inbred lines should, however, be done, including the effect of location and the

pollination methods on tryptophan content, as significant differences have been reported when genotypes were evaluated under multi-location trials.

Significant genotypic differences were observed for all traits measured in different environments. Genotype effects were larger than environment and GEI effects for most of the traits measured. The existence of genetic variability amongst the QPM inbred lines will allow for selection for further improvement in the breeding programme. The large genetic variation encountered under low N environments creates an opportunity to exploit the QPM inbred lines even more effectively in order to develop hybrids that are adaptable or tolerant to these environments. The results of across environment analysis revealed that in some instances both low and optimum N environments discriminated the inbred lines similarly, which gave an indication of the possibility of genetic improvement through selection of genotypes that will perform well in both low N and optimal conditions. As expected, an increase in N fertiliser caused an increase in the values of the majority of the traits, while a decrease in N levels resulted in decreases. The inbred lines performed well for the majority of the traits under both optimum and stress environments, which indicated the tolerance of the germplasm available in the country for low N environments. These materials will be beneficial to farmers since they usually plant maize under low N conditions.

For the all the traits GEI was significant except for endosperm hardness across all environments, indicating the differential response of the QPM inbred lines to contrasting N levels, which also indicated the possibility of developing hybrids or the formation of specific populations for each environment in the maize breeding programme.

The AMMI and GGE analysis were efficient in analysing and interpreting GEI effects which was shown by the large GEI extracted by both analyses. GGE biplots were easy to interpret because of their superior ability to visualise the results and superiority to partition G + GEI as compared to the AMMI biplots. Both methods were similar in the identification of genotype performance for QI, tryptophan and protein content; and similar for determining stability of inbred lines for the majority of the traits. The ideal genotypes were KO54W, Hans Male, KO54W, SO503W and CML144 for QI, protein, tryptophan, oil and starch content respectively. AMMI identified the highest yielding

environments while the GGE biplots were able to identify the ideal environments which are most discriminating and representative of all the environments. Both high yielding and ideal environments from the two methods were optimum N environments. GGE biplots for "which won where" identified BO163W, KO54W and SO713W as vertex genotypes for tryptophan content, while Hans Male and RO559W were superior in all environments for protein content. KO54W and SO713W won in seven environments for QI. Hans Male was superior at Cedara and Towoomba while SO503W was superior at TWL, TSH, PH and TSL for oil content. CML144 won in seven environments for starch content. As for further using GGE biplots for identification of mega-environments future studies need to be done which include year interactions in order to identify and validate mega-environment results.

It is encouraging to see that QPM varieties have been improved to have grain quality similar to non-QPM varieties which is extremely important for the maize industry. The maize industry follows strict standards for acceptable grain quality traits; therefore it is good to see that QPM genotypes evaluated in this study meet the standards set for non-QPM genotypes. This brings scientists a step closer to bringing better nutrition to millions of people who rely on maize. In SA farmers are interested in producing QPM varieties; however what is missing is linking QPM programmes to the existing maize industry, because currently farmers that produce QPM are faced with the problem that their produce is mixed with non-QPM at the millers and secondly there is no price incentive involved in producing this nutritious maize.

Genetic variation observed for tryptophan content in the genotypes can be useful in the QPM breeding programmes, with newly developed synthetics to be tested across more environments to determine their stability. The reason for non-significant protein, starch content and endosperm hardness differences between genotypes might be due to the fact that the commercial QPM hybrids available have been improved for those traits, especially protein content and endosperm hardness, so that they perform better or similar to non-QPM varieties. This shows the amount of dedication and effort given to the development of QPM varieties, and this is confirmed by the newly developed QPM synthetics from the ARC-GCI which are also performing similar for all grain quality traits except for tryptophan content and quality index value, for which they perform better than

non-QPM. Overall Obatampa-SR was the best performer under low N and optimum N environments for endosperm hardness (2.24; 2.28), QI (0.73; 1.14), protein (9.98; 10.07), tryptophan (0.07; 0.12), oil (5.10; 5.27) and starch content (70.15). The best five varieties for tryptophan and QI under low and optimum N environments were Syn15QW, Syn9QW, QS7705, QS7707 and QS7715.

Correlated traits make it easier for simultaneous selection of grain quality traits. Interdependent relationship was observed for most grain quality traits in this study. Endosperm hardness was significantly positively correlated with QI, whilst protein was significantly correlated with the majority of the traits. Most of the correlations were significant. Tryptophan content and QI, and starch and oil content were positively and negatively highly correlated at almost 100% and 70% respectively at low, optimum and across N environments. However to come up with conclusive recommendations, further studies with more locations and a large number of QPM genotypes are required under low and optimum N environments to determine the stability of genotypes for grain quality traits, thus seeking a deeper understanding of the relationships and GEI, in the view of increasing the efficiency of breeding programmes to N stress conditions.

In a QPM breeding programme the existence of genetic variability for grain quality traits is of major importance, since it will allow for selection of those traits. F_2 progeny differed significantly for all traits, showing the existence of large variability amongst QPMs. The significance of GCA and SCA mean squares suggests the importance of both additive and non-additive variance for the majority of the traits. Contribution of SCA (lines x testers), was higher than that of GCA for lines and testers for most traits, with contribution of between 29-83% to the sum of squares; except for endosperm hardness. For endosperm hardness GCA for tester was the highest contributor to total sum of squares by 50%. Larger GCA of females than males showed the importance of maternal effects in most of the traits when QPM and non-QPM inbred lines were crossed. Donors identified from this study will be further utilised in future breeding programmes for development of QPM with enhanced grain quality traits and for the conversion of well-adapted normal maize genotypes into QPM counterparts. GCA tester effects revealed that the best donors were CML144, SO503W and SO607W, while RO452W was identified as a tester with good SCA effect for the majority of the traits. In terms of GCA effects the best inbred lines

which can easily be converted to QPM for the majority of the traits were J80W and T1162W. The following normal x QPM inbred combinations were good and therefore recommended for further advancement to homozygous/permanent QPM inbred lines for the ARC breeding programme; K64R x SO503W, T1162W x RO452W, K64R x RO452W and K64R x SO713W. As for protein content SO713W and CZL01005 were the best specific combiners. However the performance of those testers also needs to be confirmed, by crossing them to more inbred lines.

In conclusion, the present study has provided relevant information on pollination parent studies, combining ability and maternal effects related to QPM. The study further showed that QPM hybrids developed in South Africa are performing similar to commercially released non-QPM hybrids when evaluated under low and optimum N conditions. Correlated traits can be further exploited for improvement. Genotypes with good grain quality traits have been identified for release and further use in QPM improvement programmes. The results obtained from this study will benefit various stakeholders such as farmers, and breeders from both private and public institutions.

Summary

Genetic improvement of QPM can assist to better the livelihoods of resource poor communities and farmers in SA who rely on maize. QPM germplasm from the ARC-GCI was evaluated to generate information to assist breeders in improving the breeding programme through designing efficient selection procedures that will reduce the time and cost required to develop lines with improved nutritional quality; in the process benefiting small-scale farmers. Pollen parent effect was determined by selfing and cross-pollinating 12 QPM inbred lines for tryptophan content. The two pollination methods did not differ significantly for tryptophan content. Single ANOVAs detected genotypic differences for grain quality traits, while combined ANOVAs across eight environments detected highly significant genotype, environment and GEI effects for all traits except endosperm hardness. The AMMI and GGE biplot analyses explained more than 60% of GEI variation. GGE biplots were the best for visualisation of environments and cultivar performance. AMMI and GGE biplots were similar in identifying high performing genotypes for all traits except for oil content, and the most stable genotypes except for starch and tryptophan content. The GGE biplots identified the ideal genotypes as KO54W, KO54W, Hans Male, SO503W and CML144 for QI, tryptophan, protein, oil and starch content respectively. GGE biplots identified Cedara optimum N as the most representative and discriminating environment for QI, tryptophan and starch content, Tshiombo optimum N for protein and Potchefstroom optimum N for oil content. Twenty QPM and non-QPM genotypes were evaluated under low and optimum N conditions in two locations and highly significant environment effects were observed for all traits, whilst significant genotypic differences and GEI were observed for tryptophan content and QI. Most of the traits improved with increased N level. QPM varieties performed significantly better than non-QPM varieties for tryptophan content and QI in all environments. Tryptophan content was highly significantly correlated with QI ($r = 0.95$, 0.98 and 0.97), while starch content was significantly negatively correlated with oil content ($r = -0.72$, -0.65 and -0.67) in low, optimum and across N environments. Line x tester analyses of QPM inbred lines showed highly significant GCA mean squares of lines and testers for most traits and highly significant SCA mean squares for all traits except endosperm hardness. Contribution of SCA (lines x testers), was higher than that of GCA for lines and testers for most traits, with contribution of between 29-83% to the sum of

squares; except for endosperm hardness. For endosperm hardness GCA for tester was the highest contributor to total sum of squares by 50%. Larger GCA of females than males showed the importance of maternal effects in most of the traits. Among the testers CML144, SO607W and SO503W had the best GCA for the majority of the traits. Four crosses viz., K64R x SO503W, T1162W x RO452W, K64R x RO452W and K64R x SO713W were promising for endosperm hardness, tryptophan content, QI and protein content, respectively. In general RO452W was the best specific combiner. The present study provides novel results on pollination methods, GEI, AMMI and GGE analyses, line x tester analysis, and correlation of grain quality traits of QPM germplasm in SA.

Keywords: QPM, GEI, AMMI, GGE biplot, stability, pollination effect, line x tester analysis, combining ability

Opsomming

Genetiese verbetering van QPM kan bydra tot die verbetering van lewenskwaliteit van hulpbronarm gemeenskappe in SA wat afhanklik is van mielies. QPM kiemplasma van die LNR-SGI is geëvalueer om inligting te genereer wat telers kan help met die verbetering van teelprogramme deur effektiewe seleksieprosedures te ontwerp wat die tyd en koste vir die ontwikkeling van lyne met verbeterde voedingswaardes sal verminder, wat gelyktydig ook bestaansboere sal bevoordeel. Stuifmeelouer effekte is bepaal deur die self- en kruisbestuiwing van 12 QPM ingeteelde lyne vir triptofaan inhoud. Die twee bestuiwingsmetodes het geen betekenisvolle verskille vir triptofaan inhoud getoon nie. Enkel ANOVAs het genotipiese verskille getoon vir graankwaliteitseienskappe, terwyl gekombineerde ANOVAs oor agt omgewings hoogs betekenisvolle verskille getoon het vir genotipe, omgewing en GEI effekte vir alle eienskappe behalwe endosperm hardheid. Die AMMI en GGE “biplot” analises het meer as 60% van die GEI variasie verklaar. GGE “biplots” was die beste vir die visualisering van omgewings and cultivar prestasie. AMMI en GGE “biplots” was soortgelyk vir die identifikasie van hoogs produktiewe genotipes vir alle eienskappe behalwe stysel en triptofaan inhoud. GGE “biplots” het die mees ideale genotipes geïdentifiseer as KO54W, KO54W, Hans Male, SO503W en CML144 vir QI, triptofaan, proteïen, olie en stysel inhoud onderskeidelik. GGE “biplots” het Cedara optimum N as die mees verteenwoordigende en diskriminerende omgewing geïdentifiseer vir QI, triptofaan en stysel inhoud, Tshiombo optimum N vir proteïen, en Potchefstroom optimum N vir olie inhoud. Twintig QPM en nie-QPM genotipes is geëvalueer onder lae en optimum N omstandighede in twee omgewings, en hoogs betekenisvolle omgewingseffekte is gesien vir alle eienskappe, terwyl betekenisvolle genotipiese verskille en GEI gesien is vir triptofaan inhoud en QI. Meeste van die eienskappe het verbeter onder hoër N toestande. QPM genotipes het betekenisvol beter as nie-QPM genotipes gepresteer ten opsigte van triptofaan en QI in alle omgewings. Triptofaaninhoud was betekenisvol gekorreleer met QI (r=0.95, 0.98 en 0.97), en styselinhoud was betekenisvol negatief gekorreleer met olie inhoud (r=-0.72, -0.65 en -0.67) in lae, optimum en oor N omgewings. Lyn x toetser analises van QPM ingeteelde lyne het hoogs betekenisvolle GCA gemiddelde kwadrate van lyne en toetsers vir meeste eienskappe getoon, en hoogs betekenisvolle SCA gemiddelde kwadrate vir alle eienskappe behalwe endosperm hardheid. Bydrae van SCA (lyne x toetsers) was hoër as

die van GCA vir lyne en toetsers vir meeste eienskappe, met bydraes tussen 29-83% van die som van kwadrate, behalwe vir endosperm hardheid. Vir endosperm hardheid was GCA vir die toetser die hoogste bydraer tot totale som van kwadrate van 50%. Groter GCA van wyfies as mannetjies het die belangrikheid van moedereffekte in meeste van die eienskappe getoon. Van die toetsers gebruik het CML144, SO607W en SO503W die beste GCA gehad vir die meeste eienskappe. Vier kruisings nl. K64R x SO503W, T1162W x RO452W, K64R x RO452W en K64R x SO713W het potensiaal getoon vir endosperm hardheid, triptofaan inhoud, QI en proteïen, inhoud, onderskeidelik. Oor die algemeen was RO452W die beste spesifieke kombineerder. Hierdie studie het nuwe data gegenereer ten opsigte van bestuiwings metodes, AMMI en GGE analises, lyn x toetser analise, en korrelasie van graankwaliteitseienskappe van QPM kiemplasma in SA.

Sleutelwoorde: QPM, GEI, AMMI, GGE biplots, stabiliteit, bestuiwingseffekte, lyn x toetser analise, kombineervermoë