INFLUENCE OF ENVIRONMENTAL CONDITIONS ON NUTRITIONAL VALUE OF QUALITY PROTEIN MAIZE

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DECLARATION

"I, Hilda Chilekeni Shawa, declare that the Master's Degree research dissertation that I herewith submit for the Master's Degree qualification Magister Scientiae Agriculturae at the University of the Free State is my independent work, and that I have not previously submitted it for a qualification at another institution of higher education."

Hilda Chilekeni Shawa

DEDICATION

This study is dedicated to my loving husband (Elwin Shawa) and our beautiful daughters, Roselyn and Imellah for your endurance of hard times you went through during my absence. To my only sister, Pelani, thank you for being there for my family and the support you gave us. God bless you all.

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LIST OF ABBREVIATIONS AND SYMBOLS

AACC	American Association of Cereal Chemists
AOAC	Association of Official Analytical Chemists
ANOVA	Analysis of variance
AW	Atomic weight
BCP	Biofortification Challenge Programme
Ca	Calcium
CIAT	International Centre for Tropical Agriculture
CGIAR	Consultative Group on International Agricultural Research
CIMMYT	International Maize and Wheat Improvement Centre
Cu	Copper
CV	Coefficient of variation
Da	Daltons
DAFF	Department of Agriculture, Forestry and Fisheries
DDH_2O	Double distilled water
DF	Degrees of freedom
EDTA	Ethylenediaminetetraacetic acid
Env	Environment
FAO	Food and Agriculture Organisation of the United Nations
Fe	Iron
g	Gram(s)
g 100 g^{-1}	Gram per 100 grams
g kg^{-1}	Gram per kilogram
GDP	Gross domestic product
GEI	Genotype and environment interaction
Gen	Genotype
ha	Hectare(s)
HPLC	High performance liquid chromatography
H_2SO_4	Sulphuric acid
I	Iodine
IDC	Industrial Development Corporation
IFAD	International Fund for Agricultural Development
IFPRI	International Food Policy Research Institute

IP1	Monophosphate
IP2	Diphosphate
IP3	Triphosphate
IP4	Tetra phosphate
IP5	Penta phosphate
IP6	Hexa phosphate
IOA	In on Africa
K	Potassium
Kcal 100 g^{-1}	Kilocalorie per 100 g
kDa	Kilo Daltons
Kg	Kilograms
Kg ha^{-1}	Kilograms per hectare
Kg ha^{-1} K	Kilogram per hectare potassium
Kg ha^{-1} N	Kilogram per hectare nitrogen
Kg ha^{-1} P	Kilogram per hectare phosphorus
Low N	Low nitrogen
lpa	Low phytic acid
LSD	Least significant difference
M	Molar
m	Meters
masl	Metres above sea level
MDGs	Millennium Development Goals
MEF	Ministry of Environment and Forests
µg g^{-1}	Microgram per gram
µl	Microlitres
µm	Micrometres
Mg	Magnesium
mg	Milligrams
mg g^{-1}	Milligram per gram
mg 100 g^{-1}	Milligram per 100 grams
mg kg^{-1}	Milligram per kilogram
mg ml^{-1}	Milligram per millilitre
ml	Millilitre
$mlmin^{-1}$	Millilitre per minute

mM	Micro Mole
min	Minutes
mm	Millimetres
Mn	Manganese
MR	Molar ratio
MRFe	Phytic acid to iron molar ratio
MRZn	Phytic acid to zinc molar ratio
MS	Mean squares
MW	Molecular weight
N	Nitrogen
NEPAD	New Partnership for Africa's Development
NH_4	Ammonium
NHO_3	Nitric acid
NIR	Near Infrared Reflectance
nm	Nanometre
NO_3	Nitrate
NO_x	Nitrogen Oxides
NRC	National Research Council
OD	Optical Density
OECD	Organisation for Economic Co-operation and Development
Opt N	Optimum nitrogen
OPVs	Open pollinated varieties
P	Phosphorus
PC	Principal Component
PCA	Principal Component analysis
pH	Power of hydrogen
Phy	Phytic acid
ppm	Parts per million
PPMC	Pearson's product moment correlation
PVDF	Polyvinylidene difluoride
QPM	Quality protein maize
RP-HPLC	Reversed phase-high performance liquid chromatography
rpm	Revolutions per minute
sec	Seconds

SDGs	Sustainable Development Goals
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
SA	South Africa
SSA	Sub-Saharan Africa
Spectra AA 300	Atomic Absorption Spectrophotometer
TCA	Trichloroacetic acid
TFA	Trifluoacetic acid
ton ha^{-1}	Tonnes per hectare
Try	Tryptophan
UFS	University of the Free State
UN	United Nations
UNICEF	United Nations Children's Fund
UNSCN	United Nation System Standing Committee on Nutrition
USA	United States of America
USDA	United States Department of Agriculture
UV	Ultraviolet light
V8	Collar of 8^{th} leaf visible
WFP	World Food Programme
WHO	World Health Organisation
Zn	Zinc
α	Alpha
β	Beta
^{o}C	Degrees Celsius
δ	Delta
$	Dollar
γ	Gamma
%	Percent
2ME	2-mercaptoethanol
o2	Opaque 2
o2o2	Homozygous recessive
O2O2	Homozygous dominant
v/v	Volume per volume
w/v	Weight per volume

ABSTRACT

Maize is a staple crop to many people and it provides calorie, minerals and proteins to people in developing countries and globally. Quality protein maize (QPM) has improved nutritional quality but environmental conditions may have an effect on grain yield and nutritional content, especially under low nitrogen (N) conditions. The main objective of this study was to determine the influence of different production environments on nutritional quality and grain yield in QPM hybrids. This was done by grain yield assessment and nutritional quality analysis of QPM hybrids from CIMMYT-Zimbabwe, which were produced in different sites under low and optimum N conditions. The results for single analysis of variance (ANOVA) indicated that genotypes were significantly different for grain yield and all nutritional characteristics under low and optimum N. This was true except for oil content, phytic acid to iron molar ratio (MRFe) and phytic acid to zinc molar ratio (MRZn) at Harare (low N) and grain yield at Gwebi, phytic acid content, MRFe and MRZn at Glandel (optimum N). Combined ANOVA across optimum N locations were significantly different for genotypes, locations and genotype by environment interaction for all the traits, except for location effect for protein content. Negative correlations under both low and optimum environments were observed between α and γ zeins, β and γ zeins, and grain yield and Fe content. Principal component analysis biplots identified genotypes TH15938 and TH151082 to have high oil, phytic acid, γ zein and tryptophan contents in all environments. These characteristics were also positively correlated. Generally, low N reduced grain yield and nutritional quality characteristics. However, some specific genotypes were less sensitive to low N as it maintained grain yield (TH15976) and nutritional quality such as tryptophan (TH151082 and TH15895), oil (TH15938), total protein (Local check 1), Fe (TH15889) and Zn (Local check 1 and TH15851) contents. Alpha zein and phytic acid contents were reduced in most genotypes under low N conditions, suggesting increased tryptophan content, improved nutritional quality and micronutrient bioavailability under such conditions.

Keywords: QPM, non-QPM, nutritional quality, micronutrient bioavailability, micronutrient deficiency, GEI, genetic variation

CHAPTER 1

GENERAL INTRODUCTION

Maize (*Zea mays* L.*)* plays an important role in people's diet and is considered as a nutrient carrier for man and animal (Huang *et al*., 2004). It is the third most important cereal crop worldwide in terms of production, following wheat and rice (Karasu, 2012). Maize grain contains carbohydrates, proteins and vitamins such as thiamine, niacin and riboflavin and minerals like phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), manganese (Mn), copper (Cu), iron (Fe) and zinc (Zn) (M'mboyi *et al*., 2010; Kataria, 2014). Maize is also the primary source of protein and energy for low-income people (Sule Enyisi *et al*., 2014), where it provides 50 - 60% of the dietary protein requirement, especially in developing countries (Showemimo, 2004). Besides being a staple, maize is an industrial crop in developed countries and is processed into different products such as ethanol, starch, oil, corn syrup and corn gluten meal (Erickson *et al*., 2005).

The increase in the global population, poverty levels and demand for biofuel are likely to increase demand for maize and maize products (Phalafala, 2013; Ngaboyisonga and Njoroge, 2014). Normal maize is generally limited in the essential amino acids; lysine and tryptophan, which are vital for human and monogastric nutrition (Prasanna *et al*., 2001; Krivanek *et a*l., 2007; Sofi *et al*., 2009; Ngaboyisonga *et al*., 2012). This deficiency is detrimental to the preschool children as well as expectant women's health and exposes them to a high risk of malnutrition (Pixley and Bjarnason, 2002), especially in communities which depend on maize as their main calorie source. National Research Council (NRC, 1988) also reported that maize represents 15% of crop protein that is produced annually in the world and 19% of calories in the world's food crop despite it being of low nutritional value.

Protein quantity and quality are very important for nutrition. Improving the nutritional quality of maize is therefore very important to meet the nutritional requirements of people who are dependent on maize. About 45% of the deaths in children under the age of five years, globally is attributed to malnutrition (Black *et al*., 2014), can be reduced through proper nutrition. According to the World Health Organisation (WHO), malnutrition due to protein deficiency, is a major problem in Africa (WHO, 1999). Unfortunately, in the face of persistent and recurrent hunger and food crisis, research focuses on increasing food production by breeding for high yield, but nutritional value is often overlooked (Kandianis *et al*., 2013).

Zein storage proteins, which are deficient in essential amino acids, occupy the largest portion of normal maize storage proteins negatively affecting the crop's nutritional value (Vasal, 2000; Krivanek *et al*., 2007; Sofi *et al*., 2009). Hence the introduction of quality protein maize (QPM), which has improved nutritional value, with double the amount of lysine and tryptophan compared to normal maize (Mertz *et al.*, 1964). QPM contains a mutant gene, *opaque* 2 (*o2*) that is responsible for reduction of alpha (α) zeins, which contain no lysine and tryptophan. It has an increased amount of non-zein proteins such as albumin, glutelin and globulin that contain other essential amino acids (Krivanek *et al*., 2007; Sofi *et al*., 2009; Ngaboyisonga *et al*., 2012; Bello *et al*., 2014). Vivek *et al*. (2008) reported that protein quantity in QPM and common maize is not very different, but rather the level of lysine and tryptophan is increased in QPM, thus improving the nutritional quality of QPM.

Discovery of the *o*2 mutant gene in maize in the early 1960 has opened a way to improve maize nutritional value (Vietmeyer, 2000). The International Maize and Wheat Research Institute (CIMMYT) capitalised on this discovery and developed maize cultivars, which were high yielding with improved nutrition, called QPM (Masindeni, 2013). The introduction of QPM genotypes targeted nutritional challenges faced by the majority of people who rely on maize as a source of food in their diet, without much diversification. NRC (1988) showed that the introduction of QPM paid off, since malnutrition related diseases such as kwashiorkor and pellagra greatly decreased. QPM diets reduce severe protein deficiency in children (Prasanna *et al*., 2001; Ngaboyisonga *et al*., 2012) and this shows that QPM can be used in combating high incidences of malnutrition.

In past studies, QPM genotypes produced under low nitrogen (N) soil conditions showed a significant decrease in grain yield and nutritional quality (CIMMYT, 2003; Zaidi *et al*., 2008; Ngaboyisonga *et al*. 2012; Anjorin and Ogunniyan, 2014; Gerde *et al*., 2017). According to Blumenthal *et al*. (2008), low N reduces zein protein accumulation in maize endosperm and hence results in low grain yield. Bänziger *et al*. (2000) reported that low N reduces kernel size in addition to promoting ear abortion, especially if N levels significantly drop at flowering stage. QPM grown under low N conditions has been reported to have soft kernels due to reduced endosperm modifiers (Blumenthal *et al*., 2008). This reduces adoption of QPM by the end users who prefer vitreous/hard kernels. Report by OECD/FAO (2016) has shown that smallholder farmers produce 80% of maize in Sub-Saharan Africa (SSA). These farmers produce the crop in poor soils which are characterised by low N. Most small-scale farmers are

resource poor and hence are unable to supplement N through chemical fertilisers, which are generally expensive. Therefore, determining the genetic variability for grain yield, protein quantity and quality in QPM hybrids and how it is influenced by low N conditions, is vital to breeders for selection and/or developing new cultivars with enhanced levels of these traits. Again, it is important to validate different research findings on what happens under low N to zein accumulation in endosperm and grain yield per se.

Vitamin A and micronutrient deficiency such as Fe and Zn deficiencies are major challenges which affect about half of the world's population (Nestel *et al*., 2006). Most people from developing countries acquire Fe and Zn nutrients from the staple foods in their diet (CIAT, IFPRI, 2002). Staple foods include maize, beans, rice and wheat, and have high levels of phytic acid. Phytates in seed and grain cereals inhibits absorption of minerals like Fe, Zn, Mg and Ca. Phytates present in the diets prepared from cereal grains and other crops makes minerals insoluble and unavailable for absorption. Therefore, this makes most people who depend on cereals as their staple food, suffer from micronutrient deficiency as phytates hinder mineral absorption (Lim *et al*., 2013). Hambidge *et al*. (2004) noted genetic variation for the content of phytates in maize, which lead to development of cultivars with low phytic acid and hence increased mineral bioavailability such as Fe and Zn. As such, it is important to determine mineral genetic variability for Fe and Zn as well as anti-nutritional factor such as phytates in QPM hybrids, and to determine the effect that low N soil conditions has on it.

Studies by various researchers such as Nestel *et al*. (2006), Bouis *et al*. (2011), Chakraborti *et al*. (2011), Qin *et al.* (2012) and Kandianis *et al*. (2013) showed that micronutrient deficiency could be resolved and prevented through crop biofortification. Saltzman *et al*. (2014) defines biofortification as an enhancement of nutrient content in staple food crops such as maize, wheat and rice, with the aim of combating micronutrient deficiency. According to Chakraborti *et al*. (2011), Qin *et al*. (2012) and Kandianis *et al*. (2013), genetic variation and high heritability for mineral content are a prerequisite for successful biofortification. However, Bänziger and Long (2000) and Kandianis *et al*. (2013), reported a negative association between micronutrient content and grain yield in maize. Chakraborti *et al*. (2011) on the other hand reported a positive relationship between Fe, Zn and grain yield, making the simultaneous improvement of these traits feasible.

Majority of farmers in developing countries including SSA are small holder farmers that are poor and cannot supplement their crops with chemical fertilisers to increase production and nutritional quality since it is expensive. The impact of low N on nutritional quality on QPM hybrids including zeins, total protein, oil content, tryptophan, Fe, Zn, phytic acid and its bioavailability have not been studied extensively, hence this study. But QPM is nutritionally improved maize, the question that arises is in what ways are the nutritional traits and grain yield in QPM hybrids are affected by different N conditions, including low N.

1.1 Research objectives

The objectives of this study were to determine, under different N conditions:

(a) The effect of different N conditions on grain yield, tryptophan and oil content of the tested QPM hybrids

(b) Genetic variability for Fe, Zn and phytic acid content for determination of mineral bioavailability in QPM hybrids

(c) The impact of environmental conditions on protein quality and quantity of QPM hybrids

(d) The relationship between grain yield and nutritional characteristics under different N conditions.

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CHAPTER TWO

POTENTIAL CONTRIBUTION OF QUALITY PROTEIN MAIZE TO FOOD AND NUTRITIONAL SECURITY

2.1 Introduction

The world population, which is estimated at 7.3 billion, is growing at an exponential rate and is expected to reach 8.5 billion by 2030 (UN DESA, 2015). According to the United Nations Children's Fund (UNICEF, 2016), half of this population lives on less than $2.50 a day, while 1.3 billion people live in extreme poverty, spending less than $1.25 a day. This economic disposition has implications on food and nutritional security, as well as the economic activities of the poor majority (Ashley, 2016). As many of these people produce their own food, which comprises of maize, wheat or rice as their primary staple. Additionally, due to economic challenges, most people use recycled seed, which are grown on highly degraded soil due to continuous mono-cropping and lack of soil improvement programmes. This is the major reason for poor quantity and quality of food produced by these farmers (Morris *et al*., 1999). It is not surprising then that world hunger statistics confirm that one out of nine people have no food, while a further 12.90% are undernourished, the majority of which live in developing countries (Kirk, 2016). The purpose of this review was to assess the dynamics behind high malnutrition levels and the reasons for maize biofortification. The review further assessed the potential importance of QPM in comparison to other similar approaches such as nutrient supplementation and food fortification, which have currently been used to combat malnutrition.

2.2 Maize overview

2.2.1 Taxonomy, origin and distribution

Maize (*Zea mays* L.) is a plant that belongs to the grass family Poaceae and is a native to Mexico. The crop is believed to have originally been discovered in the Mesoamerican highland region 6000 years ago. The crop was introduced to South Asia and Spain, and then later the crop spread to other areas (MEF, 2010). Currently, maize is grown and utilised across the whole world, even though production and utilisation levels are different (Ranum *et al*., 2014). However, optimum maize production is attained in areas where daily temperatures range between 15 - 30^{o}C with precipitation that ranges between 400 - 650 mm per season. Deep, well-drained and fertile soil, which is rich in nutrients such as N, P and K, is paramount for high maize production (Farnham *et al*., 2003).

2.2.2 Maize production and utilisation

2.2.2.1 Maize production

Currently, the main producers of maize are the United States of America (USA), China, Brazil, European Union countries, Argentina, Mexico, Ukraine, India, Canada and Russia (Macauley, 2015; FAO, 2018). Almost 717 million metric tons of maize are produced every year worldwide. The USA, China and Brazil produce approximately 78% of all maize annually (Ranum *et al*., 2014). The African continent's contribution to world production is negligible and is estimated at 6.80%, where South Africa (SA), Nigeria and Egypt are the largest maize producers (Daly *et al*., 2016). World maize production is declining in general (FAO, 2018; USDA, 2018). From 2017 to 2018, maize production was estimated to have dropped by 1% and the reduction is expected to increase to 1.9% in the 2018/2019 season (USDA, 2018).

2.2.2.2 Maize utilisation

Maize as feed crop

A major use of maize in the world is for animal feed, which accounts for 65% of total maize usage (Zhang *et al.*, 2012). It is the world's number one feed grain in addition to maize stalks, which are utilised as hay or silage. Research shows that feed made from maize grain has better nutritional attributes as it has a high conversion ratio of dry substance to meat, milk and eggs compared to other grains (Erickson *et al*., 2005). The crop has a high net energy content amongst cereals, which makes it preferred over other crop plants, and it is easily consumed by animals due to its low fibre content the grains low fibre content, easily consume it. In feed formulation, a large proportion (>50%) of the ingredients is maize grain, hence a relation has been established between increased maize production and increased livestock production (Erickson *et al*., 2005; Zhang *et al*., 2012; Dei, 2017).

Biofuel production

Biofuel extraction is one of the many industrial applications of maize due to its abundance, high content of starch and relative ease with which it is converted to ethyl alcohol (ethanol) (Ranum *et al*., 2014). About 38% of maize in the USA is used for ethanol production (Hay, 2015). The Food and Agriculture Organisation of the United Nations (FAO) have even considered the potential of financing small-scale farmers to promote local biofuel production. However, increased biofuel production entails potential reduction of food availability (Hay, 2015).

Maize as food source

The utilisation of maize differs from country to country and region to region. It was noted by Du Plessis (2003) that in developed countries, maize is mainly produced for animal feed and industrial applications, hence consumed as second-cycle produce in the form of meat, eggs and dairy products. In developing and under-developed countries, maize is mainly produced for direct human consumption.

According to Zhang *et al.* (2012), 15% of the world production of maize is consumed as food and Ranum *et al*. (2014) reported that it is a staple in 22 countries in the world. Sixteen of those countries are in Africa, where an individual, consumes over 50 g of maize on a daily basis. Maize consumption is even higher in countries such as Malawi, Zambia, Lesotho, Zimbabwe, Kenya and SA (Ranum *et al*., 2014). In Africa, maize provides over 50% of the daily-required calories, while in Malawi and Zambia, maize accounts for more than 80% of the calorie intake (Byerlee, 1994). In total, maize is a staple food for over 24 million households in Africa (Ranum *et al*., 2014).

In 2013, the USA only used about 12.74% of the crop yield for food, seed and other industrial applications. The larger portion of maize harvested were for animal feed and ethanol production. In SA, one of the developing countries, white and yellow maize is produced in the ratio of 57:43. White maize is primarily produced for human consumption and yellow maize for animal feed (DAFF, 2012).

2.2.3 Population increase and maize production

In 2016, the population in sub-Saharan Africa (SSA) was more than 950 million, representing 13% of the world's population. This population is expected to reach 2.1 billion inhabitants in the year 2050 (FAO, IFAD, WFP, 2015). As such, the demand for maize is likely to increase, which will eventually result in an increase in the number of undernourished people (OECD/FAO, 2016). As maize lack certain essential amino acids and cannot provide a balance diet as staple food. Apart from food, the demand for maize will also increase for other uses such as feed and biofuel purposes. In SSA and other developing countries, maize production will need to more than double to meet the projected demand in 2050, while in developed countries an increase of one third will be enough to service the increased demand (FAO, 2017).

2.2.4 Maize production and utilisation in South Africa

Maize is the most important grain crop in SA, and is produced in diverse environments. Most of the maize (83%) is produced in the Free State, Mpumalanga and North West provinces (Brand South Africa, 2017). Maize production varies within and between seasons but on average, 8 million tons of maize grain is produced annually on 3.1 million hectares (ha) of land (Farmers Weekly, 2015). According to the South African Maize Crop Quality Report (SAGL, 2018) of the 2016/2017 season, the 10-year average is just more than 11 million tons (Figure 2.1). The 2016/2017 season resulted in an all-time high record crop yield, after a severely drought affected season. This makes SA one of the largest producers and consumers of maize in Africa, and the largest producer and consumer in the sub-Saharan region.

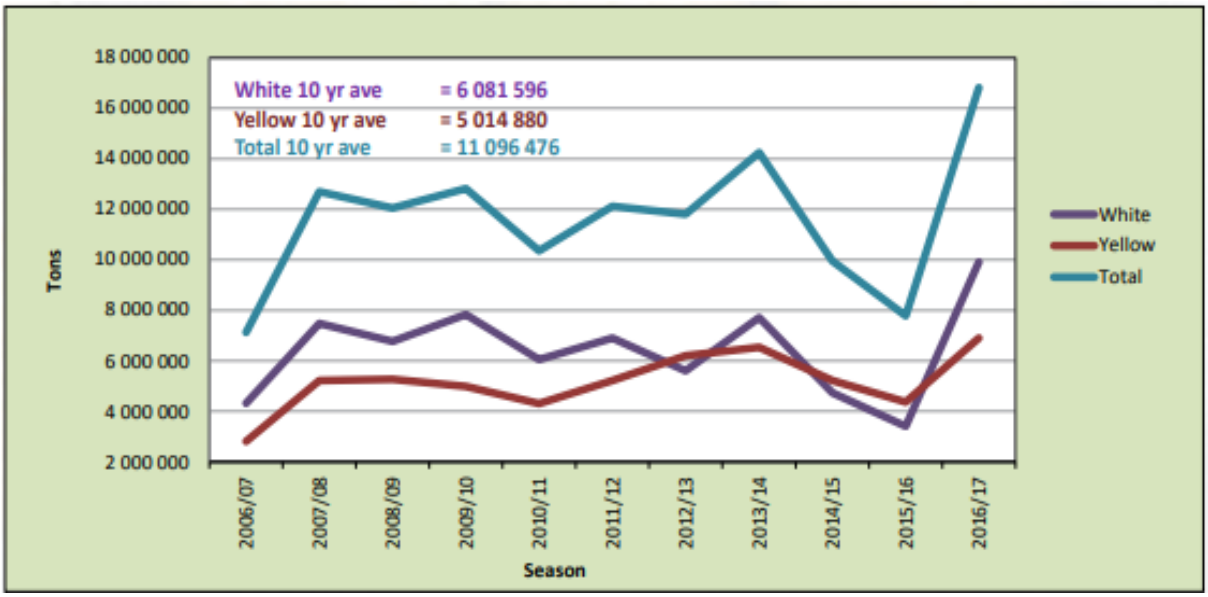

Figure 2.1 Commercial maize production in SA from 2006/2007 to 2016/2017 (SAGL 2018)

There have been variations in maize production in SA. Drought and long dry spells result in grain yield and production reduction. In 2015, for instance, maize production declined by 35.20% compared to the 2014 season due to drought stress. The decrease in maize production did not only affect food availability and prices but reduced maize exports by 58.60% and increased maize imports by 23% (IDC, 2016). However, Van Der Walt and Mokone (2016) reported that maize production was expected to increase by 26.50% in the 2017/2018 season. This was still not enough to fulfil the country's demand, and maize imports would continue to meet the demand.

2.3 Soil status in sub-Saharan Africa

SSA is one of the major producing areas for maize in Africa, with SA topping the list (Dei, 2017). Most of the countries within this region are food insecure, as measured by maize availability, as the majority of the population in the region depend on maize as their staple food. Climate change as exhibited by high temperature, long dry spells and drought, has been reported to affect maize grain yield and production (Abdalla, 2007; IDC, 2016; SAGL, 2018). Apart from climate, soil nutrient condition has an effect on maize yield and productivity (Langa, 2005). According to Sanchez (2002) and Ertiro (2018), low N is one of the abiotic factors that are among the major causes of reduced maize productivity in SSA.

2.3.1 Nutrient status

Crop productivity and subsequent production, is affected by soil conditions such as soil fertility, which is a function of biomass, nutrient content and soil texture (Tully *et al*., 2015). Based on that description, Henao and Baanante (1999) and Tully *et al*. (2015) described most soils in SSA as poor and N deficient. Soils in SSA are highly weathered, which is typically of humid, sub-humid and semi-arid regions soils. The soils are naturally are susceptible to accelerated soil erosion, crust compaction and drought. Their N levels and water holding capacities are low. M'mboyi *et al.* (2010) concluded that poor soils are a common phenomenon in Africa.

Amongst many reasons, the growing human population have a negative impacted on crop production. Firstly, the demand for food has increased. Yet the increase in population reduces the production unit area, especially in SSA where growth in agricultural crop-output is achieved through expanding area of production, compared to Asia and South America where production increases are due to intensification and mechanisation, respectively (NEPAD, 2014). Consequently, more land is cleared, which results in deforestation. The need for more food also results in continuous cropping without fallowing, resulting in continuous mining of soil nutrients such as N to the extent that 4.4 million tons of N is mined per year and only 0.8 million tons are replenished yearly, resulting in a negative nutrient balance (Henao and Baanante, 1999; Bationo *et al*., 2006). This has a great impact on the crop yield and productivity in general.

2.4 Maize kernel chemical composition

2.4.1 Starch

The largest proportion of the maize kernel (72%) consists of starch, which is primarily made up of glucose. Starch is the most common storage form of glucose in plants (Boyer and Shannon, 2003). It is an energy reserve for higher plants, and comprises of branched and unbranched glucose polymers. Thus, starch extraction is one of the major uses of maize, after livestock feed (Erickson *et al.,* 2005; Phalafala, 2013). Mauro *et al.* (2003) and Wilson *et al*. (2004) observed that maize starch is made of 21% amylose, which are unbranched chains of α (1 - 4) linkage glucose and 79% amylopectin, which are highly branched with α (1 - 6) linkage glucose.

2.4.2 Oil content

Normal maize kernels contain around 4% oil while high oil content cultivars have about 6% oil (Singh *et al*., 2014). A high proportion (> 80%) of the oil is found in the germ, 12% in the aleurone layer and 5% in the endosperm (Yang *et al*., 2012; Singh *et al*., 2014). Therefore, there is a high positive correlation between oil content and germ size (Yang *et al*., 2012), and research show that oil content is negatively correlated with grain yield (Yang *et al*., 2012; Singh *et al*., 2014). Oil from maize comprises poly-unsaturated fatty acids, and it is important for animal and human nutrition (Blumenthal *et al*., 2008). According to Singh *et al*. (2014), oil content in maize grain is influenced by both genetic makeup and environmental conditions.

2.4.3 Maize protein quantity and quality

Total protein

Protein is the second largest component in maize kernel after starch. Normal maize contains 6 - 12% protein, a large proportion of which is found in the endosperm and germ (Shukla and Cheryan, 2001). Maize endosperm contains less protein (7 - 10%) than the germ (17 - 18%) (Ai and Jane, 2016). According to Sofi *et al.* (2009), embryo (germ) protein is of superior quality compared to that of the endosperm. Protein content in QPM kernels, on the other hand, ranges between 6 - 14%, depending on the genotype and environment (Prasanna *et al*., 2001). According to Radovul *et al.* (2010) protein content in normal maize and QPM is the same, but the presence of high lysine and tryptophan content in QPM makes them nutritionally superior to normal maize.

Zein proteins

Zeins are storage proteins (Prasanna *et al*., 2001; Sofi *et al*., 2009) and comprise α, beta (β), gamma (γ) and delta (δ) zeins (Esen, 1986; Wallace *et al*., 1990; Sofi *et al*., 2009). In general, β and γ zeins are rich in methionine (Shewry and Halford, 2002) and are also called zein 2 while α and δ zeins are called zein 1 (O'Kennedy, 2011). The largest proportion of maize endosperm (50 - 70%) is composed of these four types of zein proteins (Shewry and Halford, 2002). The content of α-zein is almost double that of β and γ zeins in vitreous endosperm (O'Kennedy, 2011).

Glover (1992) described zein as a mixture of polypeptides with varying sizes of molecular masses of 27 kilo Dalton (kDa), 22 kDa, 19 kDa, 16 kDa, 14 kDa and 10 kDa. According to Young-Min *et al*. (2001) and O'Kennedy (2011), zein proteins are categorized into higher or lower molecular weight subunits and occur in different quantities and solubilities. The molecular weight and structural differences determine the behavior of zein proteins, such as their solubility in alcohol and other aqueous solutions (Lending and Larkins, 1998). The amount of amino acids in maize seeds is the determining factor of quality, which is influenced by zein protein accumulation and abundance. Alpha, γ and δ zeins are further divided in sub-groups due to differences within the groups because of molecular mass, structure, amino acid sequences and solubility that causes them to separate and resolve differently when using sodium dodecyl sulphate - polyacrylamide gel electrophoresis (SDS-PAGE) and high-performance liquid chromatography (HPLC). Alpha zein has molecular masses of 19 kDa and 22 kDa when separated using SDS-PAGE (Esen, 1986; Wallace *et al*., 1990). When α zeins are separated using reversed phase – HPLC (RP-HPLC), several peaks appear with the sub-classes of 19 kDa being the first to be eluted and then 22 kDa appearing in the last stages of the chromatogram (Dombrick-Kurtzman and Beitz, 1993; Harvey, 2007). The α zeins are known to contain high concentrations of cysteine and less glutamine, leucine, alanine and proline (Esen, 1986).

Gamma zein is another class of prolamins with a molecular mass of 16 kDa and 27 kDa due to different lengths of polypeptides, which cause differences in mobility when separated using SDS-PAGE (Miclaus *et al*., 2011). Separation of γ zeins using RP-HPLC shows that they are divided into two sub-classes, hence resolved as two peaks of 27 kDa and 16 kDa, which appear second and third in the chromatogram, respectively (Dombrick-Kurtzman and Beitz, 1993; O'Kennedy, 2011). It is the second largest fraction after α zein and accounts for 20% of the

total zeins found in maize endosperm (Lawton, 2002). Gamma zeins with molecular mass of 27 kDa have high concentrations of proline (25%) (Sofi *et al*., 2009) and cysteine (Harvey, 2007).

Beta zeins are prolamins with a molecular mass of 14 kDa (Wallace *et al*., 1990; Larkins and Lopez, 1992) and are rich in methionine (Larkins and Lopez, 1992; Shewry and Halford, 2002). Delta zeins, on the other hand, are the smallest among the zein proteins with a molecular mass of 10 kDa (Wallace *et al*., 1990), and are rich in sulphur containing amino acids (Larkins and Lopez, 1992). According to Swarup *et al*. (1995) δ zeins that have a molecular mass of 10 kDa are rich in methionine and limited in lysine and tryptophan, while 18 kDa δ zein are rich in lysine and tryptophan. The elevated presence of 18 kDa δ zein results in the improved availability of these essential amino acids in some maize genotypes, because δ zeins have high content of lysine and tryptophan compared to other zein types (Harvey, 2007). Therefore, genetic variation for zeins is of great importance in breeding, as it provides the opportunity for developing genotypes with increased essential amino acid (Azevedo *et al*., 2003; Pollak and Scott, 2005) and improved maize nutritional quality.

Glutelins

Glutelins are among the non-prolamin proteins that are soluble in dilute acid/base solutions. These are the second largest protein fraction (34%) in maize endosperm, following zeins (Sofi *et al*., 2009). It is considered as cross-linked zein proteins and QPM contains 17% more glutelin than non-QPM genotypes (Bjarnason and Vasal, 1992). Lawton and Wilson (2003) found that glutelins contain relatively lower amounts of lysine and tryptophan compared to albumins and globulins. Glutelin content in maize endosperm is influenced by genotype and grain size (Konopka *et al*., 2007).

Globulins

Globulins are non-zein proteins that make up 3% of the maize endosperm and contain essential amino acids; lysine and tryptophan (Sofi *et al*., 2009; Lawton and Wilson, 2003). These proteins readily dissolve in dilute salt solution and have a molecular weight range between 150 and 190 kDa. Normal maize has a low globulin content of about 0.15% compared to QPM that contains about 0.39% globulin (Vivek *et al*., 2008). In maize endosperm, the concentration of globulin is directly proportional to the kernel size and there is a positive relationship between globulin content and size of the grain kernel (Konopka *et al*., 2007).

Albumins

Albumins are water-soluble proteins and constitute 3% of maize endosperm proteins in normal maize and 13.20% in QPM (Sofi *et al*., 2009). Albumins and globulins contain 5 - 7% lysine, and these contribute 6 - 12% to total protein in maize kernels (Lawton and Wilson, 2003). Albumins and globulins are non-zein proteins hence contain high amounts of essential amino acids, which are well balanced (Vasal, 2002). However, these non-zein proteins are present in small quantities in the maize endosperm, compared to zein proteins, but still affect the content of essential amino acids available in the grain kernel.

Amino acids

Amino acids are linked to form proteins, and hence are referred to as protein building blocks. There are 20 different amino acids, which are required by the human body to function normally. The human body is able to synthesize some of these amino acids while others are obtained from food. On the basis of this, amino acids are categorized into non-essential and essential amino acids. Common maize is considered to be of poor nutritional quality due to limitation in some of the essential amino acids such as lysine and tryptophan (Toro *et al*., 2003). Large genetic variations exist among different maize genotypes for amino acid composition. This variation has been exploited by different researchers in breeding for QPM that has enhanced levels of lysine and tryptophan (Krivanek *et al*., 2007; Vivek *et al*., 2008; Sofi *et al*., 2009).

Lysine

Lysine is one of the limiting essential amino acids in common maize. Common maize has high amounts of zein and is hence low in lysine, and as such is considered to be of poor quality (Toro *et al*., 2003). QPM on the other hand, have reduced amounts of zeins and have about double the amount of lysine compared to common maize (Prasanna *et al*., 2001; Sofi *et al*., 2009). Lysine content ranges between 3.30 - 4.00% in QPM and around 1.30% in non-QPM (Prasanna *et al*., 2001). Vasal (1999) noted lysine content in QPM hybrids ranging between 3.80 - 4.50%. According to Sofi *et al*. (2009), lysine is genetically controlled, and the *o*2 gene that confers higher lysine concentration can be introduced in maize to develop genotypes with high levels of lysine. A negative correlation was reported between zein and lysine, and therefore grains with large amounts of zein have less lysine (Krivanek *et al*., 2007). Yu *et al*. (2004) found that integration of potato pollen, which is a lysine rich protein (*sb*401) was capable of increasing lysine concentration by 16.10 - 54.80% while the total protein level increased between 11.60 and 39% in transgenic maize genotypes.

Tryptophan

Tryptophan is one of the essential amino acids that is limiting in most cereal crops. Maize endosperm contains small quantities of tryptophan and it is deficient in most varieties (Sofi *et al*., 2009). Different amounts of tryptophan have been reported in conventional maize and QPM genotypes (Vasal, 1999; Vivek *et al*., 2008), depending on the genotype background. The amount of tryptophan in normal maize and QPM is 0.40% and 0.90%, respectively (Vivek *et al*., 2008). Vasal (1999) reported tryptophan ranging from 0.90 - 1.10% in QPM varieties. Variation exists in the amount of tryptophan present in white QPM hybrids and sub-tropical QPM hybrids ranged from 0.80 - 1.00% and 0.90 - 1.00%, respectively (Prasanna *et al*., 2001).

2.4.4 Minerals

In addition to starch and proteins, maize provides minerals to consumers. The distribution of the minerals within the grain varies, with the germ part of the seed being relatively rich in minerals (75%) compared to the maize endosperm (Masindeni, 2013). Minerals play a significant role in growth, development and reproduction of plants such as maize (Battal *et al*., 2003). Insufficient soil nutrients do not only affect plant growth but also the concentration of the respective nutrients in the edible portion of the plant. This results in nutritional disorders amongst individuals whose diet primarily consists of the crop in question (Imtiaz *et al*., 2010). Nube and Voortman (2006) reported that minerals present in the soil and their availability to the plants influence micronutrient content in maize grain. According to White and Broadley (2005), soil mineral imbalances result in cereal grains that mostly have inadequate amounts of minerals like Fe, Zn, Cu, Ca, Mg, iodine and selenium, which consequently have an effect on the health of the consumer.

Zinc

Minerals such as Zn play various roles in the plant physiological processes such as oxidation-reduction reactions, metabolism and enzymatic reactions (Hafeez *et al*., 2013), in addition to synthesis of carbohydrates and proteins (Sajedi *et al*., 2009). Zn deficiency in the soil therefore results in yield and quality reduction in crops (Hafeez *et al*., 2013). Chen *et al.* (2017) reported yield reduction of 30% in most staple crops such as wheat, maize and rice that were produced in Zn deficient soils. Genetic variation exists in maize genotypes for Zn content (Bänziger and Long, 2000). Various studies found different ranges of grain Zn content in QPM, inbred lines and conventional maize (Menkir, 2008; Queiroz *et al*., 2011; Phalafala, 2013). Breeders, in

development of cultivars with enhanced grain Zn content, can explore available genetic variation in maize.

Iron

Plants require Fe in small quantities but this mineral is vital for growth and reproduction, and various processes that take place in the plant. The mineral is required for formation of chlorophyll and normal enzyme functioning in the plant (Hochmuth, 2011). According to Sajedi *et al*. (2009), Fe is responsible for boosting different types of enzymes that are crucial for photosynthesis and respiration processes. It is essential for the electron transfer system in mitochondria and plant chloroplasts (Hochmuth, 2011). Grains with low Fe content are produced from soils, which are deficient in Fe and hence affect nutritional status of individuals who depend on the crop for food. Fe deficiency affects billions of people worldwide and the problem can be reduced through breeding for high Fe content in staple crops (Menkir, 2008). Messias *et al*. (2015) reported that an increase in Fe fortified fertilizer could increase the element content in the grain. However, soil application has proved less effective since Fe has a low mobility in the soil. Therefore, foliar application is preferred. Frossard *et al*. (2000) further observed that NPK application enhanced Ca, Zn and Fe absorption in the soil.

Significant variation exists in maize cultivars for Fe content in the grain (Bänziger and Long, 2000; Agrawal *et al*. 2012), although Bänziger and Long (2000) suggested that environment contributes more to variation in mineral content in maize than genotype. In their study re-evaluating promising genotypes for high Fe and Zn in different places in Zimbabwe including N stress and non-stress N conditions, resulted in drastic decrease in minerals grain content compared to the grain mineral content observed at the first evaluation. Different Fe content were observed in QPM genotypes and local varieties by Chakraborti *et al*. (2011); Queiroz *et al*. (2011) and Phalafala (2013).

2.4.5 Phytic acid and mineral bioavailability

Phytic acid in grain cereals and legume seed is stored in the form of P and occurs in the largest quantity compared to other minerals (Gibson *et al*., 2010). Phytic acid (phytates) constitutes 50 - 80% of total P in cereal grains and legume seeds (Wu *et al.,* 2009; Queiroz *et al*., 2011). The total P content in cereal grains varies due to growing conditions, harvesting techniques and age of the crop (Garcia-Estepa *et al*., 1999; Coulibaly *et al*., 2011). Genetic makeup and environmental factors are major determining factors for phytic acid content in grains (Ortiz-Monasterio *et al.*, 2007). Coulibaly *et al.* (2011) reported high phytic acid content in foods,

which are produced in soils with high amounts of P fertilisers. In grains, phytic acid consists of naturally occurring salts present as mono and divalent metal ions like Mg^{2+}, Ca^{2+} and K^{+} that accumulate in the grain kernel during the maturation period (Wu *et al*., 2009; Coulibaly *et al*., 2011). But the presence of phytic acid in cereal grains and legume seeds bind micronutrients like Fe, Zn and Ca, making them insoluble salts and hence unavailable for absorption (Wu *et al*., 2009; Goudia and Hash, 2015). Deficiency of these minerals is one of the main causes of malnutrition. Coulibaly *et al.* (2011) found that the release of enzymes such as pepsin, trypsin and amylase are inhibited by phytic acid. These enzymes are responsible for food digestion, and the inhibition can contribute in creating malnutrition.

Gibson *et al*. (2010) observed that Zn absorption is not affected by phytic acid (myo-inositol phosphates) such as tetra phosphates (IP4), tri-phosphates (IP3), di-phosphates (IP2) and mono-phosphates (IP1) whereas Fe absorption is not affected by IP2 and IP1, and this explains why Zn is more available in the diet compared to Fe. The hydrolysis of higher phytates to lower inositol phosphates such as IP4, IP3, IP2 and IP1 by phytase enzymes through different processes makes minerals available for absorption. Hexa phosphate (IP6) can be degraded into lower inositol phosphate compounds through germination, storage and fermentation (Garcia-Estepa *et al*., 1999). Generally, mutant maize *lpa* contain less phytic acid and Raboy *et al*. (2000) observed total phytates of 3.40 mg g^{-1} in wild type mutants while *lpa* mutants contained a lower content of 1.10 - 2.60 mg g^{-1} in maize grains with higher mineral bioavailability reported in *lpa* than in wild type mutants.

2.5 Quality protein maize

Teklewold *et al*. (2015) defined QPM as maize genotypes whose lysine and tryptophan levels in the endosperm of the kernels are twice that of common maize. Lysine and tryptophan are essential amino acids that the body cannot synthesize hence the need to provide in the in food. QPM originated from manipulation of a naturally occurring mutant gene, *o*2, which occurs as homozygous recessive (*o*2*o*2) in QPM but homozygous dominant (*O*2*O*2) in common maize. Other components of QPM development include manipulation of enhancers of the *o2* containing endosperm, to increase lysine and tryptophan levels, and manipulation of genes that modify the *o*2 to confer either soft endosperm or hard endosperm (Prasanna *et al*., 2001; Vivek *et al*., 2008). Other than the two essential amino acids, the traits of QPM are not different from the common maize and visual distinction is not possible unless biochemical analysis is performed (Vivek *et al*., 2008). The maintained properties of common maize in QPM means

that QPM would be a cheap source of protein, given that farmers can grow, manage, harvest and consume it in the same way they do common maize (Teklewold *et al*., 2015).

2.5.1 Genetic basis of quality protein maize

Negative correlation has been observed between α zein, lysine and tryptophan content (Blumenthal *et al*., 2008) and the different forms of the same gene, *o2*, control both. The dominant *O2* gene promotes the synthesis of α zein, which occupies the larger part of maize endosperm but deficient in lysine and tryptophan, while the recessive *o2* gene down regulates synthesis of α zein and promotes production of non-zeins which are rich in lysine and tryptophan (Gibbon and Larkins, 2005), leading to an increase in these essential amino acids.

Endosperm hardness is controlled by γ zeins, which at a molecular level are controlled by modifier genes called endosperm modifiers. The *o2* mutant gene and endosperm modifiers convert soft or opaque mutant endosperm to become hard/vitreous without altering its nutritional quality (Sofi *et al*., 2009). QPM grains that have *o2* endosperm-modifiers contain twice as much γ zein as QPM genotypes that do not contain the *o2* gene (Vasal, 2002). However, Dombrick-Kurtzman and Beitz (1993), indicated that endosperm texture is a polygenic trait. Microscopically, the soft grain endosperm looks opaque and the degree of opaqueness indicates whether the grain is a homozygous mutant, a homozygous non-mutant or heterozygous. Therefore, breeders can select grains that have high levels of lysine and tryptophan by using a light table. This can be used to classify grains on a 1 - 5 scale, and class 2/3 is selected based on the generation stage, as these are still segregating and likely to produce different kinds of kernels in the coming generation. However, class 3 is deamed to have homozygous recessive *o2o2* and good modification, and is usually it is selected in early generation (F_2). Score 2 is used for selection when modifiers are towards fixation in the genotype usually in F_3/F_4 (Vivek *et al*., 2008).

Another genetic system that is manipulated in common maize for QPM development is the amino acid modifier gene (amino acid modifiers). Amino acid modifiers/enhancer are responsible for regulating the amount of amino acids lysine and tryptophan (Vivek *et al*., 2008). The average tryptophan and lysine in common maize and QPM is 1.60 - 2.60 g 100 g^{-1} and 2.60 - 4.50 g 100 g^{-1}, respectively (Krivanek *et al*., 2007). According to Vivek *et al*. (2008), if amino acids lysine and tryptophan in developed QPM are not properly monitored, it is possible to

come up with QPM cultivars with amino acid amount equivalent to common maize since the maximum value in common maize is the minimum value in QPM as ranges overlap.

QPM genotypes are thus superior to common maize in terms of nutrition and biological value. QPM lysine content varies from 3.30 - 4.00 g 100 g^{-1} due to genetic differences, while common maize has around 1.30 g 100 g^{-1} lysine. QPM therefore has 30% and 55% more lysine and tryptophan, respectively than common maize (Prasanna *et al*., 2001). Biological value defines the N quantity that the body needs and absorbs from the food consumed. Biological value accounts for the proportion of consumed proteins that is made available to the body for metabolic processes. Common maize has a biological value of 45% while different QPM has biological value of above 80% (Ignjatović-Micić *et al*., 2014). According to Glover (1992), QPM has high net protein utilization of more than 71% while normal maize has a net protein utilisation of 61.50%.

2.6 Malnutrition

2.6.1 Definition

Novelo (2016) defined malnutrition as micronutrient deficiencies, excesses or imbalances in a person's intake of energy and/or nutrients and it results in stunted growth or wasting. Sobotka *et al*. (2006) defined malnutrition as a state of nutrition, which is a deficiency or excess of energy, protein and other nutrients, which causes measurable adverse effects on tissue/body form, body function and clinical outcome. Based on the two definitions, malnutrition includes not only protein-energy deficiency but also other nutrient deficencies such as micronutrients.

2.6.2 Malnutrition statistics and distribution

The International Food Policy Research Institute (IFPRI) reported that malnutrition is a global concern and is experienced in every country but at varying intensity (IFPRI, 2014). About 870 million people, representing around 12.50% of the world population, are undernourished of which 825 million people are from developing and under-developed countries (Akhtar *et al*., 2013; IFPRI, 2015). Novelo (2016) broke the statistics down based on gender and showed that 462 million adult men, 209 million children and 528 million (29% of women of reproductive age), are malnourished.

2.6.3 Causes of malnutrition

There are diverse multi-factorial and interlinked causes of malnutrition (Abdalla, 2007). However, poverty, food shortage and poor bioavailability of minerals are the primary causes of food insecurity and hence malnutrition (IOA, 2012). These factors are worse in Africa, especially in East and West Africa and it occurs in a vicious cycle.

2.6.3.1 Food shortage

Food shortage is defined in terms of availability and access. According to Cleaver *et al*. (2004), major malnutrition cases occur because people produce less food than they need or are unable to obtain adequate food due to insufficient financial resources. In SSA, most countries experience acute food insecurity compared to developing countries. Little has been achieved in an effort to be food secure by 2015, midway through achieving the Millennium Development Goals (MDGs) and more than one third of the people in Africa are still chronically hungry. Research shows that around 23 million people in 11 African countries of East and West Africa have food shortages and are prone to malnutrition (IOA, 2012). This is due to high poverty levels, large populations, political instability and climate change that have negative impacts on crop production, hence causing high rates of malnutrition (IOA, 2012).

2.6.3.2 Lack of access to quality food

Another major cause of malnutrition is lack of economic access to adequate nutritious foods (Abdalla, 2007) and some of these are fresh fruits and vegetables, legumes, meat, fish and milk. Alternatively, the less privileged turn to and over depend on food or drinks, which have poor nutritional quality due to their affordability and availability (Evans *et al*., 2015). This results in malnutrition rates that are higher in developing and under-developed countries.

In developing countries, protein deficiency is a major form of malnutrition and has been attributed to inadequate intake of proteins (WHO, 1999), and it is rampant in African countries. This is because the majority of the people in Africa depend on maize as their primary staple food to meet their calorie and protein dietary requirements, with limited food diversification (NRC, 1988). Common maize is limited in essential amino acids such as lysine and tryptophan.

Besides protein deficiency, micronutrient deficiency is another common problem, especially amongst childbearing women (Bhutta *et al*., 2009), adolescents, infants and children under the age of five (Akhtar *et al*., 2013). The most commonly reported micronutrient deficiencies are for Zn, Fe, iodine and vitamin A. Similarly, micronutrient deficiency is higher in developing

countries than in developed countries. According to WHO (2012), worldwide 530 000 women pass away every year due to maternal related problems. About 99% of these deaths occur in developing countries, where 56% are from SSA and 80% of these deaths are reported to be due to malnutrition, which can be avoided.

2.6.3.3 Mineral bioavailability

The availability of nutrients in nutrient rich food sources does not guarantee their availability to the body, especially in plant-based food sources. Several factors influence mineral bioavailability such as the chemical form of the element, age of a person, individual's body requirement, presence of other minerals and organic compounds such as phytic acid. However, amongst all the factors, phytic acid content is the main cause of reduced mineral bioavailability in plant-based diets (Gibson *et al*., 2006). The presence of phytates in cereal grains and legume seeds affects the absorption of nutrients such as Fe, Zn and Ca in the body. Minerals become insoluble in the digestive system making them unavailable for absorption and creating micronutrient deficiency (Gibson *et al*., 2010). Improving mineral absorption is important to reduce micronutrient deficiency.

2.6.4 Consequences of malnutrition

There are many effects of malnutrition, including higher morbidity, higher mortality, lower cognitive ability, lower work productivity, impaired growth, impaired reproduction and loss in gross domestic product (GDP), amongst others (Welch, 2002). Severe vitamin A deficiency could result in permanent blindness, illness and death due to infections, while Zn deficiency causes compromised immune systems that result in infections, retarded growth and development, and delay in sexual maturity, amongst others. Lack of Fe in the diet results in susceptibility to infections, stunted growth and mental impairment while iodine deficiency causes goitre problems, reproductive failure and brain damage (Welch, 2002). According to Black *et al*. (2014), malnutrition related problems accounts for 45% of children deaths in the world and this can be avoided with good nutrition. In a report by WHO it is estimated that in Africa, stunted growth has increased by 17% from 2000 - 2016 while in most other countries, the number has dropped substantially. In Asian and Latin American countries, the numbers have dropped by 35% and 44%, respectively. For instance, in West Africa, statistics show that the number of stunted children has increased by 4 million between the years 2000 - 2016. Again, West Africa also has the highest number of wasting children under the age of five years (WHO, 2017).

2.6.5 Approaches to addressing malnutrition

2.6.5.1 Global and regional actions against malnutrition

Countries and organisations all over the world realise and appreciate the overall effect of malnutrition on human health and global, regional and countries' economic development. Policies, declarations and treaties have therefore been established and set forth as benchmarks in an effort to eradicate malnutrition.

(a) Universal declaration on the eradication of hunger and malnutrition

This declaration was adopted on 16 November 1974 by the World Food Conference, which convened under the General Assembly resolution 3180 (XXVIII) of 17 December 1973 but was endorsed by the General Assembly resolution 3348 (XXIX) of 17 December 1974. Amongst many, the declaration recognised that a grave food crisis afflicts the majority of people in developing countries, where two thirds of the world population live. This group produces only one third of the world's food and have the highest number of the world's hungry and ill-nourished. The declaration further recognised the social and economic implications associated with malnutrition and its potential infringement of the most fundamental principles and values associated with the right to life and human dignity as in the Universal Declaration of Human Rights. The declaration therefore called upon all people expressing their will as individuals, and through their governments, and non-governmental organizations, to work together to bring about the end of the age-old scourge of hunger. It called upon all donor countries to accept and implement the concept of forward planning of food aid and make all efforts to provide commodities and/or financial assistance that would ensure adequate quantities of grains and other food commodities (The World Food Conference, 1974).

(b) Abuja declaration on fertiliser for an African Green Revolution

The African Union adopted this declaration in 2006 in Abuja (Nigeria). The declaration was revitalised on the commitment made by the African Ministers of Agriculture in the year 2006, Abuja Declaration on Fertilizers for an African Green Revolution, which originally pledged to increase fertilizer use from 8 kg ha^{-1} to 50 kg ha^{-1} by the year 2015. During this event, the strong accent on fertilisers was replaced by a more holistic approach of sustainable soil management in order to ensure that soil systems are optimised to perform their multiple natural functions and contribute to the efficient use of fertilisers applied.

These declarations recognise the agricultural sector's crucial role in the fight against persistent hunger and poverty eradication. The declarations further recognise that in Africa, agriculture

is the backbone for most economies and provides employment (60%), a source of income (90%), GDP (20%) and foreign currency (20%). Despite the institution of these declarations, African countries are currently even worse off in terms of food insecurity and malnutrition (Sustainable Development Goals, 2016).

(c) UN decade of action on nutrition

In an effort to ensure that all people have access to healthier food and more sustainable diets, as a road map to eradicating all forms of malnutrition worldwide, the United Nations General Assembly, adopted a resolution proclaiming the UN Decade of Action on Nutrition from 2016 to 2025. The resolution aimed at catalysing policy commitments that would result in measurable action to address all forms of malnutrition (UNSCN, 2016).

Before the 2016 resolution, several other approaches were used to address malnutrition. Ortiz-Monasterio *et al*. (2007) and Kandianis *et al.* (2013) reported that nutrient supplementation and food fortification were used as rapid and effective methods in the absence of other options. It was later realised that the approaches did not reach the majority of the poor in rural communities. Secondly, the recurrent nature of the approaches made them unsustainable (Nestel *et al*., 2006). There had been efforts to identify low cost and sustainable approaches that could not only effectively address malnutrition but also reach out to the majority of the poor as well as addressing the issue once and for all, hence the introduction of biofortification.

2.7 Biofortification

Biofortification is defined as the process of breeding nutrients into food crops. According to HarvestPlus (2007) and Manjeru (2017), the Biofortification Challenge Programme (BCP), which is a Consultative Group on International Agricultural Research (CGIAR), was established in July 2003 with the aim of "adding food quality to its agricultural production research paradigm". The process provides a relatively cost-effective, sustainable and long-term means of delivering more micronutrients (Nestel *et al*., 2006; Bouis *et al*., 2013). The biofortification strategy seeks to put the micronutrient dense trait into those varieties that already have preferred agronomic and other traits, such as high yield and disease resistance (Chakraborti *et al*., 2011). It has been noted that biofortification lowers the number of severely malnourished people who require treatment by complementary interventions. Besides, this approach ensures that their nutritional status is maintained. Biofortification provides a feasible

means of reaching malnourished rural populations who have limited access to commercially marketed fortified foods and supplements (Bouis *et al*., 2011; Qin *et al.,* 2012).

2.7.1 Maize biofortification

Maize biofortification involves the enhancement of essential micronutrients such as Fe and Zn plus vitamin A (Chakraborti *et al*., 2011; Kandianis *et al*., 2013). This is achieved through conventional breeding as in QPM or biotechnology techniques and agronomic approaches through ferti-fortification (Hefferon, 2015).

2.7.1.1 Conventional breeding

Nutrient enhancement can be achieved through breeding, usually conventional plant breeding (genetic biofortification) (Reddy *et al*., 2014). Conventional breeding is the most popular approach for developing crops with increased nutritional quality, such as QPM (Krivanek *et al.* 2007; Reddy *et al*., 2014). The success of the approach depends on whether the nutrient in question is genetically controlled, the genetic diversity and heritability. For instance, it has been reported that Fe and Zn content vary in QPM and non-QPM and are genetically influenced (Badu-Apraku *et al*., 2013). The same has also been reported by Mulualem (2015) and Welch and Graham (2004) and they further noted that Fe and Zn are positively correlated and hence concurrent breeding for both is possible in maize. However, Bänziger and Long (2000) as well as Chen *et al.* (2017), noted an inverse relationship between grain yield and Zn/Fe content, which makes simultaneous breeding for grain yield and high Zn/Fe content almost impossible. Lung'aho *et al.* (2011) noted that Zn and Fe content are also influenced by environmental factors.

2.7.1.2 Transgenic biofortification

Bouis and Saltzman (2017) noted that genetic variation does not exist for some micronutrients and, as such, conventional breeding may not be successful. Secondly, conventional breeding involves extensive field evaluations and phenotyping and it takes time to produce results, which is sometimes associated with linkage drag i.e. transferring of unwanted traits. Biofortification can lead to enhanced nutrient levels but may not achieve sufficient bioavailability. Hence the application of transgenics in biofortification which involves desired and specific gene sequence transfer and introgressing it into a broad range of desired background such as for high yield and disease tolerant germplasm (Visarada *et al*., 2009). The major advantages of this approach are the possibility of gene exchange amongst unrelated species. Genes, such as those coding

for Zn and Fe synthesis could be identified, cloned from other organisms such as bacteria and introduced in crops such as maize. Genetic modification by transgenic means has been employed in many different crops to enhance their macronutrient and micronutrients content (Hirschi, 2009; Trijatmiko *et al*., 2016). However, the introduction of alien genes is associated with issues of instability of traits, transferability to weed plants and health risks to humans. As a result, despite its immense achievements and usefulness, regulatory hurdles limit the use of transgenic biofortification (Hirschi, 2009; Yashveer *et al*., 2014).

2.7.1.3 Fertiliser biofortification (agronomic approach)

Fertiliser biofortification, also known as ferti-fortification, is an agronomic approach to enhancement of nutrients in crops (Nazir *et al.*, 2016). Specific fertilisers are applied to the plant through either soil or foliar application in a manner that makes specific elements readily available for uptake by the plant De Valença *et al.* (2017) reported that increasing micronutrient availability to plants would eventually increase micronutrient content in plant tissues. Low Zn content has been reported in grains, which were harvested from Zn deficient soils. Ferti-fortification in cereals using Zn increased grain Zn content (De Valença *et al.*, 2017; Nazir *et al.*, 2017). It has been reported that application of Zn fertilizer through foliar feed at flowering stage of cereal crops increased Zn content in grains. According to De Valença *et al*. (2017), ferti-fortification is fast, simple and produces immediate and effective results. However, interactions amongst micronutrients in the soil may interfere with absorption of the fortified nutrient.

2.7.2 Why biofortifying maize

Biofortified maize can drastically reduce malnutrition. Biofortification forms part of the long-term effort for sustainable development goals (SDGs), which aim to address malnutrition in the world by 2030 - “End hunger, achieve food security, improved nutrition and sustainable agriculture”. SDGs considers nutrition as “a fundamental right of all the humanity.” In order to achieve good nutrition for all and to accelerate and sustain the progress made in nutrition, all SDGs have to work together (UNSCN, 2015).

2.8 The effect of environment on grain yield and nutritional quality

The genetic composition and the environment affect the phenotypic appearance of a plant. The environment comprises the rhizosphere and atmosphere, and hence factors such as precipitation, temperature, relative humidity, water regime, nutrient quantity and balance affect

the life of plants. One of the most important factors of growth and development in crop plants is N (Leghari *et al*., 2016).

2.8.1 Nitrogen

N is one of the elements that are required in large amounts by plants for growth and reproduction. Ammonium (NH_4) and nitrate (NO_3) are the two readily usable inorganic forms of N that exist in the soil. According to Lambers and Bassham (2017), N is critical for synthesis of compounds that are necessary for different metabolic pathways. Chlorophyll is one of the most important compounds that play an important role in the food chain, through photosynthesis that need N. It is a crucial component of amino acids and hence of proteins, which play structural, catalytic, energy transfer and genetic roles in organisms (Leghari *et al*., 2016; Lambers and Bassham, 2017). N deficiency therefore has a number of implications on grain yield and nutritional quality.

2.8.1.1 Effects of low N on grain yield and nutritional quality in maize

Grain yield

N plays a significant role in plant growth and development and its reduction greatly decreases grain yield (Shah *et al*., 2016), and grain nutritional quality and mineral content (Gu *et al*., 2015). Reduction in N decreases photosynthetic processes as well as reproductive development (flowering) exhibited by high flower abortions and hence results in reduced grain yield (Seebauer *et al*., 2010). Therefore, increasing N application reduces flower abortions, increases number of kernels and hence increases grain yield (Oktem *et al*., 2010; Seebauer *et al*., 2010).

Generally maize is sensitive to N levels (Karasu, 2012). According to Khan *et al*. (2009), the lowest grain yield was reported in low N fertiliser treatment and grain yield increased with increased N application. Increased N fertilizer application increases zein protein accumulation in the endosperm, which compromises on essential amino acid accumulation, leading to decreased lysine and tryptophan content (Blumenthal *et al*., 2008). N deficiency at the V8 maize growth stage reduces grain yield by 22% while no effect was observed when deficiency occurred at silking stage (Khan *et al*., 2009). Imran *et al*. (2015) showed that in low N soil conditions, N application increased maize grain yield by 43 - 68%. Gerde *et al.* (2017) also reported that increased N supplies increased maize grain yield and kernel hardness.

Total protein and zein proteins

One of the factors that affect maize grain quality is protein content, which is affected by genetic makeup, management practices and the environment, especially N content in the soil (Cheetham *et al*., 2006). Generally, grains produced in low N environments have low protein content (Karasu, 2012). Zaidi *et al*. (2008) found that grain protein, lysine and tryptophan decreased by 17.00%, 12.50% and 15.60%, respectively, in low N conditions. Ngaboyisonga and Njoroge (2014) also observed reduced total protein content and tryptophan content in low N compared to optimum N soil environments. Therefore, an increase in the amount of N supply is likely to increase the protein content in the maize kernel (Oktem *et al*., 2010; Seebauer *et al.,* 2010). Blumenthal *et al.* (2008) noted reduced biological value in maize produced in areas with a high N content, since the grain contains relatively less lysine and tryptophan.

Khan *et al.* (2014) reported that storage proteins are influenced by genetic makeup rather than by the growing environment. However, O'Kennedy (2011) noted differences in zein content from maize plants, which were grown under varying N levels. Low N has an effect on gene expression, especially for α zein storage protein accumulation in maize kernels, but no effects were observed in β zein protein (Gerde *et al*., 2017). Wu and Messing (2012) observed high accumulation of α zein protein in Illinois high protein maize genotypes, which also had reduced essential amino acid concentration compared to common maize in low N soil conditions. The reduced content of α zein is vital as it increases the proportion of lysine and tryptophan, improving maize grain nutritional quality.

Tryptophan and oil content

Tryptophan content in maize grain is among the most important nutritional traits, which breeders need to take note of in addressing malnutrition. According to Duarte *et al*. (2005) and Medici *et al*. (2009), additive gene effects rather than non-additive effects determine essential amino acids content. Low tryptophan and lysine content were observed in QPM and checks in low N compared to optimum N soil conditions (Wegary *et al*., 2011; Ngaboyisonga *et al*., 2012). However, some QPM genotypes maintained the essential amino acids content in low N environments (Wegary *et al*., 2011). In contrast, Tsai *et al*. (1992) noted decreased tryptophan levels in maize endosperm grown in optimum N soil conditions.

Maize oil is of great importance for human and animal nutrition as source of energy. It contains unsaturated fatty acids that have many health benefits for human beings (Blumenthal *et al*, 2008). Ferro *et al*. (2006) and Liu *et al*. (2008) noted that low N significantly reduced grain oil

content. Lower oil content was found in maize kernels grown under low N than optimum N conditions (Duarte *et al*., 2004; Liu *et al*., 2008), although the variation in oil content amongst genotypes was insignificant. Genetic variation and positive correlation were observed between tryptophan and oil content (Duarte *et al*., 2004), which is important for simultaneous improvement of traits in genotypes.

Micronutrient content

Mineral content in maize kernels is affected by growing conditions and genetic factors (Imtiaz *et al*., 2010; Chakraborti *et al*., 2011). Imtiaz *et al.* (2010) reported that lack of micronutrients in the growing environment results in compromised grain quantity and quality in terms of mineral content. Micronutrient content increase with increased N supply and *vice-versa*.

Iron content in maize grain kernel under low N conditions

Kandianis *et al*. (2013) noted that stressful growing conditions such as low N, drought and their interaction negatively affected Fe content in maize kernels. According to Oktem *et al.* (2010), low Fe content in maize kernels were seen in low N environments compared to optimum N conditions. The N stress in maize lowers grain Fe content (Oktem *et al*., 2010; Xue *et al*., 2014).

Zinc content in maize grain kernel under low N conditions

Low N conditions reduce grain mineral accumulation such as Zn, Fe and Cu in the grain kernel. Lower mean average of grain Zn content was reported under low N soil conditions (Xue *et al*., 2014). Oktem *et al.* (2010) found reduced Zn grain content in sweet corn kernels grown under low N conditions. According to Wang *et al*. (2017), grain Zn content increased with an increase in N application in wheat and high mean value of Zn content was reported in a Zn and N fertilizer treatment, using foliar application. Genetic variation exists that can be explored by breeders in development of new cultivars with high grain Zn content under low N environment.

Phytic acid (micronutrient inhibitor)

Phytic acid is the major anti-nutritional inhibitor and it is abundant in cereal grains such as maize, rice and wheat (Lim *et al*., 2013). Apart from genetic makeup and environmental conditions, phytic acid content is also influenced by soil type and irrigation (Coulibaly *et al*., 2011). N level has a large effect on the accumulation of P in the grain (Feil *et al*., 1992; Ning *et al*., 2009). Generally, there is a positive relationship between N in the soil and phytic acid content in the edible plant tissues (Manzeke *et al*., 2012; Erdal *et al*., 2014). Manzeke *et al*.

(2012) recorded low phytic acid in grain kernels in unfertilized treatments in different locations, suggesting that fertilisers have an effect on grain phytic acid accumulation.

2.9 Conclusions

Common maize is limited in essential amino acids and yet it is the primary staple for the majority of the poor in rural communities. In addition, maize generally has low concentrations of minerals such as Fe and Zn, but is high in phytic acid content, the anti-nutritional factor that inhibits nutrient availability. It is thus not surprising that there are so many malnutrition cases in developing countries due to overdependence on cereal grains as staple food. In an effort to address malnutrition, CIMMYT has developed QPM, which has double the amount of lysine and tryptophan than that in common maize. However, most small-scale farmers produce maize under low N conditions. It is therefore imperative to determine the effect of soil conditions on the nutritional attributes of QPM hybrids, to assure that the technology is effective.

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CHAPTER THREE

THE INFLUENCE OF DIFFERENT PRODUCTION ENVIRONMENTS ON TRYPTOPHAN AND OIL CONTENT AND GRAIN YIELD IN QUALITY PROTEIN MAIZE HYBRIDS

3.1 Abstract

Micronutrient malnutrition is a major problem for the majority of people in developing countries. The problem is higher among urban and rural communities, which depend on maize as their main staple. This study investigated the effect of environmental conditions on tryptophan and oil content as well as grain yield in QPM hybrids. Genotype, environment and, genotype and environment interaction effects were significant for all measured characteristics. Highly significant differences were observed between genotypes for tryptophan and oil content, and grain yield under most production environments. In general, values were lower under low N compared to optimum N conditions for all characteristics, with the largest effect on grain yield. Therefore, it is concluded that environmental conditions have an effect on tryptophan content, oil content and grain yield in QPM hybrids.

3.2 Introduction

Maize (*Zea mays* L.) has diverse uses worldwide and is mostly used as a source of food, animal feed and for industrial applications (Milander, 2015). The crop is produced globally because of its ability to adapt in a wide range of growing conditions (Macauley, 2015). In SSA the crop is produced by large numbers of people and it is a major staple crop (Nuss and Tanumihardjo, 2011). Maize contains macro and micronutrients that are necessary for growth and development in humans and animals. According to Sofi *et al*. (2009), storage proteins, the zeins, in maize are found in the endosperm, and are soluble in alcohol. Superior quality and quantity protein are found in the germ part of mature maize compared to the immature endosperm.

Common maize contains low levels of essential amino acids tryptophan and lysine and has poor nutritional quality (Sofi *et al*., 2009), although most people depend on it as staple food. In countries where maize is a source of protein in human diets, QPM can be used instead of common maize due to its increased levels of lysine and tryptophan, which are twice the amount present in common maize (Tandzi *et al*., 2017). Protein deficiency, common in the lives of most people in Africa that obtain their protein from a maize diet, can be minimized through the

use of QPM (Nuss and Tanumihardjo, 2011; Ngaboyisonga *et al*., 2012). Various researchers reported the benefits of QPM over common maize for food and feed, respectively (Scrimshaw, 2006; Krivanek *et al*., 2007; Gunaratna *et al*., 2008). The research findings showed that pigs fed on a QPM diet weighed 18 kg more compared to those fed on common maize in El Salvador (Vivek *et al*., 2008). Reduction in stunting growth was reported in children weaned on QPM compared to normal maize porridge in Ghana (Vivek *et al*., 2008). According to Nuss and Tanumihardjo (2011) increased growth and weight were reported in children fed on QPM porridge by 9 and 12% respectively, compared to conventional maize.

Opaque 2 gene present in QPM genotypes reduce the amount of zein proteins synthesized, hence increase the levels of lysine and tryptophan in grain endosperm (Krivanek *et al*., 2007; Vivek *et al*., 2008; Sofi *et al*., 2009). It is reported that lysine and tryptophan levels are positively correlated in maize kernel (Krivanek *et al*., 2007; Nurit *et al*., 2009; Masindeni, 2013). This suggests that these traits can be improved simultaneously.

According to Thomison *et al.* (2002) and Moro *et al*. (2012), grain yield decreases as the oil content increases and *vice versa*. This makes cultivar development of high grain yield and high oil content difficult due to their negative correlation (Tandzi *et al*., 2017). Maize genotypes differ significantly in grain yield, and grain protein content, which may be due to genetic makeup or environmental conditions (Duarte *et al*., 2004). According to Mittelmann *et al.* (2003), grain protein, oil content and grain yield are mainly determined by genetic makeup, and are less influenced by soil conditions. Zaidi *et al*. (2008) noted a significant reduction in grain yield and grain quality characteristics such as total protein, lysine and tryptophan content in QPM and non-QPM under different production environments, including N stressed conditions. Breeding QPM genotypes for high oil content, protein content and grain yield are necessary to address malnutrition mainly in vulnerable groups such as pregnant women and preschool children. QPM promises to reduce the malnutrition gap due to its improved nutritional profile.

Generally, maize is grown under low N conditions in most tropical regions which are characteristically known to have poor soil fertility (Monneveux *et al*., 2006). Masindeni (2013) reported that most farmers in developing countries produce maize under low N conditions as chemical fertilizers are expensive and most people live below the poverty line. Research has been done on the influence of low N on grain quality and other characteristics in common maize. However, only a few studies have been conducted on the influence of low N conditions

on grain quality and grain yield in QPM hybrids (Gissa, 2008; Wegary *et al*., 2011; Ngaboyisonga *et al*., 2012). Therefore, this study aimed at determining the effect of low N and optimal conditions on tryptophan content, oil content and grain yield in QPM hybrids.

3.3 Materials and methods

3.3.1 Planting locations

The trials were planted during the rainy season in 2016/2017 in Zimbabwe at different sites under low N stress and optimum N soil conditions. The trials were grown under rain fed conditions, but irrigation was given when necessary to establish the trials. Harare lies at latitude 17º 46' S and longitude 31º 02' E at an altitude of 1406 meters above sea level (masl). Glandel is located at latitude 17º 36'S, and longitude 31º 06'E at an altitude of 1147 masl. Bindura is located at latitude 17º 18'S and longitude of 31º 02' at an altitude of 1480 masl. Gwebi is located at latitude 17º 04'S and longitude 31º 19'E at an altitude of 1428 masl.

3.3.2 Planting materials

Ten QPM hybrids and two non-QPM hybrids local checks were used in this study (Table 3.1). These 12 hybrids were a sub-sample of 220 entries that were evaluated in Zimbabwe at different locations.

Table 3.1 List of 10 QPM hybrids and two non-QPM hybrids evaluated at different locations in Zimbabwe

Genotype	Entry #	Type
VH06760	1	QPM
TH15938	2	QPM
TH15889	3	QPM
TH151141	4	QPM
SC 627	5	Non-QPM
TH151144	6	QPM
Local check 1	7	Non-QPM
TH15851	8	QPM
TH15895	9	QPM
TH15976	10	QPM
TH151022	11	QPM
TH151082	12	QPM

3.3.3 Experimental design and field management

The trials were planted in an alpha lattice design (Patterson and Williams, 1976), with two replications. Each plot comprised of 4 m long rows that were spaced 0.75 m apart and the plant spacing within the rows were 0.25 m and the plant population per hectare was 53 333. Low N stress conditions in Harare was achieved by depleting N through growing summer maize and irrigated winter wheat continuously for seven years. This was done to simulate the production conditions practiced by the majority of smallscale farmers in the area. Optimum soil N conditions were achieved through application of fertilizers at the recommended rate of 250 kg ha^{-1} N, 83 kg ha^{-1} P and 111 kg ha^{-1} K. At all optimal sites, basal fertilizer application was done in the form of NPK and additional N application was done four weeks after seed emergence. Pre-emergence herbicides; Gramoxone, Basagram and 2, 4-D were applied to control weeds and after that hand weeding was done to make sure that the fields were relatively free of weeds. Insecticides; Carbaryl and Karate were applied to control stalk borer and cutworms, respectively. Carbaryl was applied at planting stage while Karate was applied at planting and seedling emergence stages.

3.3.4 Tryptophan analysis

3.3.4.1 Sample defatting

Well dried maize kernels with uniform size were finely ground using a 1KA, A10 Yellowline (Merck Chemicals Pty Ltd) analysis grinder. Milled samples were sieved (1 mm) and (2 g) were placed into 50 ml Falcon tubes for defatting (Folch *et al*., 1957). A 10 ml chloroform: methanol 2:1 (v/v) mixture were added. The samples were shaken well and allowed to stand overnight in a refrigerator (4^{o}C). After the overnight stand, the samples were removed and washed three times with fresh 10 ml mixture of chloroform and methanol. The samples were left on filter paper until they were completely dry and stored in the cold room (4^{o}C) until tryptophan was measured.

3.3.4.2 Tryptophan determination

The defatted samples were analysed for tryptophan content using a colorimetric method based on glyoxilic acid reaction with the tryptophan present in the flour in the presence of ferric chloride and sulphuric acid (H_2SO_4) according to the protocol of Nurit *et al*. (2009).

Papain and sodium acetate solution was prepared freshly for the number of samples to be analysed at one time. The chemicals and reagents were prepared as follows: 1 mg ml^{-1} papain

was dissolved in 0.165 M sodium acetate solution. DL-Tryptophan was used as a standard and was prepared in 0.1 M sodium acetate at pH 7 on weekly basis by dissolving 10 mg in 100 ml sodium acetate and was stored in the refrigerator at 4^{o}C. A 30 N of H_2SO_4 stock solution was prepared by adding 833.30 ml of H_2SO_4 (96%) concentration slowly into a bottle with 166.70 ml of double distilled water (DDH_2O) making a volume of 1000 ml while placed on ice and stirred continuously with a magnetic stirrer. The mixture was allowed to cool at room temperature before use. A 7 N of H_2SO_4 stock solution was made by mixing 35 ml 30 N of H_2SO_4 and 115 ml of DDH_2O to prepare 150 ml 7 N of H_2SO_4 while the bottle was placed on ice and stirred continuously with the magnetic stirrer and it was cooled at room temperature. Thereafter, four reagents (A-D) were prepared. Reagent A consisted of 0.1 M glyoxilic acid and this was prepared by adding 0.9205 g glyoxilic acid to a 100 ml flask. Then 50 ml of 7 N H_2SO_4 was added and shaken slowly until all the glyoxilic acid was completely dissolved and the 100 ml flask was filled with 7 N H_2SO_4; reagent B, 1.8 mM ferric chloride, was prepared by dissolving 0.050 g of ferric chloride in 100 ml of reagent A, and this was prepared on daily basis; reagent C was 30 N of H_2SO_4 and reagent D (colorimetric reagent) was prepared by mixing 20 ml of ferric chloride and 20 ml of 30 N H_2SO_4. The reagent was prepared on daily basis, an hour before use and had to be protected from direct light and oxygen.

For the analysis 0.08 g defatted samples were placed in a 15 ml Falcon tube and was digested using 3 ml papain and sodium acetate solution. Blank tubes that contained papain and sodium acetate solution were used as a control in the analysis and were used to zero the spectrophotometer. The mixture was vortexed for 3 - 5 sec, thereafter incubated in the waterbath at 64^{o}C for 16 hours. After the 16 hours incubation, samples were cooled to room temperature and were vortexed before being centrifuged at 3600 g for 10 min to obtain a clear supernatant. From each sample, 1 ml of the supernatant was carefully transferred into glass tubes and 3 ml of the colorimetric reagent was added to the test tubes. The samples were thoroughly vortexed for 3 - 5 sec and then placed in a waterbath at 64^{o}C for 30 min for colour development. Samples were allowed to cool to room temperature and optical density reading was done at 560 nm using a spectrophotometer.

For the standard curve dilution, known concentrations of tryptophan of 0, 10, 15, 20, 25 and 30 μg ml^{-1} were prepared from the tryptophan stock solution in 0.1 M sodium acetate (pH 7). From each concentration, 1 ml was transferred into a 15 ml glass tube and colorimetric reagent was added (3 ml) into each test tube and vortexed for 3 - 5 sec before incubating in the

waterbath at 64^{o}C for 30 min for colour development. Thereafter, samples were taken out of the waterbath and allowed to cool at room temperature and optical density readings were done on the spectrophotometer. The readings obtained were used in drawing a calibration curve with the slope having a unit of OD_{560nm} x ml μg^{-1}. Tryptophan percentage was calculated from the following formula, according to protocol of Nurit *et al*., (2009):

$$\%Try = \frac{OD_{560nm}}{slope} \times \frac{hydrolysis\ volume}{sample\ weight} \times 100$$

3.3.5 Oil content analysis

The oil content was analysed using a Near Infrared Reflectance (NIR), Infratec, 1241 Analyzer, V5.11SPI, 2010 FOSS at CIMMYT in Zimbabwe. Seed samples (200 g) from each genotype were scanned five times at spectra between 900 - 2000 nm to determine oil content in the grain (Twumasi-Afriyie *et al*., 2016).

3.3.6 Grain yield

Dry maize cobs were de-husked and shelled. Grain weight per plot was adjusted to 12.50% grain moisture content were converted to ton ha^{-1}.

3.3.7 Data analysis

The collected data were analysed using AGROBASE Gen II software (Agronomix 2016). Separate ANOVA was done for the four locations. Combined ANOVA was conducted for the three optimum environments. Variance contribution percentages were calculated using sum of squares for each main effect as a percentage of the total sum of squares.

3.4 Results

3.4.1 Analysis of variance for tryptophan (%) and oil content (%) and grain yield (ton ha^{-1})

3.4.1.1 Tryptophan content

There were significant differences between genotypes for tryptophan content at all locations. Genotype effect was highly significant ($P \leq 0.001$) for all four locations (Table 3.2). Genotype contributed 98.06%, 99.56%, 99.72% and 99.57% to total variation for tryptophan content at Harare, Glandel, Bindura and Gwebi, respectively.

The percentage of tryptophan in the maize grain ranged from 0.043 - 0.109% with an average of 0.079% for QPM hybrids and a range of 0.054 - 0.066% for non-QPM hybrids with an average of 0.060% at Harare, the low N site (Table 3.3). For Glandel, the tryptophan content in QPM hybrids ranged from 0.079 - 0.117% with an average value of 0.102%, while the range was from 0.042 - 0.044% with an average of 0.043% for the non-QPM hybrids. The tryptophan content at Bindura ranged from 0.058 - 0.107% with an average of 0.088% and 0.039 - 0.055% with an average of 0.047% in QPM hybrids and non-QPM hybrids, respectively. At Gwebi, the tryptophan content in QPM hybrids ranged from 0.071 - 0.153% with an average of 0.103% and for non-QPM it ranged from 0.063 - 0.064% with an average of 0.064%. The lowest tryptophan content was observed at Bindura (0.039%), while the highest tryptophan content was observed at Gwebi (0.153%), both in an optimum N environment. Tryptophan content in QPM hybrids was almost double that of the non-QPM hybrids in all the trials. QPM hybrids at Harare had the lowest average tryptophan content (0.079%) and Gwebi the highest (0.103%), while Bindura and Glandel had averages of 0.088% and 0.102%, respectively. Hybrids chande in rank order due to GEI. Overall performance show that QPM hybrids had higher tryptophan content under low and optimum N trials compared to non-QPM hybrids. Tryptophan content in the hybrids in four locations is shown in Figure 3.1.

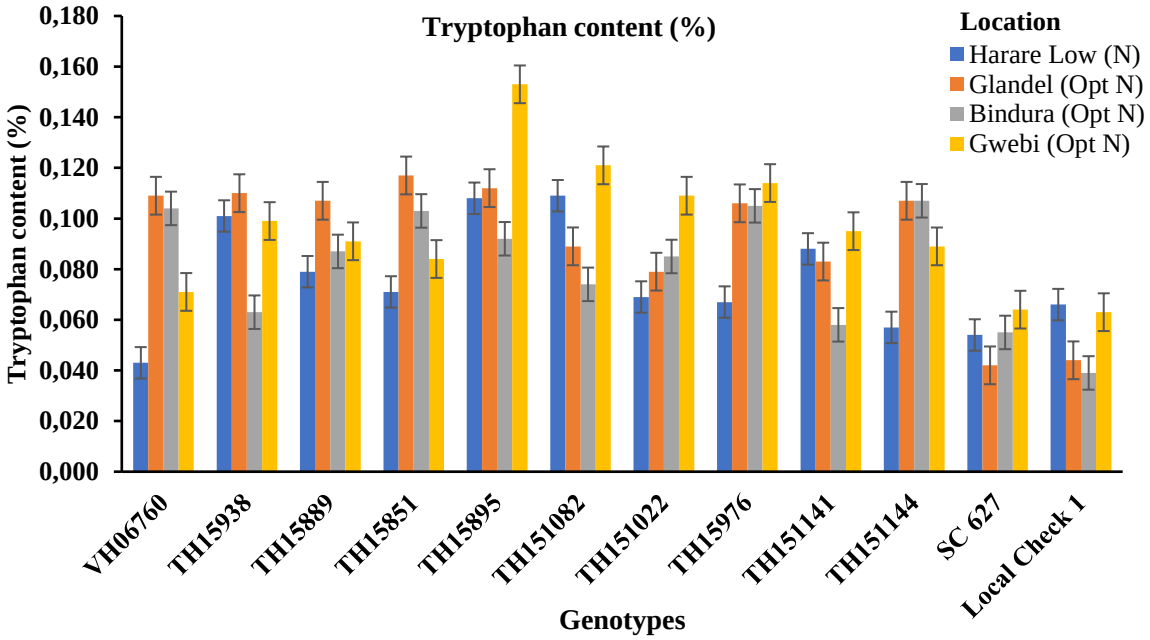

Figure 3.1 Tryptophan content (%) in maize hybrids at four locations

Table 3.2 Analysis of variance for tryptophan content (%) in maize hybrids at four locations

		Harare (Low N)		Glandel (Opt N)		Bindura (Opt N)		Gwebi (Opt N)	
Source	**DF**	**MS**	**% Variation**	**MS**	**% Variation**	**MS**	**% Variation**	**MS**	**% Variation**
Genotype	11	9.19***	98.06	13.41***	99.56	10.60***	99.72	13.40***	99.56
Block	1	0.09	0.09	0.00	0.00	0.01	0.01	0.06	0.04
Residual	11	0.17	1.86	0.06	0.04	0.03	0.02	0.05	0.04
CV (%)		5.49		2.66		1.99		2.42	

***P≤0.001; DF = Degrees of freedom; MS = Mean squares; Low N = Low nitrogen; Opt N = Optimum nitrogen; CV = Coefficient of variation

Table 3.3 Mean values and rankings of tryptophan content (%) in maize hybrids at four locations

Genotype	**Harare (Low N)**		**Glandel (Opt N)**		**Bindura (Opt N)**		**Gwebi (Opt N)**	
	Mean	**Rank**	**Mean**	**Rank**	**Mean**	**Rank**	**Mean**	**Rank**
VH06760	0.043	12	0.109	4	0.104	3	0.071	10
TH15938	0.101	3	0.110	3	0.063	9	0.099	5
TH15889	0.079	5	0.107	5	0.087	6	0.091	7
TH15851	0.071	6	0.117	1	0.103	4	0.084	9
TH15895	0.108	2	0.112	2	0.092	5	0.153	1
TH151082	0.109	1	0.089	8	0.074	8	0.121	2
TH151022	0.069	7	0.079	10	0.085	7	0.109	4
TH15976	0.067	8	0.106	7	0.105	2	0.114	3
TH151141	0.088	4	0.083	9	0.058	10	0.095	6
TH151144	0.057	10	0.107	6	0.107	1	0.089	8
QPM hybrid mean	**0.079**		**0.102**		**0.088**		**0.103**	
SC 627	0.054	11	0.042	12	0.055	11	0.064	11
Local Check 1	0.066	9	0.044	11	0.039	12	0.063	12
Non-QPM hybrid mean	**0.060**		**0.043**		**0.047**		**0.064**	
Grand mean	**0.076**		**0.092**		**0.081**		**0.096**	
LSD $_{(0.05)}$	**0.007**		**0.004**		**0.003**		**0.005**	

LSD = Least significant difference; Low N = Low nitrogen; Opt N = Optimum nitrogen

3.4.1.2 Oil content

The effect of genotype on oil content was significant (P≤0.001) for Glandel and Gwebi, and (P≤0.01) for Bindura. Percentage contribution of genotype to total variation for oil content ranged from 77.59 - 91.61% for the four sites (Table 3.4). Oil content in QPM hybrids ranged from 5.15 - 6.75% with an average of 5.92% whereas the values for non-QPM hybrids ranged from 4.90 - 5.15% with an average of 5.03% at the low N site (Harare) (Table 3.5). Under optimum N environments at Glandel, Bindura and Gwebi, the oil content ranged from 5.28 - 7.40%, 5.40 - 6.45% and 4.95 - 6.40% in QPM hybrids and 4.15 - 5.50%, 5.15 - 5.60% and 4.20 - 5.35% in non-QPM hybrids, respectively. Hybrids TH15938 (6.75%) and SC 627 (4.50%) had the highest and lowest oil content at Harare, respectively, while TH151144 had the highest oil content at Glandel and Gwebi (7.40% and 6.40%), respectively and the lowest was Local check 1 at both locations. At Bindura, the highest and the lowest oil content was from TH151082 (6.45%) and Local check 1 (5.15%), respectively. In general, oil content in the QPM hybrids was higher than one or both checks at all environments (Figure 3.2)

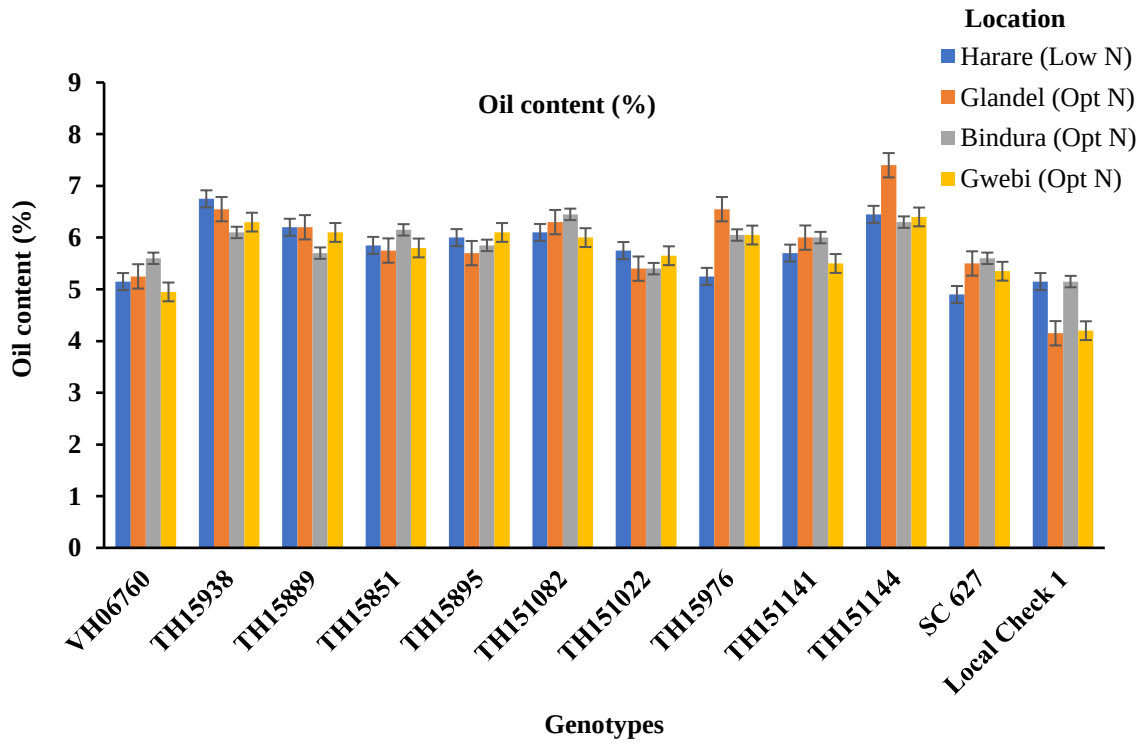

Figure 3.2 Oil content (%) in maize hybrids at four locations

Table 3.4 Analysis of variance for oil content (%) in maize hybrids at four locations

	DF	Harare (Low N)		Glandel (Opt N)		Bindura (Opt N)		Gwebi (Opt N)	
Source		**MS**	**% Variation**	**MS**	**% Variation**	**MS**	**% Variation**	**MS**	**% Variation**
Genotype	11	0.64	77.59	1.31***	91.02	0.29**	83.92	0.79***	91.61
Block	1	0.04	0.04	0.09	0.59	0.05	1.30	0.003	0.03
Residual	11	0.19	22.38	0.12	8.38	0.05	14.91	0.07	8.39
CV (%)		7.46		5.89		3.90		4.73	

***P≤0.001; **P≤0.01; DF = Degrees of freedom; MS = Mean squares; Low N = Low nitrogen; Opt N = Optimum nitrogen; CV = Coefficient of variation

Table 3.5 Mean values and rankings of oil content (%) in maize hybrids at four locations

Genotype	Harare (Low N)		Glandel (Opt N)		Bindura (Opt N)		Gwebi (Opt N)	
	Mean	**Rank**	**Mean**	**Rank**	**Mean**	**Rank**	**Mean**	**Rank**
VH06760	5.15	11	5.25	12	5.60	9	4.95	11
TH15938	6.75	1	6.55	2	6.10	4	6.30	2
TH15889	6.20	3	6.20	5	5.70	8	6.10	3
TH15851	5.85	6	5.75	7	6.15	3	5.80	7
TH15895	6.00	5	5.70	8	5.85	7	6.10	4
TH151082	6.10	4	6.30	4	6.45	1	6.00	6
TH151022	5.75	7	5.40	9	5.40	11	5.65	8
TH15976	5.25	9	6.55	3	6.05	5	6.05	5
TH151141	5.70	8	6.00	6	6.00	6	5.50	9
TH151144	6.45	2	7.40	1	6.30	2	6.40	1
QPM hybrid mean	**5.92**		**6.11**		**5.96**		**5.89**	
SC 627	4.90	12	5.50	10	5.60	10	5.35	10
Local Check 1	5.15	10	4.15	12	5.15	12	4.20	12
Non-QPM hybrid mean	**5.03**		**4.83**		**5.34**		**4.80**	
Grand mean	**5.76**		**5.90**		**5.86**		**5.70**	
LSD $_{(0.05)}$	**0.77**		**0.62**		**0.41**		**0.48**	

LSD = Least significant difference; Low N = Low nitrogen; Opt N = Optimum nitrogen

3.4.1.3 Grain yield

Genotype effect for grain yield differ significantly at Glandel (P≤0.001) and Bindura (P≤0.01, and Harare (P≤0.05) (Table 3.6). Grain yield mean values for QPM hybrids ranged from 2.70 - 4.61 ton ha^{-1} with an average of 3.61 ton ha^{-1} while non-QPM hybrids ranged from 2.93 - 3.59 ton ha^{-1} with an average of 3.26 ton ha^{-1} at the low N location, Harare. The mean values for Glandel, Bindura and Gwebi ranged from 2.71 - 10.10 ton ha^{-1}, 2.29 - 5.32 ton ha^{-1} and 5.63 - 7.70 ton ha^{-1}, with averages of 7.16, 4.04 and 6.71 ton ha^{-1}, respectively for QPM hybrids. For the non-QPM hybrids, the values ranged from 2.70 - 7.40, 4.27- 6.39 and 5.33 - 5.54 ton ha^{-1} with averages of 5.05, 5.33 and 5.44 ton ha^{-1}, respectively (Table 3.7). Bindura had the lowest grain yield of 2.29 ton ha^{-1} (TH15851) and Gwebi had the highest grain yield of 7.70 ton ha^{-1} (TH151144). Generally, QPM hybrids yielded more than non-QPM hybrids in all locations, except at Bindura where Local check 1 was the highest grain yielder. This may be due to the effective QPM breeding programmes and probably the checks used were not good yielder and not good representative of the commercial hybrids. Overall performance shows that grain yield was lower under low N conditions for most genotypes. However, TH15851 did better at Harare (low N) than under optimum N at Bindura, TH15976 also did better at Harare than Glandel and Bindura (Figure 3.3).

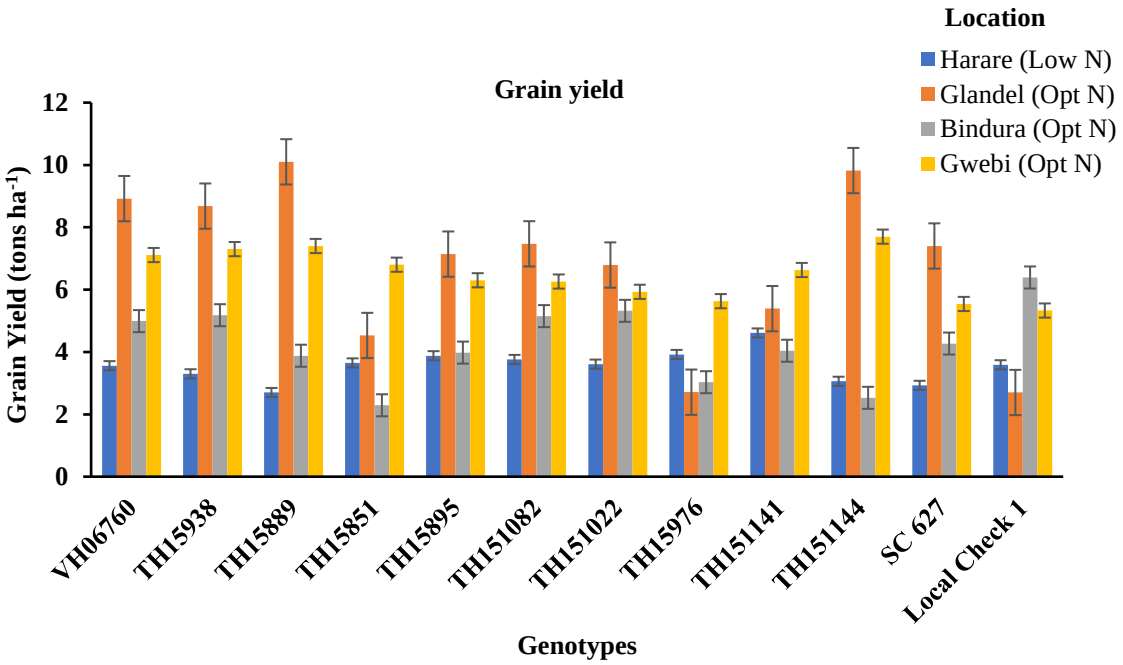

Figure 3.3 Grain yield (ton ha^{-1}) for maize hybrids at four locations

Table 3.6 Analysis of variance for grain yield (ton ha^{-1}) in maize hybrids at four locations

		Harare (Low N)		Glandel (Opt N)		Bindura (Opt N)		Gwebi (Opt N)	
Source	**DF**	**MS**	**% Variation**	**MS**	**% Variation**	**MS**	**% Variation**	**MS**	**% Variation**
Genotype	11	0.51*	63.43	12.68***	88.75	3.00**	86.52	1.21	62.39
Block	1	1.74	19.51	9.67	6.14	0.06	0.157	1.59	7.45
Residual	11	0.14	17.06	0.73	5.09	0.46	13.33	0.59	30.15
CV (%)		10.49		12.54		15.99		11.80	

***P≤0.001; **P≤0.01; *P≤0.05; DF = Degrees of freedom; MS = Mean squares; Low N = Low nitrogen; Opt N = Optimum nitrogen; CV = Coefficient of variation

Table 3.7 Mean values and rankings for grain yield (ton ha^{-1}) in maize hybrids at four locations

	Harare (Low N)		Glandel (Opt N)		Bindura (Opt N)		Gwebi (Opt N)	
Genotype	**Mean**	**Rank**	**Mean**	**Rank**	**Mean**	**Rank**	**Mean**	**Rank**
VH06760	3.56	8	8.92	3	4.99	5	7.11	4
TH15938	3.30	9	8.68	4	5.18	3	7.30	3
TH15889	2.70	12	10.10	1	3.88	9	7.40	2
TH15851	3.65	5	4.53	10	2.29	12	6.80	5
TH15895	3.88	3	7.14	7	3.98	8	6.30	7
TH151082	3.76	4	7.47	5	5.15	4	6.26	8
TH151022	3.61	6	6.79	8	5.32	2	5.93	9
TH15976	3.92	2	2.71	11	3.03	10	5.63	10
TH151141	4.61	1	5.39	9	4.04	7	6.63	6
TH151144	3.06	10	9.82	2	2.53	11	7.70	1
QPM hybrid mean	**3.61**		**7.16**		**4.04**		**6.71**	
SC 627	2.93	11	7.40	6	4.27	6	5.54	11
Local Check 1	3.59	7	2.70	12	6.39	1	5.33	12
Non-QPM hybrid mean	**3.26**		**5.05**		**5.33**		**5.44**	
Grand mean	**3.55**		**6.81**		**4.25**		**6.48**	
LSD $_{(0.05)}$	**0.67**		**1.53**		**1.22**		**1.37**	

LSD = Least significant difference; Low N = Low nitrogen; Opt N = Optimum nitrogen

3.4.2 Combined analysis of variance for tryptophan (%) and oil content (%) and grain yield (ton ha^{-1}) in maize hybrids across optimum N environments

The effects of genotype, environment and, genotype and environment interaction (GEI) were highly significant for all measured characteristics (Table 3.8). Genotype had the largest effect on tryptophan and oil content, whereas the GEI effects played a major role in grain yield. The genotype contribution to total variation was 63.63%, 73.64% and 24.33% for tryptophan content, oil content and grain yield, respectively. The environment contribution was 6.44%, 1.87% and 30.03% for tryptophan content, oil content and grain yield, respectively. GEI contributed 29.56%, 14.93% and 35.68% to variation for tryptophan content, oil content and grain yield, respectively.

Tryptophan content under optimal N environments ranged from 0.049 - 0.119%, with high values observed in QPM hybrids (Table 3.9). For oil content, values ranged from 4.50 - 6.70% and Local check 1 (4.50%) had the lowest oil content and QPM TH151144 (6.70%) had the highest oil content. The averages for grain yield ranged from 3.79 - 7.09 ton ha^{-1} in all tested hybrids. Non-QPM hybrids mean values were lower than that of QPM hybrids, although not significantly different. However, both non-QPM hybrids had higher grain yield than lowest yielding QPM TH15851 and QPM TH15976. QPM TH15889 had the highest grain yield across optimal N sites (Figure 3.4).

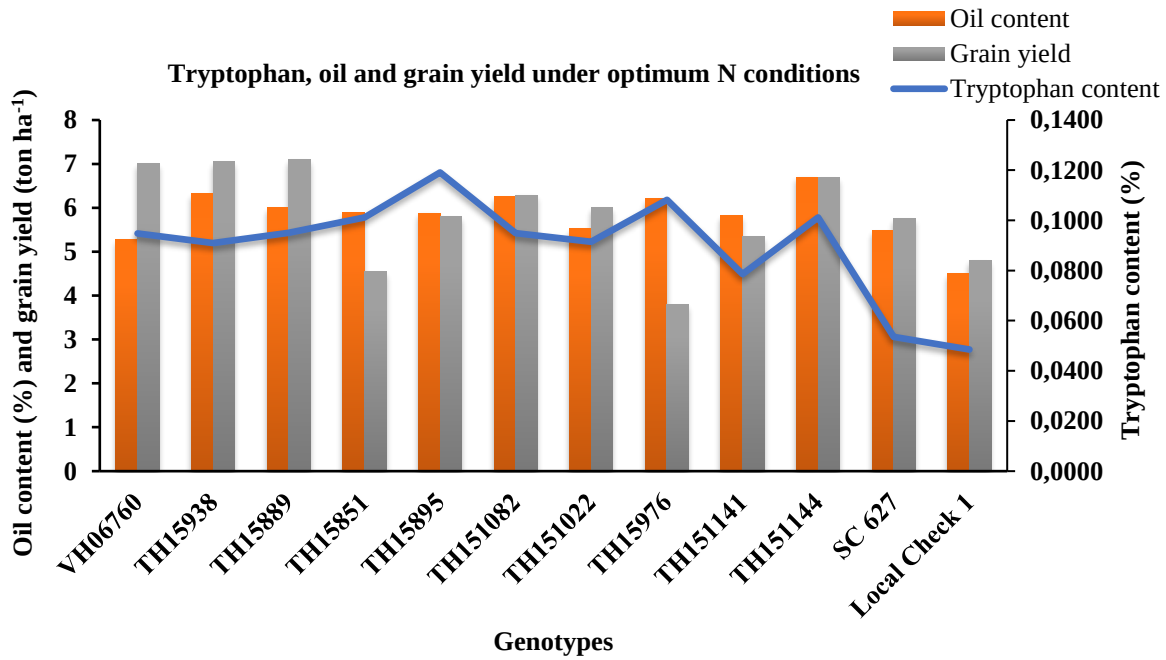

Figure 3.4 Performance of maize hybrids for tryptophan (%) and oil content (%) and grain yield (ton ha^{-1}) across optimum N environments

Table 3.8 Combined analysis of variance for tryptophan (%) and oil content (%) and grain yield (ton ha^{-1}) in maize hybrids across optimum N environments

		Tryptophan content (%)		Oil content (%)		Grain yield (ton ha^{-1})	
Source	**DF**	**MS**	**% Variation**	**MS**	**% Variation**	**MS**	**% Variation**
Genotype	11	25.55***	63.63	1.99***	73.64	6.85***	24.33
Env	2	14.22***	6.44	0.28*	1.87	46.49***	30.03
Gen * Env	22	5.94***	29.56	0.20**	14.93	5.02***	35.68
Residual	33	0.05	0.35	0.08	0.27	0.59	6.31
CV (%)		2.41		4.92		13.16	

***P≤0.001; **P≤0.01; *P≤0.05; DF = Degrees of freedom; MS = Mean squares; Env = Environment; Gen = Genotype; CV = Coefficient of variation

Table 3.9 Mean values and rankings for tryptophan (%) and oil content (%) and grain yield (ton ha^{-1}) across optimum N environments

Genotype	**Type**	**Tryptophan content (%)**		**Oil content (%)**		**Grain yield (ton ha^{-1})**	
		Mean	**Rank**	**Mean**	**Rank**	**Mean**	**Rank**
VH06760	QPM	0.094	7	5.27	11	7.01	3
TH15938	QPM	0.091	9	6.32	3	7.05	2
TH15889	QPM	0.095	5	6.00	6	7.09	1
TH15851	QPM	0.101	4	5.90	6	4.54	11
TH15895	QPM	0.119	1	5.88	7	5.81	7
TH151082	QPM	0.095	6	6.25	2	6.29	5
TH151022	QPM	0.091	8	5.52	9	6.01	6
TH15976	QPM	0.108	2	6.22	4	3.79	12
TH151141	QPM	0.079	10	5.83	8	5.35	9
TH151144	QPM	0.101	3	6.70	1	6.68	4
QPM hybrid mean		**0.097**		**6.01**		**5.96**	
SC 627	Non-QPM	0.054	11	5.48	10	5.75	8
Local Check 1	Non-QPM	0.049	12	4.50	12	4.80	10
Non-QPM hybrid mean		**0.052**		**4.99**		**5.28**	
Grand mean		**0.089**		**5.82**		**5.85**	
LSD $_{(0.05)}$		**0.002**		**0.28**		**0.75**	

LSD = Least significant difference; ton ha^{-1} = Tons per hectare

3.4.3 Comparison of maize hybrids for tryptophan and oil content (%) and grain yield (ton ha^{-1}) under low and optimum N conditions

Performance of hybrids were generally reduced under low N conditions in all traits. However, some individual hybrids had higher means under low N such as TH15938, TH151141, TH151082 and Local check 1 for tryptophan. For oil content, TH15938, TH15889, TH15895, TH151022 and Local check 1 had higher means under low N than under optimal N conditions. QPM TH15976 had higher grain yield under low N than across optimum N conditions. Mean values for tryptophan content in QPM and non-QPM hybrids ranged from 0.043 - 0.109% and 0.054 - 0.066%, respectively under low N environment, whereas across optimum N locations, the range was 0.079 - 0.119% and 0.049 - 0.054% in QPM and non-QPM hybrids, respectively. Oil content under low N conditions varied from 5.15 - 6.75% and 4.90 - 5.15% in QPM and non-QPM hybrids, respectively, while across optimum N conditions, the range was from 5.27 - 6.70% and 4.50 - 5.48% in QPM and non-QPM hybrids, respectively. As for grain yield in QPM and non-QPM hybrids under low N conditions, the values ranged from 2.70 - 4.61 ton ha^{-1} and 2.93 - 3.59 ton ha^{-1}, respectively. Across optimum environments, grain yield ranged from 3.79 - 7.09 ton ha^{-1} and 4.80 - 5.75 ton ha^{-1} in QPM and non-QPM hybrids, respectively.

Table 3.10 Mean values for tryptophan (%) and oil content (%) and grain yield (ton ha^{-1}) in maize hybrids under low and optimum N environments

Genotype	Type	Tryptophan Content (%)		Oil content (%)		Grain yield (ton ha^{-1})	
		Low N	Opt N	Low N	Opt N	Low N	Opt N
VH06760	QPM	0.043	0.094	5.15	5.27	3.56	7.01
TH15938	QPM	0.101	0.091	6.75	6.32	3.30	7.05
TH15889	QPM	0.079	0.095	6.20	6.00	2.70	7.09
TH15851	QPM	0.071	0.101	5.85	5.90	3.65	4.54
TH15895	QPM	0.108	0.119	6.00	5.88	3.88	5.81
TH151082	QPM	0.109	0.095	6.10	6.26	3.76	6.29
TH151022	QPM	0.069	0.091	5.75	5.52	3.61	6.01
TH15976	QPM	0.067	0.108	5.25	6.22	3.92	3.79
TH151141	QPM	0.088	0.079	5.70	6.00	4.61	5.35
TH151144	QPM	0.057	0.101	6.45	6.70	3.06	6.68
QPM hybrid mean		**0.079**	**0.097**	**5.92**	**6.01**	**3.61**	**5.96**
SC 627	Non-QPM	0.054	0.054	4.90	5.48	2.93	5.75
Local Check 1	Non-QPM	0.066	0.049	5.15	4.50	3.59	4.80
Non-QPM hybrid mean		**0.060**	**0.052**	**5.03**	**4.99**	**3.26**	**5.28**
Grand mean		**0.076**	**0.089**	**5.76**	**5.82**	**3.55**	**5.85**
LSD $_{(0.05)}$		**0.007**	**0.002**	**0.77**	**0.28**	**0.67**	**0.75**

LSD = Least significant difference; Low N = Low nitrogen; Opt N = Optimum nitrogen

3.5 Discussion

Nutritional quality characteristics of maize such as oil, total protein, lysine and tryptophan content are of great importance for people in developing countries where maize is the staple food, with the aim of addressing micronutrient deficiency and also for the maize industry as a whole. Grain yield has been a major focus in the development of QPM genotypes, to ensure grain yield equal to or even better than normal maize varieties (Tandzi *et al*., 2017), in addition to other challenges observed in breeding for QPM. Grain nutritional quality and grain yield are affected by different factors such as genetic makeup and environmental factors. Some grain quality characteristics are negatively correlated with grain yield (Zaidi *et al*., 2008). Therefore, monitoring the nutritional quality and grain yield in QPM genotypes in different environments is very important to meet the intended purpose and expectation by the users for animal feed and human consumption.

This study showed highly significant differences between the hybrids for all measured characteristics at all four locations, but no significant differences were seen between hybrids for oil content under low N conditions, which indicates that the response of tested hybrids in this environment was similar. Genotype made a large contribution to variation in oil and tryptophan content and less so to grain yield under optimum N conditions. This indicated the higher heritability of oil and tryptophan content compared to that of grain yield.

Ngaboyisonga *et al.* (2012) evaluated 41 maize single cross hybrids and one local check for tryptophan content under optimum and low N, and drought conditions in different locations. They found higher average tryptophan content with the mean being higher under low N (0.113%) compared to optimum N conditions (0.092%). The current study disagrees with these findings as the mean for tryptophan under optimum N conditions was higher than under low N conditions (0.089% and 0.076%), respectively. This could be due to the specific hybrids used, or the effect of environmental conditions. Worku *et al*. (2007) reported significant genotypic differences in a study involving QPM and non-QPM genotypes for tryptophan content in different environments. Higher tryptophan values were recorded under optimal N than low N conditions for both QPM and non-QPM cultivars. Significant differences were observed between environments for tryptophan trait in present study which agrees with the findings of Worku *et al.* (2007) and Ngaboyisonga *et al*. (2012).

Sentayehu (2008) and Wegary *et al.* (2011) reported highly significant genotype, environment and GEI effects for tryptophan content. High GEI indicates that genotype performance changes from one environment to another as they are not stable. They reported that QPM genotypes maintained their nutritional quality under low N conditions that commonly occur in Africa. Generally, tryptophan content was reduced under low N conditions. The results in our study were in line with the findings of Wegary *et al*. (2011) because QPM hybrids such as TH15938 (0.101%), TH151141 (0.088%) and TH151082 (0.109%) maintained tryptophan levels under low N conditions. However, higher averages were observed in QPM hybrids under optimum N conditions than in non-QPM hybrids under low N conditions.

Ignjatović-Micić *et al*. (2014) reported tryptophan average above 0.075% in their study for maize landraces and introduced populations. Tryptophan content ranged between 0.072 - 0.086% in landraces and 0.071 - 0.088% in introduced populations. Ignjatović-Micić *et al*. (2009) observed tryptophan content in QPM cultivars ranging between 0.101 - 0.136% and in non-QPM the range was between 0.071 - 0.076%. Studies conducted by various researchers observed higher tryptophan levels in QPM genotypes under optimum and low N conditions than in common maize varieties (Gissa 2008; Wegary *et al*., 2011; Masindeni 2013). These studies agree with present findings as high average tryptophan content was seen in QPM hybrids under low and optimum N conditions compared to non-QPM hybrids in both soil environments. This was expected, as common maize contains a high α zein content that is devoid of tryptophan and lysine, compared to QPM varieties, hence a higher level of these amino acids. According to Ngaboyisonga *et al*. (2012), low N conditions in conjunction with drought stress affected grain development, despite an increase in tryptophan level in the grain. Worku *et al*. (2007) suggested that N availability in the soil has an effect on tryptophan content and grain protein content, where an increase in N application increased tryptophan level and low N reduced tryptophan and protein content in grain endosperm. High tryptophan content was reported in nutritionally enhanced cultivars (QPM) compared to non-QPM varieties in a study by Pachón *et al*. (2009), and this is in line with present study findings. Duarte *et al*. (2004) found higher tryptophan content in QPM kernels than normal maize, and this presence of high tryptophan levels makes QPM superior to non-QPM and improves its nutritional content. Typtophan content was high in QPM hybrid TH15895 in both soil conditions in the current study.

Genetic variation was observed in the current study for oil content, which can be explored to develop cultivars with enhanced oil content. Oil content was not significantly different under low N conditions, which suggests that genotypes reacted in a similar way in this environment. Significant differences were observed between hybrids under optimum N conditions, indicating different response of genotypes to the environment. Duarte *et al*. (2004) found oil content in QPM hybrids and normal maize ranging from 2.88 - 4.33% which was statistically not different. Duarte *et al*. (2005) found oil content in Brazilian maize genotypes varying between 4.90 - 5.15% and 4.50 - 5.40% in low and optimum N environments, respectively. Nuss and Tanumihardjo (2010) found that QPM genotypes contained oil in kernels that ranged from 3.50 - 6.00% with an average of 4.50% and the current results for oil content in QPM hybrids fall in the same range. Mittelmann *et al*. (2003) observed high oil content in QPM compared to common maize in their study. The present study agrees with this as the oil content mean for QPM hybrids was relatively higher than non-QPM hybrids, however, the difference was not significant.

Masindeni (2013) reported the highest oil content in QPM inbred lines of 6.23% under optimum N and 6% for low N conditions, however, the difference was insignificant. In the present study, highest oil content was seen in QPM hybrids under low and optimum N trials. Ignjatović-Micić *et al*. (2015) reported oil content above 4.60% in landraces and introduced populations, which were similar to the current research findings obtained in QPM and non-QPM hybrids. Vasquez-Carrillo *et al*. (2011) found mean oil content in landraces of 4.90%, in white hybrids 3.70% and yellow hybrids 3.20%, proving the presence of variation for oil content in maize genotypes. Significant GEI was observed in the present study, and similar findings were reported by Lambert *et al*. (1998), Vasquez-Carrillo *et al*. (2011), Masindeni (2013) and Ignjatović-Micić *et al*. (2015).

Research showed that maize mutants such as *o*2 have higher germ size than common maize, that results in higher oil content (Mittelmann *et al*., 2003; Singh *et al*., 2014). Maize kernels contain lower amount of fat, at about 5 g 100 g^{-1} in relation to soybean and sunflowers seeds that have 20 g 100 g^{-1} and 51 g 100 g^{-1}, respectively (Nuss and Tanumihardjo, 2010). However, maize oil is high in unsaturated fats that are healthy for human consumption compared to oils from other sources (Nuss and Tanumihardjo, 2010; Ignjatović-Micić *et al*., 2015). On average, maize germ oil contains palmitic acid (11%), linoleic acid (60%) and oleic acid (24%) which makes it highly competitive on the market (Nuss and Tanumihardjo, 2010; Singh *et al*., 2014). Khan *et al*. (2016)

reported significant differences in maize genotypes in their study with higher oil content found in hybrids and high N conditions than local varieties. This is similar to the current study results, showing higher oil content in QPM hybrids than in checks. In this study, the highest oil content was seen in the low N environment for QPM TH15938 (6.75%) and TH151144 (6.70%) under optimum N conditions, and this suggests that oil content trait is more genetically influenced than environment such as N availability.

Significant differences were observed for grain yield between the hybrids under low and optimum N conditions. Generally, grain yield was lower in QPM and non-QPM hybrids under low N compared to optimum N conditions, except TH15976 that performed better in Harare (low N) than Glandel and Bindura which are optimum N sites. Marković *et al*. (2017) reported similar findings of lower grain yield under low N compared to optimum N conditions for the two years of the study. They found an average grain yield of 5.70 ton ha^{-1} and 6.26 ton ha^{-1} under low N in two seasons, respectively, whereas 11.70 ton ha^{-1} and 11.78 ton ha^{-1} were realized under optimum N conditions in the same two seasons, respectively. Similar trends of low grain yield under low N and higher yield under optimal conditions were observed in the current study.

Duarte *et al*. (2004) obtained high grain yield of 8.53 ton ha^{-1} in normal single cross hybrids which was higher compared to 7.23 ton ha^{-1} in QPM single crosses, indicating lower yield for QPM hybrids. This was not the case in the current study, as there were no significant yield differences between QPM and non-QPM hybrid averages in all soil conditions. Langa (2005) reported high variability in maize grain yield between genotypes and large GEI effects. These findings agree with the current research findings for variations were observed between tested hybrids for grain yield and varying performance of the hybrids across optimum N environments were reported. Khan and Shah (2010) and Tadesse and Kim (2015) noted variations in grain yield with the lowest average mean value seen in the low N compared to optimum conditions. Khan *et al*. (2016) observed an increase in kernel grain weight with an increase in N fertilizer, leading to higher grain yield and also protein content in optimum N environment. According to Worku *et al*. (2007), high grain yield in optimum N environments is expected, as starch and protein are accumulated in endosperm as carbon and N, respectively. These results are in line with the current study that observed lowest average values under low N conditions compared to optimum N environments. Slightly higher average grain yield was obtained for QPM hybrids compared to non-QPM hybrids

in this study, and this is also in agreement with the results reported in other studies (Vasal, 2000; Olakojo *et al*., 2007; Worku *et al*., 2007).

Badu-Apraku *et al*. (2015) reported high genotype and environmental effects on grain yield under low N, drought stress and Striga infestation conditions in early maturing yellow QPM inbreds, and normal maize. These results are consistent with the current study where significant genotype variations were observed for grain yield under all conditions. This indicates that it is possible to select for higher grain yield in QPM hybrids under both low N and optimal conditions. As in the current study, Haruna *et al*. (2017) noted significant GEI for grain yield in a study involving intermediate maturity top cross maize hybrids with the yield ranging from 3.30 - 4.70 ton ha^{-1}.

Setimela *et al*. (2017) evaluated 22 QPM hybrids under on-farm and on-station conditions with non-QPM checks for grain yield, tryptophan, lysine and other characteristics. They found significant genotype and GEI effects for grain yield, tryptophan, lysine and other characteristics in tested cultivars. QPM hybrids had higher values for grain yield, tryptophan and lysine than non-QPM checks. These findings are consistent with current findings that QPM hybrids yielded more and had higher tryptophan content than non-QPM hybrids. The present study showed that QPM TH151141 and TH15889 had high grain yield under low and optimum N conditions, respectively.

3.6 Conclusions

In summary, this study showed that environmental conditions had a significant effect on tryptophan content, oil content and grain yield. Generally, genotypes under low N conditions gave relatively lower mean values compared to optimum N conditions for all the traits. However, grain yield was the most sensitive to low N conditions, with only hybrid TH15976 giving high grain yield under low N than in optimal environments. For tryptophan and oil content, some genotypes were less sensitive to low N such as TH15938, TH151141, TH151082 and Local check 1, and TH15938, TH151022, TH15889, TH15895 and Local check 1, respectively, as they gave high tryptophan and oil content under low N conditions. Highly significant differences were observed between hybrids for all three measured characteristics, indicating the presence of genetic variation that gives an opportunity for selection and improvement for these characteristics under both optimum and low N conditions.

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CHAPTER FOUR

THE EFFECT OF DIFFERENT ENVIRONMENTS ON IRON, ZINC AND PHYTIC ACID CONTENT IN QUALITY PROTEIN MAIZE HYBRIDS

4.1 Abstract

Bioavailability of minerals such as Fe and Zn in cereal grains is greatly affected by phytic acid content. Poor mineral absorption from plant-based foods causes micronutrient deficiencies, especially in people that depend on maize as staple crop. This study aimed at determining the genetic variability of Fe, Zn and phytic acid content and the molar ratios of phytic acid to minerals in QPM hybrids under different growing conditions. Analysis of variance (ANOVA) showed significant differences between genotypes for all the traits at all locations except for phytic acid at Glandel and Zn molar ratio at Harare. Combined ANOVA showed significant effects of genotype, environment and their interaction for all measured traits. Genetic variations observed in genotypes is important for breeding genotypes with high mineral content and reduced phytic acid content which show higher mineral bioavailability that will lead to reduced micronutrient deficiencies to consumers.

4.2 Introduction

Maize contains different quantities of minerals, with each playing a specific role in plant growth and development (Battal *et al*., 2003). Primary, secondary and trace elements are necessary for the plant to complete its normal life cycle. The trace elements are important, as they activate different plant functions in the metabolic system of the plant necessary for crop development (Bareja, 2012). According to Sajedi *et al*. (2009) the plant requires Zn to synthesise protein, carbohydrate, nucleic acid, lipids and saccharides. Hemalatha *et al*. (2007) suggested that magnesium (Mg) is also vital for photosynthesis and protein metabolic processes in maize plants. On the other hand, Fe is one of the major components of myoglobin and cytochromes within the plant and it is important for energy transfer.

Macronutrients and micronutrients are required in certain amounts to have a significant impact on human nutrition, which is necessary for good health in human beings. Different minerals are necessary for human metabolic processes. For normal body functioning, Fe is necessary for hemoglobin formation that transports oxygen to all body tissues and cells. Fe deficiency leads to

anemia that has a negative effect on mental development, especially in children. It also causes high incidences of disease and death in pregnant and child bearing age women and infants. The deficiency also has an effect on human work performance and increases susceptibility to infections (Fairweather-Tait and Hurrell, 1996). Zn is vital for immune system development and catalyzing different enzymatic functions in the body so that it can function normally (Fageria *et al*., 2012). Roohani *et al*. (2013) reported that Zn deficiency results in growth failure such as development of the skeleton, central nervous system, gastrointestinal, immune and reproductive system. Inadequate mineral intake and overdependence on plant-based food sources that are inefficiently absorbed, are major causes of micronutrient deficiency (Platel and Srinivasan, 2016).

Most people in low-income countries rely on plant-based foods to obtain energy and minerals like Fe, Zn and Ca (Gibson *et al*., 2010). The Fe and Zn deficiency are major cause of illnesses and diseases in children, especially in developing countries, and it affects one third of the global population and is ranked fifth and sixth as the major causes of illness and death (WFP, 2007). As a result, current maize research is often conducted with the objective of developing new cultivars with high yield and enhanced micronutrients necessary for human and monogastric animal requirements, with zero supplementation. This is crucial in combating micronutrient deficiency in people that depend on maize as their major food crop. Raboy (2009) and Gibson *et al*. (2010) reported that most cereal grains contain high concentrations of phytic acid that inhibit mineral absorption.

Bioavailability of minerals in cereal grains and legume seeds is influenced by phytic acid concentration, food processing, and the presence of tannin and fibers (Gibson *et al*., 2010). Minerals such as Fe and Zn can become unavailable in the digestive system as they are chelated by phytic acid, which is an anti-nutritional factor (Raboy, 2009). Research shows that bioavailability in maize greatly improves with small decreases in the amount of phytic acid (Hemalatha *et al*., 2007; Beavers *et al*., 2015). Minerals in cereal grains and pulses are absorbed by humans in different amounts, based on phytic acid content (Platel and Srinivasan, 2016).

As such, it becomes very important to determine mineral content in different genotypes to identify those with high mineral content (Phalafala, 2013). In addition, determining the bioavailability of Fe and Zn in newly developed QPM hybrids is important before variety release in the current

situation where micronutrient deficiency is a global issue, especially to those that depend on maize as staple food. Variation is necessary for selection and development of genotypes with enhanced mineral content. The aim of this study was to determine the effect of different growing environments on Fe, Zn and its bioavailability in QPM hybrids.

4.3 Materials and methods

4.3.1 Planting locations

As described in Chapter 3 (3.3.1)

4.3.2 Planting materials

As described in Chapter 3 (3.3.2)

4.3.3 Experimental design and field management

As described in Chapter 3 (3.3.3)

4.3.4 Mineral extraction

The extraction of Zn and Fe minerals was done according to the dry-ashing method outlined by the AOAC (2000). Maize flour samples weighing 2 g were placed in porcelain crucibles. The samples were ashed in a furnace at 550^{o}C for 3 - 4 hours and then allowed to cool. Nitric acid (HNO_3) (55%) (1 ml) was added to the cooled samples for digestion and then placed in a hot sandbath to evaporate until completely dry. Samples were placed in the furnace for another hour at 550°C for further ashing and it was then allowed to cool, after which 10 ml of 1:2 HNO_3 was added for further digestion of the samples and then desiccated. After desiccation, the samples were transferred into 100 ml volumetric flasks and filled to the mark with distilled water. Fe and Zn content were measured in triplicate using an Atomic Absorption Spectrophotometer (Spectra AA 300).

4.3.5 Phytic acid determination

Phytic acid extraction was done according to Dragićević *et al*. (2011). Finely ground maize samples (0.25 g) were placed in a glass tube. Phytic acid was extracted with 10 ml of 5% Trichloroacetic acid (TCA) solution which was added into the glass tube containing the sample and mechanically shaken for 1 hour, and vortexed at 10 min intervals. After this, 5 ml extract was

transferred into a 15 ml tube and centrifuged at 12000 g for 20 min. Then a dilution gradient of the supernatant was made in order to obtain a standard curve. The supernatant was centrifuged for 10 min at 12000 g, double distilled water (DDH_20) was used as blank. Absorbance readings were measured at 500 nm for samples and Wade reagent, a solution that was made by a mixture of ferric chloride and sulfosalicylic acid.

4.3.6 Phytic acid to mineral molar ratios

The Fe and Zn mineral bioavailability was estimated using the phytic acid/zinc and phytic acid/iron molar ratios, according to the equations of Norhaizan and Norfaizadatul (2009) and Queiroz *et al*. (2011):

MR = [(Phy /MW Phy)/ (Mineral/AW Mineral)], where MR = Molar ratio; Phy = Phytic acid in the sample; MW Phy = Phytic acid molecular weight (660 Da); Mineral = Fe or Zn in the sample; AW Mineral = Fe (56 Da) or Zn (65 Da) atomic weight.

4.3.7 Data analysis

As described in Chapter 3 (3.3.7)

4.4 Results

4.4.1 Analysis of variance for Fe, Zn, phytic acid, and their molar ratios in four environments

4.4.1.1 Iron content

Genotype effects were significant ($P \leq 0.01$) for Fe content at Harare, Glandel, Gwebi and Bindura ($P \leq 0.05$) (Table 4.1). Genotype contributed 65.69%, 39.20%, 70.35% and 83.13% to total variation in Fe content at Harare, Bindura, Glandel and Gwebi, respectively. Genotype performance differed between environments, resulting in changes in genotype rankings in the tested environments. The Fe content ranged from 10.88 - 28.60 mg kg^{-1} at Harare, 23.50 - 43.18 mg kg^{-1} at Bindura, 16.65 - 37.55 mg kg^{-1} at Glandel and 6.33 - 24.20 mg kg^{-1} at Gwebi in QPM hybrids. In non-QPM hybrids, the range varied from 15.55 - 18.88 mg kg^{-1}, 29.70 - 34.05 mg kg^{-1}, 18.68 - 21.28 mg kg^{-1} and 12.48 - 24.05 mg kg^{-1} for Harare, Bindura, Glandel and Gwebi, respectively. QPM hybrid Fe averages were 16.72 mg kg^{-1}, 33.35 mg kg^{-1}, 24.16 mg kg^{-1}, and 16.54 mg kg^{-1} for Harare, Bindura, Glandel and Gwebi, respectively. Non-QPM averages were 17.22 mg kg^{-1}, 31.88 mg kg^{-1}, 19.98 mg kg^{-1} and 18.27 mg kg^{-1} for Harare, Bindura, Glandel and

Gwebi, respectively (Table 4.2 and Figure 4.1). However, all locations showed higher Fe content averages in non-QPM compared to QPM, except at Glandel. The hybrids VH06760, TH15851, TH151141 and TH15889 recorded the highest Fe content at Bindura, Glandel, Gwebi and Harare, respectively.

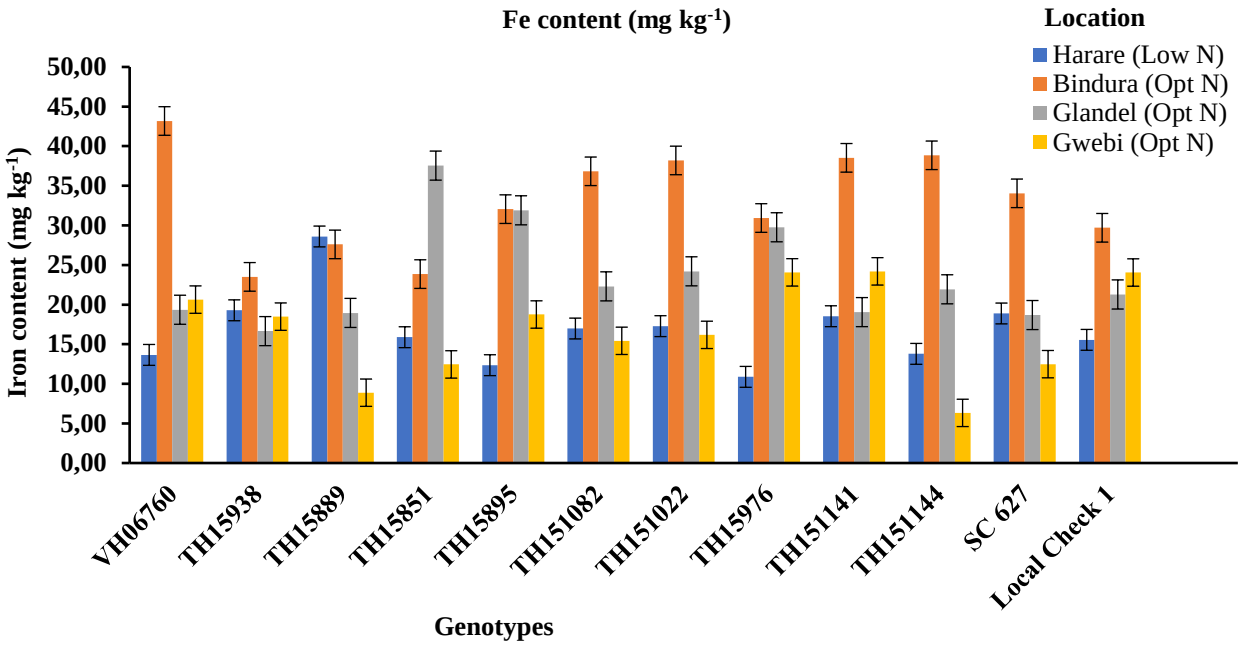

Figure 4.1 Iron content (mg kg^{-1}) in maize hybrids at four locations

Table 4.1 Analysis of variance for Fe content (mg kg^{-1}) in maize hybrids at four locations

		Harare (Low N)		Glandel (Opt N)		Bindura (Opt N)		Gwebi (Opt N)	
Source	**DF**	**MS**	**% Variation**	**MS**	**% Variation**	**MS**	**% Variation**	**MS**	**% Variation**
Genotype	11	41.69**	65.69	80.78**	70.35	74.03*	39.20	71.59**	83.13
Block	1	1.04	0.15	183.98	14.56	433.08	20.80	38.56	4.06
Residual	11	21.69	34.17	17.32	15.08	75.45	39.95	11.02	12.79
CV (%)		27.72		17.74		27.58		19.74	

**P≤0.01; *P≤0.05; DF = Degrees of freedom; MS = Mean squares; Low N = Low nitrogen; Opt N = Optimum nitrogen; CV = Coefficient of variation

Table 4.2 Mean values and rankings for Fe content (mg kg^{-1}) in maize hybrids at four locations

Genotype	**Harare (Low N)**		**Glandel (Opt N)**		**Bindura (Opt N)**		**Gwebi (Opt N)**	
	Mean	**Rank**	**Mean**	**Rank**	**Mean**	**Rank**	**Mean**	**Rank**
VH06760	13.65	10	19.35	8	43.18	1	20.63	4
TH15938	19.28	2	16.65	12	23.50	12	18.48	6
TH15889	28.60	1	18.95	10	27.60	8	8.88	11
TH15851	15.88	7	37.55	1	23.85	7	12.45	10
TH15895	12.35	11	31.90	2	32.05	9	18.75	5
TH151082	16.98	6	22.30	5	36.83	5	15.43	8
TH151022	17.28	5	24.20	4	38.20	4	16.18	7
TH15976	10.88	12	29.76	3	30.93	10	24.07	2
TH151141	18.53	4	19.05	9	38.53	3	24.20	1
TH151144	13.78	9	21.93	6	38.85	2	6.33	12
QPM hybrid mean	**16.72**		**24.16**		**33.35**		**16.54**	
SC 627	18.88	3	18.68	11	34.05	6	12.48	9
Local Check 1	15.55	8	21.28	7	29.70	11	24.05	3
Non-QPM hybrid mean	**17.21**		**19.98**		**31.88**		**18.27**	
Grand mean	**16.80**		**23.47**		**31.49**		**16.82**	
LSD $_{(0.05)}$	**8.36**		**7.47**		**15.59**		**5.96**	

LSD = Least significant difference; Low N = Low nitrogen; Opt N = Optimum nitrogen

4.4.1.2 Zinc content

Genotype effect for Zn content was significant (P≤0.01) at Harare and Bindura, and Glandel and Gwebi (P≤0.001) (Table 4.3). Genotype contributed 55.01%, 51.85%, 75.39% and 74.25% to variation in Zn content at Harare, Bindura, Glandel and Gwebi, respectively. Zn mean values for QPM ranged from 15.40 - 21.30 mg kg^{-1}, 18.23 - 28.98 mg kg^{-1}, 18.20 - 31.43 mg kg^{-1} and 19.53 - 28.75 mg kg^{-1} at Harare, Bindura, Glandel and Gwebi locations, respectively. In non-QPM, Zn content ranged from 15.95 - 23.83 mg kg^{-1}, 19.80 - 19.81 mg kg^{-1}, 19.88 - 26.43 mg kg^{-1} and 21.85 - 24.17 mg kg^{-1} at Harare, Bindura, Glandel and Gwebi, respectively (Table 4.4 and Figure 4.2). On average, Gwebi had the highest Zn content of 25.01 mg kg^{-1} for QPM hybrids whereas non-QPM at Glandel had the highest Zn content of 23.16 mg kg^{-1}. Hybrid TH15851 had the highest Zn content of all the hybrids at Bindura and Glandel, while at Gwebi and Harare, QPM TH15976 and Local check 1 had the highest values, respectively. The hybrid with the lowest Zn content was TH151144 at Glandel, Gwebi and Harare, and TH15889 at Bindura.

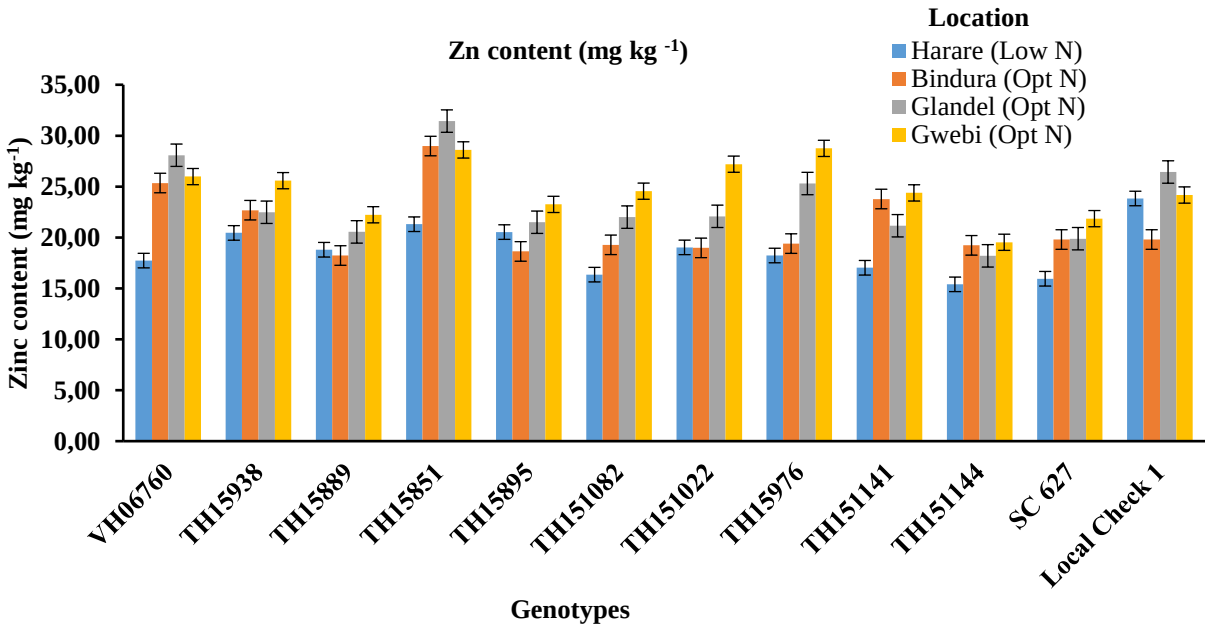

Figure 4.2 Zinc content (mg kg^{-1}) in maize hybrids at four locations

Table 4.3 Analysis of variance for Zn content (mg kg^{-1}) in maize hybrids at four locations

		Harare (Low N)		Glandel (Opt N)		Bindura (Opt N)		Gwebi (Opt N)	
Source	**DF**	**MS**	**% Variation**	**MS**	**% Variation**	**MS**	**% Variation**	**MS**	**% Variation**
Genotype	11	12.27**	55.01	29.02***	75.39	22.12**	51.85	15.23***	74.25
Block	1	0.22	0.09	19.08	20.10	177.13	37.74	1.99	0.88
Residual	11	10.02	44.91	7.37	4.51	4.44	10.40	5.10	24.86
CV (%)		16.91		11.96		9.95		9.16	

***P≤0.001; ** P≤0.01; DF = Degrees of freedom; MS = Mean squares; Low N = Low nitrogen; Opt N = Optimum nitrogen; CV = Coefficient of variation

Table 4.4 Mean values and rankings of Zn content (mg kg^{-1}) in maize hybrids at four locations

Genotype	**Harare (Low N)**		**Glandel ((Opt N)**		**Bindura (Opt N)**		**Gwebi (Opt N)**	
	Mean	**Rank**	**Mean**	**Rank**	**Mean**	**Rank**	**Mean**	**Rank**
VH06760	17.73	8	28.08	2	25.35	2	25.98	4
TH15938	20.45	4	22.68	5	22.48	4	25.58	5
TH15889	18.80	6	20.55	10	18.23	12	22.23	10
TH15851	21.30	2	31.43	1	28.98	1	28.60	2
TH15895	20.53	3	21.50	8	18.63	11	23.25	9
TH151082	16.35	10	22.00	7	19.28	8	24.55	6
TH151022	19.03	5	22.08	6	18.98	9	27.20	3
TH15976	18.23	7	25.30	4	19.40	7	28.75	1
TH151141	17.03	9	21.15	9	23.78	3	24.38	7
TH151144	15.40	12	18.20	12	19.23	10	19.53	12
QPM hybrid mean	**18.49**		**23.28**		**21.45**		**25.01**	
SC 627	15.95	11	19.88	11	19.80	6	21.85	11
Local Check 1	23.83	1	26.43	3	19.81	5	24.17	8
Non-QPM hybrid mean	**19.89**		**23.16**		**19.80**		**23.01**	
Grand mean	**18.72**		**23.25**		**21.18**		**24.67**	
LSD $_{(0.05)}$	**5.68**		**4.99**		**3.78**		**4.06**	

LSD = Least significant difference; Low N = Low nitrogen; Opt N = Optimum nitrogen

4.4.1.3 Phytic acid content

Significant (P≤0.001) differences for phytic acid content were seen at Gwebi, Harare and Bindura (P≤0.01) (Table 4.5). Contribution of genotype to variation in phytic acid were 79.10%, 83.54%, 63.75% and 89.95% for Harare, Bindura, Glandel and Gwebi, respectively. Phytic acid content in different locations ranged from 424.30 - 764.25 mg 100 g^{-1} at Harare, 414.30 - 1100.00 mg 100 g^{-1} at Glandel, 466.60 - 1007.85 mg 100 g^{-1} at Bindura and 359.40 - 826.70 mg 100 g^{-1} at Gwebi for QPM. The content in non-QPM ranged from 398.00 - 636.75 mg 100 g^{-1}, 568.20 - 792.85 mg 100 g^{-1}, 758.85 - 918.40 mg 100 g^{-1} and 369.40 - 495.85 mg 100 g^{-1} for Harare, Glandel, Bindura and Gwebi, respectively (Table 4.6 and Figure 4.3). The highest phytic acid content of 1100.00 mg 100 g^{-1} was observed in QPM hybrid TH15976 at Glandel while the lowest amount of 359.40 mg 100 g^{-1} was seen in QPM hybrid TH15938 at Gwebi. Variations in genotype ranking were observed at different locations, suggesting that genotypes responded differently to different environments.

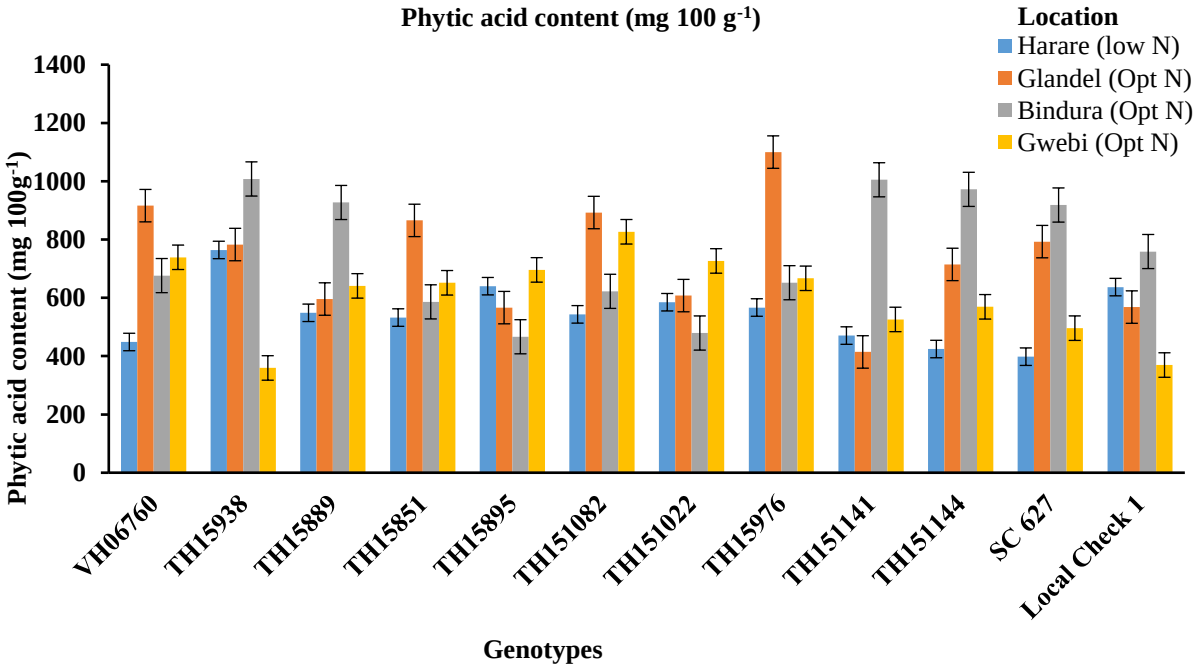

Figure 4.3 Phytic acid content (mg 100 g^{-1}) in maize hybrids at four locations

Table 4.5 Analysis of variance for phytic acid content (mg 100 g^{-1}) in maize hybrids at four locations

		Harare (Low N)		Glandel (Opt N)		Bindura (Opt N)		Gwebi (Opt N)	
Source	**DF**	**MS**	**% Variation**	**MS**	**% Variation**	**MS**	**% Variation**	**MS**	**% Variation**
Genotype	11	21592.18**	79.10	74310.14	63.75	82221.37**	83.54	41897.46***	89.95
Block	1	2064.62	0.69	7076.10	0.55	27520.05	2.54	1160.65	0.23
Residual	11	5516.76	20.21	41611.49	35.70	13700.61	13.92	4814.16	10.28
CV (%)		13.59		27.76		15.48		11.47	

***P≤0.001; **P≤0.01; DF = Degrees of freedom; MS = Mean squares; Low N = Low nitrogen; Opt N = Optimum nitrogen; CV = Coefficient of variation

Table 4.6 Mean values and rankings of phytic acid content (mg 100 g^{-1}) in maize hybrids at four locations

Genotype	**Harare (Low N)**		**Glandel (Opt N)**		**Bindura (Opt N)**		**Gwebi (Opt N)**	
	Mean	**Rank**	**Mean**	**Rank**	**Mean**	**Rank**	**Mean**	**Rank**
VH06760	448.45	10	916.20	2	676.25	7	739.10	2
TH15938	764.25	1	782.80	6	1007.85	1	359.40	12
TH15889	548.65	6	595.85	9	927.10	4	641.00	7
TH15851	532.25	8	865.65	4	586.05	10	651.65	6
TH15895	639.90	2	566.40	11	466.40	12	695.85	4
TH151082	543.35	7	892.65	3	622.30	9	826.70	1
TH151022	584.85	4	607.65	8	479.25	11	726.55	3
TH15976	566.75	5	1100.00	1	651.80	8	667.05	5
TH151141	470.50	9	414.30	12	1004.95	2	525.85	9
TH151144	424.30	11	714.60	7	972.15	3	569.25	8
QPM hybrid mean	**552.33**		**745.61**		**739.41**		**640.24**	
SC 627	398.00	12	792.85	5	918.40	5	495.85	10
Local Check 1	636.75	3	568.20	10	758.85	6	369.40	11
Non-QPM hybrid mean	**517.38**		**680.53**		**838.63**		**432.63**	
Grand mean	**546.50**		**734.76**		**755.95**		**604.80**	
LSD $_{(0.05)}$	**133.39**		**366.34**		**210.21**		**124.61**	

LSD = Least significant difference; Low N = Low nitrogen; Opt N = Optimum nitrogen

4.4.1.4 Phytic acid to iron molar ratio (MRFe)

Highly significant differences (P≤0.001) between MRFe were seen at all locations (Table 4.7). The contribution of genotype to the variation for MRFe was 92.38%, 93.62%, 91.60%, and 93.48% for Harare, Glandel, Bindura and Gwebi, respectively. MRFe in QPM hybrids ranged from 16.28 - 44.26 at Harare, 18.48 - 45.41 at Glandel, 10.84 - 28.65 at Bindura and 13.18 - 77.06 at Gwebi and the lowest MRFe of 10.84 was observed in VH06760 at Bindura. The range in non-QPM hybrids were 14.14 - 34.63, 22.73 - 35.62, 18.85 - 22.89 and 13.03 - 34.53 at Harare, Glandel, Bindura and Gwebi, respectively and the lowest MRFe of 13.03 was seen in Local check 1 at Gwebi. Molar ratios averages were lower in non-QPM in Harare and Gwebi while at Glandel and Bindura the lowest averages were observed in QPM cultivars. However, the differences between QPM and non-QPM were not significant in all locations except at Gwebi.

Table 4.7 Analysis of variance for MRFe in maize hybrids at four locations

		Harare (Low N)		Glandel (Opt N)		Bindura (Opt N)		Gwebi (Opt N)	
Source	**DF**	**MS**	**% Variation**	**MS**	**% Variation**	**MS**	**% Variation**	**MS**	**% Variation**
Genotype	11	156.65***	92.38	156.88***	93.62	73.88***	91.60	740.03***	93.48
Block	1	14.35	0.78	1.17	0.06	5.62	0.63	3.26	0.04
Residual	11	11.62	6.85	10.59	6.32	6.26	7.76	51.31	6.45
CV (%)		13.11		11.76		13.46		20.67	

***P≤0.001; DF = Degrees of freedom; MS = Mean squares; Low N = Low nitrogen; Opt N = Optimum nitrogen; MRFe = Phytic acid to iron molar ratio; CV = Coefficient of variation

Table 4.8 Mean values and rankings of MRFe in maize hybrids at four locations

Genotype	**Harare (Low N)**		**Glandel (Opt N)**		**Bindura (Opt N)**		**Gwebi (Opt N)**	
	Mean	**Rank**	**Mean**	**Rank**	**Mean**	**Rank**	**Mean**	**Rank**
VH06760	22.74	7	40.46	2	10.84	12	30.57	6
TH15938	33.66	3	45.41	1	25.61	2	13.18	11
TH15889	16.28	11	26.56	6	24.14	3	56.94	2
TH15851	26.10	6	20.64	10	11.44	11	45.24	4
TH15895	31.44	4	18.79	11	13.82	9	24.91	8
TH151082	27.97	5	27.69	5	15.12	8	49.27	3
TH151022	22.31	8	21.36	9	15.47	7	28.89	7
TH15976	44.26	1	31.78	4	13.14	10	23.80	9
TH151141	16.95	10	18.48	12	22.89	4	18.57	10
TH151144	21.50	9	22.52	8	28.65	1	77.06	1
QPM hybrid mean	**26.32**		**27.37**		**18.11**		**36.84**	
SC 627	14.14	12	35.62	3	22.89	5	34.53	5
Local Check 1	34.63	2	22.73	7	18.85	6	13.03	12
Non-QPM hybrid mean	**24.39**		**29.18**		**20.87**		**23.78**	
Grand mean	**25.99**		**27.67**		**18.59**		**34.65**	
LSD $_{(0.05)}$	**6.12**		**5.84**		**4.49**		**12.86**	

LSD = Least significant difference; Low N = Low nitrogen; Opt N = Optimum nitrogen; MRFe = Phytic acid to iron molar ratio

4.4.1.5 Phytic acid to zinc molar ratio (MRZn)

Hybrids differed significantly for MRZn at Bindura, Glandel and Gwebi ($P \leq 0.001$) (Table 4.9). Hybrids ranked differently at the four locations indicating different reaction to the different locations (Table 4.10). The contribution of genotype to variation in MRZn was 54.76%, 91.10%, 91.08% and 87.42% for Harare, Glandel, Bindura and Gwebi, respectively. Molar ratios in QPM hybrids ranged from 27.23 - 36.89, 19.35 - 50.61, 20.18 - 50.24 and 13.95 - 35.24 at Harare, Glandel, Bindura and Gwebi, respectively. In non-QPM hybrids, the variations ranged from 21.70 - 24.66, 17.81 - 50.05, 37.53 - 45.68 and 14.97 - 22.48 in Harare, Glandel, Bindura and Gwebi, respectively. Lower average MRZn were observed in non-QPM compared to QPM hybrids at all locations, except at Bindura. Reduced MRZn is an indication of higher mineral bioavailability.

Table 4.9 Analysis of variance for MRZn in maize hybrids at four locations

		Harare (Low N)		Glandel (Opt N)		Bindura (Opt N)		Gwebi (Opt N)	
Source	**DF**	**MS**	**% Variation**	**MS**	**% Variation**	**MS**	**% Variation**	**MS**	**% Variation**
Genotype	11	31.97	54.76	225.19***	91.10	178.02***	91.08	71.85***	87.42
Block	1	13.46	2.10	82.66	3.04	2.89	0.13	0.33	0.04
Residual	11	25.19	43.15	14.48	5.86	17.16	8.78	10.31	12.55
CV (%)		17.09		11.21		12.21		13.36	

***P≤0.001; Degrees of freedom; MS = Mean squares; Low N = Low nitrogen; Opt N = Optimum nitrogen; MRZn = Phytic acid to zinc molar ratio;

CV = Coefficient of variation

Table 4.10 Mean values and rankings of MRZn in maize hybrids at four locations

Genotype	**Harare (Low N)**		**Glandel (Opt N)**		**Bindura (Opt N)**		**Gwebi (Opt N)**	
	Mean	**Rank**	**Mean**	**Rank**	**Mean**	**Rank**	**Mean**	**Rank**
VH06760	28.18	8	32.10	7	23.49	11	28.04	4
TH15938	36.89	1	38.68	5	43.83	3	13.95	12
TH15889	28.28	7	28.67	6	39.32	4	28.62	3
TH15851	32.49	3	26.59	10	20.18	12	22.50	8
TH15895	31.20	4	37.74	6	25.79	10	29.50	2
TH151082	32.93	2	39.95	3	26.89	9	35.24	1
TH151022	30.30	6	27.11	9	30.09	7	26.01	5
TH15976	30.60	5	50.61	1	29.14	8	22.98	7
TH151141	27.37	9	19.35	11	34.47	6	21.27	10
TH151144	27.23	10	38.75	4	50.24	1	23.00	6
QPM hybrid mean	**30.60**		**33.96**		**32.34**		**25.11**	
SC 627	24.66	11	50.05	9	45.68	2	22.48	9
Local Check 1	21.70	12	17.81	12	37.53	5	14.94	11
Non-QPM mean	**23.18**		**33.93**		**41.61**		**18.71**	
Grand Mean	**29.36**		**33.95**		**33.93**		**24.04**	
LSD $_{(0.05)}$	**9.01**		**6.83**		**7.44**		**5.77**	

LSD = Least significant difference; Low N = Low nitrogen; Opt N = Optimum nitrogen; MRZn = Phytic acid to zinc molar ratio

4.4.2 Combined analyses of variance in maize hybrids across optimum N environments

There were highly significant differences (P≤0.001) between genotypes for all traits, except for phytic acid content that was significant (P≤0.05). Significant differences (P≤0.001) between environments were noted for all the traits. Significant GEI (P≤0.001) was evident for phytic acid content, MRFe and MRZn, and Fe and Zn (P≤0.05). Genotype contribution to total variation was 17.24%, 44.22%, 13.92%, 24.25% and 24.84% for Fe, Zn, phytic acid, MRFe and MRZn, respectively. Environment contributed 37.67%, 11.71%, 10.94%, 21.41% and 21.29% to variation in Fe, Zn, phytic acid, MRFe and MRZn, respectively. GEI contributed 18.96%, 13.42%, 54.36%, 49.12% and 46.32% to variation for Fe, Zn, phytic acid, MRFe and MRZn, respectively (Table 4.11). Mean values for Fe, Zn, phytic acid, MRFe and MRZn ranged from 17.37 - 29.51 mg kg^{-1}, 18.98 - 29.67 mg kg^{-1}, 576.22 - 806.28 mg 100 g^{-1}, 19.17 - 42.74 and 23.30 - 37.33 in QPM hybrids, respectively. In non-QPM the range was from 21.73 - 25.01 mg kg^{-1}, 20.51 - 23.46 mg kg^{-1}, 565.48 - 735.70 mg 100 g^{-1}, 18.20 - 31.01 and 23.44 - 39.40 for Fe, Zn, phytic acid, MRFe and MRZn, respectively (Table 4.12 and Figure 4.4). Genotype TH151082 had the highest content for Fe (29.51 mg kg^{-1}) and Zn (29.67 mg kg^{-1}), although it had relatively higher phytic acid content that could reduce mineral bioavailability. There was no consistent pattern in the performance of the genotypes for MRFe and MRZn as some registered high mean values in low and optimum N in QPM and non-QPM and the *vice versa*.

Table 4.11 Combined analysis of variance for Fe and Zn content (mg kg^{-1}), phytic acid content (mg 100 g^{-1}), MRFe and MRZn in maize hybrids across optimum N environments

		Fe content (mg kg^{-1})		Zn Content (mg kg^{-1})		Phytic acid content (mg 100 g^{-1})		MRFe		MRZn	
Source	**DF**	**MS**	**% Variation**	**MS**	**% Variation**	**MS**	**% Variation**	**MS**	**% Variation**	**MS**	**% Variation**
Genotype	11	107.85***	17.24	50.92***	44.22	40517.29*	13.92	320.90***	24.25	165.93***	24.84
Env	2	1295.79***	37.67	74.17***	11.71	160726.82***	10.94	1557.59***	21.41	783.95***	21.29
Gen * Env	22	59.28*	18.96	7.73*	13.42	78955.84***	54.36	324.95****	49.12	154.56***	46.32
Residual	33	34.60	16.59	190.03	15.00	20042.09	20.66	22.72	5.15	13.98	6.29
CV %		24.58		10.42		20.27		17.67		12.20	

***P≤0.001; *P≤0.05; DF = Degrees of freedom; MS = Mean squares; Gen = Genotypes; Env = Environment; MRFe = Phytic acid to iron molar ratio; MRZn =Phytic acid to zinc molar ratio; CV = Coefficient of variation

Table 4.12 Mean values and rankings for Fe, Zn content (mg kg^{-1}), phytic acid content (mg 100 g^{-1}), MRFe and MRZn in maize hybrids across optimum N environments

Genotype	**Type**	**Fe content (mg kg^{-1})**		**Zn content (mg kg^{-1})**		**Phytic acid content (mg 100 g^{-1})**		**MRFe**		**MRZn**	
		Mean	**Rank**	**Mean**	**Rank**	**Mean**	**Rank**	**Mean**	**Rank**	**Mean**	**Rank**
VH06760	QPM	27.72	4	26.47	2	777.18	3	27.29	6	27.78	8
TH15938	QPM	19.54	10	23.58	4	716.68	8	28.06	5	32.15	6
TH15889	QPM	18.48	11	20.33	11	721.32	7	35.88	2	32.20	5
TH15851	QPM	26.69	5	23.10	6	648.37	9	25.77	7	23.30	12
TH15895	QPM	17.37	12	18.98	12	752.00	5	19.17	11	31.01	7
TH151082	QPM	29.51	1	29.67	1	701.12	4	30.69	4	34.03	4
TH151022	QPM	27.83	3	21.13	9	576.22	11	21.90	9	27.74	9
TH15976	QPM	28.62	2	24.48	3	806.28	1	22.91	8	34.07	3
TH151141	QPM	23.77	7	22.75	7	601.04	10	20.08	10	25.03	10
TH151144	QPM	20.88	9	21.94	8	780.55	2	42.74	1	37.33	2
QPM hybrid mean		**24.04**		**23.24**		**708.08**		**27.45**		**30.46**	
SC 627	Non-QPM	21.73	8	20.51	10	735.70	6	31.01	3	39.40	1
Local Check 1	Non-QPM	25.01	6	23.46	5	565.48	12	18.20	12	23.44	11
Non-QPM hybrid mean		**23.37**		**21.98**		**650.59**		**24.61**		**31.42**	
Grand mean		**23.93**		**23.03**		**698.80**		**26.98**		**30.64**	
LSD $_{(0.05)}$		**5.74**		**2.35**		**69.16**		**4.66**		**3.65**	

LSD = Least significant difference; MRFe = Phytic acid to iron molar ratio; MRZn = Phytic acid to zinc molar ratio

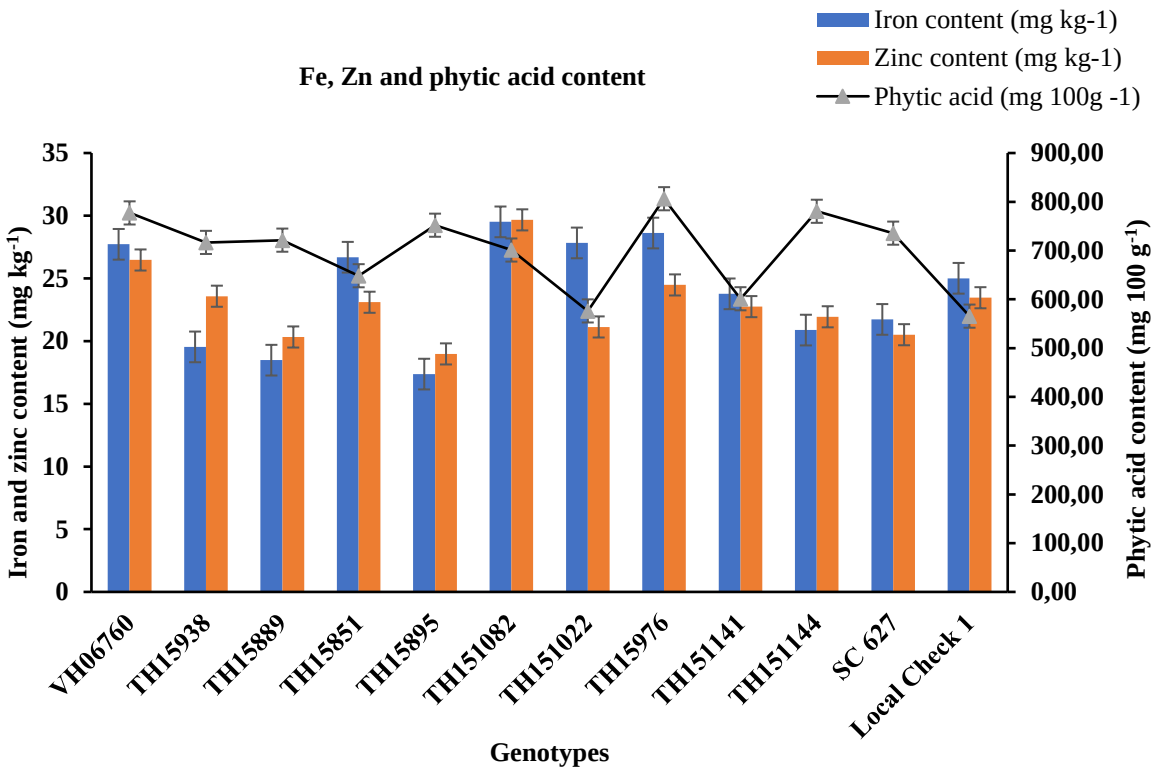

Figure 4.4 Performance of maize hybrids for Fe and Zn content (mg kg^{-1}), and phytic acid content (mg 100 g^{-1}) across optimum N environments

4.4.3 Comparison of maize hybrids for Fe and Zn content (mg kg^{-1}), phytic acid content (mg 100 g^{-1}), MRFe and MRZn under low and optimum N conditions

Generally, Fe, Zn and phytic acid were reduced in both QPM and non-QPM hybrids under low N, except for a few genotypes that outperformed their counterparts under optimum N conditions. As for MRFe and MRZn, no definite pattern was observed in QPM and non-QPM hybrids in both soil conditions (Table 4.13). Fe content under low N environment ranged from 10.88 - 28.60 mg kg^{-1} and 15.55 - 18.88 mg kg^{-1} in QPM and non-QPM, respectively, however TH15889 had higher Fe content under low N than optimum N conditions. Across optimal conditions, Fe content ranged from 17.37 - 29.51 mg kg^{-1} and 21.73 - 25.01 mg kg^{-1} in QPM and non-QPM, respectively. For Zn content, under low N conditions, the range was from 15.40 - 21.30 mg kg^{-1} and 15.95 - 23.83 mg kg^{-1} in QPM and non-QPM, respectively, but Local check 1 and TH15895 had higher mineral content under N stress than optimal N conditions. Under optimum N trials, the range was from 18.98 - 29.67 mg kg^{-1} and 20.51 - 23.46 mg kg^{-1} in QPM and non-QPM hybrids, respectively. As for phytic acid, under low N conditions values ranged from 424.30 - 764.25 mg 100 g^{-1} and 398.00 - 636.75 mg 100 g^{-1} in QPM and non-QPM hybrids, respectively, although TH15938, TH151022 and Local check 1 had high phytic acid

content under low N compared to optimal conditions. Across optimum conditions, the values ranged from 576.22 - 806.28 and 565. 48 - 735.70 mg 100 g^{-1} in QPM and non-QPM genotypes, respectively. In QPM, MRFe ranged from 16.28 - 44.26 and 14.14 - 34.63 for non-QPM hybrids under low N environment. Across optimum N trials, MRFe ranged from 19.17 - 42.74 and 18.20 - 31.01 in QPM and non-QPM hybrids, respectively. Under low N conditions, MRZn ranged from 27.23 - 36.89 and 21.70 - 24.66 in QPM and non-QPM hybrids, respectively. Across optimum N conditions, the MRZn range was from 23.30 - 37.33 and 23.44 - 39.40 for QPM and non-QPM hybrids, respectively.

Table 4.13 Mean values for Fe, Zn content (mg kg^{-1}), phytic acid content (mg 100 g^{-1}), MRFe and MRZn in maize hybrids under low and optimum N environments

Genotype	Type	Fe content		Zn content		Phytic acid content		MRFe		MRZn	
		Low N	Opt N	Low N	Opt N	Low N	Opt N	Low N	Opt N	Low N	Opt N
VH06760	QPM	13.65	27.72	17.73	26.47	448.45	777.18	22.74	27.29	28.18	27.87
TH15938	QPM	19.28	19.54	20.45	23.58	764.25	716.68	33.66	28.06	36.89	32.15
TH15889	QPM	28.60	18.48	18.80	20.33	548.65	721.32	16.28	35.88	28.82	32.20
TH15851	QPM	15.88	26.69	21.30	23.10	532.25	648.32	26.10	25.77	32.49	23.30
TH15895	QPM	12.35	17.37	20.53	18.98	639.90	752.00	31.44	19.17	31.20	31.01
TH151082	QPM	16.98	29.51	16.35	29.67	543.35	701.12	27.97	30.69	32.93	34.03
TH151022	QPM	17.28	27.83	19.03	21.13	584.85	576.22	22.31	21.90	30.30	27.74
TH15976	QPM	10.88	28.62	18.23	24.48	566.75	806.28	44.26	22.91	30.60	34.24
TH151141	QPM	18.53	23.77	17.03	22.75	470.50	601.15	16.95	20.08	27.37	25.03
TH151144	QPM	13.78	20.88	15.40	21.94	424.30	780.55	21.50	42.74	27.23	37.33
QPM hybrid mean		**16.72**	**24.04**	**18.49**	**23.24**	**552.33**	**708.08**	**26.32**	**27.45**	**30.60**	**30.49**
SC 627	Non-QPM	18.88	21.73	15.95	20.51	398.00	735.70	14.14	31.01	24.66	39.40
Local Check 1	Non-QPM	15.55	25.01	23.83	23.46	636.75	565.48	34.63	18.20	21.70	23.44
Non-QPM hybrid mean		**17.21**	**23.37**	**19.89**	**21.98**	**517.38**	**650.59**	**24.39**	**24.61**	**23.18**	**31.42**
Grand mean		**16.80**	**23.93**	**18.72**	**23.03**	**546.50**	**698.80**	**25.99**	**26.98**	**29.36**	**30.64**
LSD $_{(0.05)}$		**8.36**	**5.74**	**5.68**	**2.34**	**133.39**	**69.16**	**6.12**	**4.66**	**9.01**	**3.65**

LSD = Least Significant Difference; MRFe = Phytic acid to iron molar ratio; MRZn = Phytic acid to zinc molar ratio; Low N = Low nitrogen; Opt N = Optimum nitrogen

4.5 Discussion

Increasing the content of minerals such as Fe and Zn in staple food crops through crop biofortification may improve its nutritional value and hence reduce micronutrient deficiency. The strategy is sustainable and cheaper than food fortification and nutrient supplementation in the fight against hidden hunger (Cakmak, 2008). In developing countries, the majority of the population obtain minerals such as Fe and Zn from staple crops such as maize, rice, wheat, cassava and sweet potatoes (Ortiz-Monasterio *et al*., 2007), and these are among the target crops for enhancing minerals for Harvest Plus. According to Hotz (2009), consuming Zn biofortified staple crops increases the mineral available to the body and reduces Zn deficiency.

Cereals contain anti-nutritional factors that have negative effect on mineral bioavailability and causes micronutrient deficiency in plant-based diet dependents (Gibson *et al*., 2010). Therefore, addressing micronutrient deficiency using crop plants could be possible by enhancing grain mineral content through biofortification and enhance the bioavailability. As breeders, there is need to maintain a balance between high concentrations of minerals such as Fe and Zn, and low phytic acid, considering that in humans, phytic acid was shown to contribute to cancer inhibition and it increases seedling vigour in plants growth (Grases *et al*., 2000).

There were significant differences between hybrids for Fe and Zn content for the four locations. Across optimum locations there were variations in genotypes, locations and GEI for all the measured characteristics. This agrees with previous studies of Bänziger and Long (2000) and Menkir (2008), who reported high genotypic variations for Fe and Zn content in landraces and improved germplasm and tropical maize inbred lines. Prasanna *et al*. (2011) reported Fe ranging from 11.28 - 60.11 mg kg^{-1} and Zn ranging from 15.14 - 52.95 mg kg^{-1} in a study in which 30 sets of maize genotypes were evaluated for kernel Fe and Zn content under rain fed conditions. Queiroz *et al*. (2011) reported Fe and Zn content ranging from 12.20 - 36.70 mg kg^{-1} and 17.50 - 42.00 mg kg^{-1}, respectively in 22 tropical maize inbred lines. The current study indicated Fe mean values ranging from 15.55 -18.88 mg kg^{-1} in non-QPM and 19.28 - 28.60 mg kg^{-1} in QPM and Zn content ranged from 15.95 - 23.83 mg kg^{-1} in non-QPM and 15.40 - 21.30 mg kg^{-1} in QPM, respectively under low N conditions. This agrees with the study by Phalafala (2013), however present study mean values were on the lower side. Across optimum N environments, the Fe content means ranged from 21.73 - 25.01 mg kg^{-1} in non-QPM hybrids and 17.37 - 29.51 mg kg^{-1} in QPM hybrids, while for Zn content, the values ranged from 20.51

- 23.46 mg kg^{-1} for non-QPM varieties and 18.98 - 29.67 mg kg^{-1} for QPM hybrids, which was similar to values reported by Queiroz *et al*. (2011) and Prasanna *et al*. (2011).

Oikeh *et al*. (2003) noted variations for Fe and Zn content in early maturing tropical maize across different environments that ranged between 15.50 - 19.10 mg kg^{-1} and 16.50 - 20.50 mg kg^{-1}, respectively. Similarly, in this study variations were observed in Fe and Zn content in QPM hybrids and non-QPM varieties across different environments. The Fe content, for instance, ranged between 17.22 - 31.88 mg kg^{-1} and 16.63 - 29.53 mg kg^{-1} in non-QPM and QPM, respectively. On the other hand, Zn ranged from 19.80 - 23.16 mg kg^{-1} and 18.49 - 25.01 mg kg^{-1} in non-QPM and QPM hybrids, respectively. Various research studies by House (1999), Fiel *et al*. (2005) and Simić *et al*. (2009) reported that Fe and Zn content is affected by both the environment and genetic makeup. The difference in genotypes and environments used in the current study could have an effect on the values differences. The results of the study also differed from Maziya-Dixon *et al*. (2000) who reported high genetic variation for Fe content ranging from 13.60 - 159.43 parts per million (ppm) and Zn content ranged from 11.65 - 95.62 ppm in mid-altitude maize inbred lines and 13.60 - 133.83 ppm and 23.50 - 94.94 ppm for Fe and Zn, respectively in lowland maize inbred lines.

Significant variations in Fe and Zn content amongst entries, environments and GEI were observed by Kandianis *et al*. (2013). In their study, environmental effects such as low N and drought significantly affected Fe and Zn content in maize. Similarly, significant genotype, location and GEI was seen in the current study for kernel Fe and Zn content. Various studies by Oikeh *et al*. (2003), Simić *et al*. (2009) and Kandianis *et al*. (2013) found significant GEI for Fe and Zn in different maize varieties. On the other hand, Oikeh *et al*. (2004) noted no significant differences between locations and GEI, suggesting late maturing tropical maize genotype performance was similar in different locations and the locations were similar, which is contrary to the present study.

Significant genetic variation observed for Fe and Zn in the current study is consistent with a review of Welch and Graham (2004). Variations are important for grain mineral improvement in cultivars. In their review, low genetic variation was reported in maize compared to wheat, but greater than variation found in rice. According to Ortiz-Monasterio *et al*. (2007) the major challenge in breeding for Fe and Zn in maize is due to narrow mineral range which is associated with highly significant GEI, and this is similar to present study. The existence of GEI for the traits shows that genotype performance was not stable in different environments. These results

indicate the importance of evaluating the QPM hybrids in different environments to determine stability of mineral expression. In the current study, no genotype performed consistently in low and optimum N environments and in different locations for all the traits under investigation. Under low N conditions, genotypes TH15889 and TH15938 had the highest Fe content and Local check 1 (check) had the highest Zn content and TH15851 had the highest mean value among QPM hybrids, while for optimum N conditions, genotype TH151082 had the highest Fe and Zn content.

High genotypic variations in QPM hybrids were observed for phytic acid content under low N conditions. Across optimum conditions, significant differences were reported in phytic acid content for genotypes, locations and GEI. Similarly, in previous studies by Mladenović-Drinić *et al*. (2009) and Schelmmer *et al*. (2009) noted significant genotypic differences in different maize cultivars. According to Ruel and Bouis (1998), cereal grains, including maize, contain about 800 mg 100 g^{-1} phytic acid content, which was similar to present results. Garćia-Estepa *et al*. (1999) noted high variations in phytic acid content with an average of 1078 mg 100 g^{-1}, while Morreti *et al.* (2013) found a range of 720 - 2220 mg 100 g^{-1} phytic acid in whole maize flour. According to Michealsen *et al*. (2009), phytic acid mean values ranged from 9.80 - 21.30 mg g^{-1} in maize flour prepared from different varieties. In maize inbred lines, phytic acid content in grain kernel ranged from 479±15.80 - 1040±20.30 mg 100 g^{-1} (Queiroz *et al*., 2011).

The current study had mean values of phytic acid ranging from 576.22 - 806.28 mg 100 g^{-1} and 565.48 - 735.70 mg 100 g^{-1} in QPM and non-QPM hybrids, respectively across optimum N conditions. In the low N environment, the phytic acid ranged from 424.30 - 764.25 mg 100 g^{-1} and 398.00 - 636.75 mg 100 g^{-1} in QPM and non-QPM hybrids, respectively. Hurrell *et al*. (2003) and Nafabuanga (2006) noted higher phytic acid content in normal maize than QPM hybrids and this contradicts the current study. The lowest phytic acid was observed in the non-QPM hybrids in both low and optimum N environments, while in QPM the lowest phytic acid was in TH151144 and TH151022 under low and optimum N conditions, respectively. The differences noted in phytic acid content might be due to differences in cultivars used, climatic conditions, environmental conditions and the season that have an influence on the phytic acid content accumulation in the kernel grain (Hidvegi and Lasztity, 2003).

Mineral bioavailability is influenced by different factors such as mineral content concentration, mineral interaction and phytic acid content and Gupta *et al*. (2015) reported that phytic acid concentration is the main cause of mineral absorption inhibition in plant-based foods. Various studies showed that MRZn of >15 reduces the mineral bioavailability (Turnlund *et al*., 1984; Sandberg *et al*., 1987; Ma *et al*., 2005; Akomo *et al*., 2016). Zn absorption is high when MRZn is <15 (WHO, 1996). Hambidge *et al*. (2004) found MRZn ranging from 18 - 40 in maize hybrids and Erdal *et al*. (2014) suggested that MRZn above 20 - 30 reduce Zn mineral absorption and animals' growth. According to Pachón *et al*. (2009) lower MRZn was found in QPM maize than in common maize, although the mineral bioavailability in QPM and common maize were not significantly different. Queiroz *et al*. (2011) noted MRZn ranging from 18 - 43.50 in maize inbred lines which was similar to the present study. Studies by Norhaizan and Norfaizadatul (2009) noted significant differences in phytic acid to mineral molar ratios for Fe, Zn and Ca from different foods prepared from rice and wheat cereal grains. In their research, the ratios were all >15 indicating low Zn mineral bioavailability.

In the present study, high and significant variations in MRZn for genotypes, location and GEI were seen, indicating differences in mineral bioavailability. MRZn ranged from 23.30 - 37.33 in QPM and 23.44 - 39.40 in non-QPM in optimum N environments while under low N conditions, the range was from 27.23 - 36.89 in QPM and 21.70 - 24.66 in non-QPM hybrids, which was similar to other studies. According to Ma *et al.* (2005), MRZn ranges between 2.98 - 243.97 in maize kernels andother maize products, while Simić *et al*. (2009) found MRZn ranging from 256.80 - 425.80. MRZn ranging from 39.77 - 126.20 was reported by Yankey *et al*. (2011) in QPM and non-QPM maize genotypes, and these values were much higher compared to current study and most literature cited. In the present study, QPM TH15851 and Local check 1 across optimum N conditions and Local check 1 and SC 627 under low N conditions had high Zn bioavailability, although they were all above the critical value for maximum Zn absorption.

Fe absorption is greatly compromised when phytic acid to Fe molar ratio >1. Various studies reported poor Fe absorption in MRFe of > 1 in foods (Hallberg *et al*., 1989; Gibson *et al*., 2010; Akomo *et al*., 2016). In the present study, low Fe absorption was observed under low and optimum N conditions in tested hybrids. Significant differences were observed for genotypes which shows that the genotypes were not similar and variations noted in environment suggests that the locations differed. Mineral bioavailability in QPM hybrids can be improved due to the

presence of genetic variation that is observed. However, GEI that was observed poses a challenge to breeders as genotypes performance varies with the environment. Ma *et al*. (2005) reported MRFe ranging from 2.98 - 75.79 in maize kernel and its products. Yankey *et al.* (2011) found MRFe ranging from 7.09 -14.85 in golden crystal (normal) and Obatanpa (QPM) maize varieties, and this was much lower compared to present study. MRFe ranging from 16.30 - 45.50 was reported in tropical maize inbred lines with different backgrounds (Queiroz *et al*., 2011) and this agrees with the current study. Simić *et al*. (2009) noted MRFe between the ranges of 175.80 - 332.50 in maize which was much higher compared to the current study.

In the present study MRFe ranged from 19.17 - 42.74 and 18.20 - 31.01 in QPM and non-QPM hybrids, respectively under optimum N conditions. Under low N conditions, MRFe ranged from 16.28 - 44.26 and 14.14 - 34.63 in QPM and non-QPM hybrids, respectively. High Fe bioavailability was seen in SC 627 (check) and TH15889 under low N conditions and Local check 1 (check) and TH15895 across optimum N environments. Differences observed in molar ratios affecting mineral bioavailability might be attributed to the differences in the amount of inhibitory substances among tested hybrids (Oikeh *et al*., 2003). Ma *et al*. (2007) reported high molar ratio values for Fe and Zn in the diets consumed in rural areas in China that have low-income affecting mineral bioavailability, hence high mineral deficiency amongst poor people. This trend is similar in SSA and other developing countries as most people depend on maize with little diet diversification, hence they are susceptible to micronutrient deficiency. Sufficient genetic variation was seen in the current study for measured characteristics, suggesting the possibility of improving them through selection.

4.6 Conclusions

In summary, the study shows that low N conditions had a negative effect on Fe, Zn, phytic acid content, MRFe and MRZn. However, minerals were more bioavailable in many genotypes under low N conditions as they had relatively smaller phytic acid to mineral molar ratios than under optimum N conditions. For minerals, some specific cultivars-maintained Fe (TH15889), and Zn (Local check 1 and TH15851) levels and were less sensitive to low N conditions. Fe and Zn minerals are likely to be highly absorbed in genotypes SC 627 and Local check 1, respectively under low N conditions, while for optimal conditions, the same was true for Local check 1 (Fe) and TH15851 (Zn). Genetic variation is important for breeders to explore in development of cultivars with enhanced Fe, Zn and reduced phytic acid content, and sufficient variation was shown in this study for selection.

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CHAPTER FIVE

THE INFLUENCE OF ENVIRONMENTAL CONDITIONS ON PROTEIN QUALITY AND QUANTITY OF QUALITY PROTEIN MAIZE (QPM) HYBRIDS

5.1 Abstract

QPM nutritional quality involves breeding for high total protein content and reduced zein accumulation in maize endosperm and increase non-zein proteins, hence high levels of essential amino acids. The aim of the study was to assess the effect of different environmental conditions, including low N conditions, on protein quality and quantity in QPM hybrids. There was highly significant genotype effect for total grain protein, α, β and γ zein peak areas in all locations. The combined ANOVA across optimum N trials showed significant genotype, environment and genotype and environment interaction effects for all measured characteristics except for protein content, which was insignificant for location. Total protein content, γ and α zein peak areas were significantly reduced under low N compared to optimal conditions, except for a few genotypes that had high γ zein content, while β zein content was increased under low N conditions. Local check 1 and TH15976 had the highest protein content under low N conditions, while under optimum N conditions, SC 627(check) and TH15976 (QPM) had the highest protein content. Higher average values for α zein were observed in non-QPM than in QPM genotypes in all environments compared to other zein categories. Total protein and zein peak areas in tested hybrids were greatly influenced by environmental conditions. Lower α zein accumulation in QPM hybrids observed under low N conditions is important as it indicates higher levels of non-zeins, hence improving maize nutritional value.

5.2 Introduction

Maize is an important source of protein in most people especially in developing countries and it accounts for 15% of annual production of food crop protein in the world (Shiferaw *et al*., 2011; Dei, 2017; Zhang *et al*., 2017). Generally, low-income communities obtain their protein from maize, hence the need to develop cultivars with balanced nutritional composition (Phalafala, 2013). Nutritional limitation in conventional maize is due to high accumulation of zein proteins at the expense of non-zein proteins such as glutelins, albumins and globulins that have balanced amino acid content (Prasanna *et al*., 2001). QPM cultivars have increased amounts of non-zeins, hence the increase in essential amino acids (Prasanna *et al*., 2001; Sofi *et al*., 2009). Harvey (2007) stated that δ zeins in QPM endosperm contains higher amounts of lysine and tryptophan, improving nutritional quality of QPM compared to common maize.

Variation exists for total proteins and zein storage proteins in different maize genotypes, which affects quality of QPM and common maize (Arief, 2008). Total protein content in maize on average ranges between 6 - 14% (Prasanna *et al*., 2001). Zein in maize endosperm ranges from 40 - 70% of total protein in the seeds (Vivek *et al*., 2008), while Khan *et al*. (2014) reported the value to be 50% and above. Zein storage proteins are the major determining factor of maize nutritional quality. Suteu *et al*. (2013) identified γ and δ zein in maize inbred lines using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) from South East Europe maize cultivars, while Wu *et al*. (2009) found δ zein storage proteins which were absent in maize inbred lines from South East Europe. Wide genetic variations were noted in zein storage proteins in maize germplasm which were identified using SDS-PAGE (Khan *et al*., 2014).

QPM inbred lines and hybrids were reported to have superior nutritional value compared to open pollinated varieties (OPVs) and common maize genotypes (Bello *et al*., 2014). This is due to enhanced levels of lysine and tryptophan, hence QPM is considered important in combating malnutrition (Prasanna *et al.*, 2001; Ngaboyisonga *et al*., 2012). According to Sofi *et al*. (2009), *o*2 genotypes contain superior protein that is highly digestible compared to conventional maize and Ngaboyisonga *et al*. (2012) noted that QPM is able to correct protein deficiency, especially in children. Sofi *et al*. (2009) noted that children fed on QPM increased in weight, which was similar to those that were fed on milk in India. According to O'Kennedy (2011), reduction in the zein proportion in QPM comparison to non-zein protein resulted in increased levels of lysine and tryptophan in maize endosperm, which has an impact on maize nutritional value.

Nutritional quality traits, especially protein quality and quantity, have received limited attention, especially the response of QPM hybrids to different soil conditions. Wegary *et al*. (2011) compared protein quality in two contrasting soil environmental conditions in QPM inbred lines. Ngaboyisonga *et al*. (2012) investigated endosperm modification, total protein, lysine and tryptophan content in QPM under low N and drought stress conditions. In SA "maize quality evaluation does not involve assessment of total grain protein and zein storage proteins" (O'Kennedy, 2011). This study therefore aimed at determining the impact of growing conditions on protein quality and quantity in QPM hybrids.

5.3 Materials and methods

5.3.1 Planting locations

As described in Chapter 3 (3.3.1)

5.3.2 Planting materials

As described in Chapter 3 (3.3.2)

5.3.3 Experimental design and field management

As described in Chapter 3 (3.3.3)

5.3.4 Protein analysis

The protein content in samples was determined according to AACC Approved Method 46-30 (AACC, 2008) using a Dumas combustion analyser (TruSpec CN elemental Determinator). A number of blank samples followed by a number of ethylenediaminetetraacetic acid (EDTA) samples were analysed prior to protein determination to ensure the instrument was stable. Calibration standard EDTA chemical with known N content of 9.57% was used to calibrate the instrument. EDTA weighing 0.1 ± 0.001 g was sealed in a tin foil sample cup, twisted and then rolled into an egg shaped and placed on the loading head of the instrument and heated in the furnace. After calibration of the instrument, whole maize flour samples (1.5±0.5 g) were weighed in duplicate directly into foils with a known mass and placed on the loading head of the instrument. The encapsulated samples were purged to get rid of gasses and then dropped into a hot furnace (950^{o}C) which was flushed with oxygen for rapid and complete combustion and then analysed. Among the combustion gases are nitrogen oxides (NO_x) that were converted to N through hot copper. The N value was used to estimate protein content as: Protein % = % N x 6.25 (conversion factor).

5.3.5 Zein extraction

Zein extraction was done according to the procedure of Dombrink-Kurtzman and Beitz (1993) modified by O'Kennedy (2011). Whole maize flour (0.20 g per sample) was placed in 2 ml Eppendorf tubes and 1 ml of 70% ethanol (v/v) containing 5% 2-mercaptoethanol (2ME) (v/v) as a reducing agent, was added to break disulphide bonds, then 0.50% sodium acetate (w/v) was added for precipitation. The mixture was agitated continuously on a vortex for ±16 hours (overnight) at ambient temperature, and the suspension was centrifuged for 15 min at 6000 g revolutions per minute (rpm). The supernatant obtained was filtered through a 47 mm

membrane filter using a syringe, into glass vials and then was kept in the refrigerator at 4^{o}C for reverse phase-high performance liquid chromatography (RP-HPLC) analysis.

5.3.6 RP-HPLC analysis

RP-HPLC was performed on a Shimadzu Class VP LC system and the separation of zein protein was done using a Phenomonex Jupiter 300 C18 column (250 mm x 4.6 mm, 5 µm pore size) with a Security Guard cartridge column. The RP-HPLC separation method was adapted from Eyherabide *et al*. (1996) and Robutti *et al*. (2000). A volume of 100 µl per sample was injected and eluted with a solvent at a flow rate of 1.0 ml min^{-1} with a 50 min linear 28 - 60.50% solvent B gradient followed by a 10 min isocratic elution at 60.50% B, at a column temperature of 60^{o}C. The eluate monitoring was done by UV absorbance (210 nm). The mobile phase was a mixture of two eluents which included: (A) Filtered de-ionized water containing 0.1% (v/v) Trifluoacetic acid (TFA); (B) Acetonitrile containing 0.1% (v/v) TFA. Prior to analysis, de-ionized water was filtered through a Durapore membrane (PVDF, hydrophilic, 0.45 µm). Only distinctive peaks were considered for calculating zein area percentage.

5.3.7 Data analysis

As described in Chapter 3 (3.3.7)

5.4 Results

5.4.1 Analysis of variation for total protein and zein peak areas under different environmental conditions

5.4.1.1 Total protein

Genotypic effects were highly significant ($P \leq 0.001$) for total protein content in all locations (Table 5.1). Contribution of genotype to variation in protein content was 91.49%, 97.67%, 94.42% and 91.47% for Harare, Glandel, Bindura and Gwebi, respectively. Hybrid SC 627 had the highest total protein content at all locations, except at Harare where Local check 1 was the best, although the mean values were not statistically different from TH15976 at Bindura and Gwebi. Amongst the QPM hybrids, TH15976 had the highest protein content for all the locations, except at Glandel where the best hybrid was TH151144. The total protein content in the QPM hybrids ranged from 5.45 - 6.85%, 8.75 - 11.35%, 9.55 - 11.65% and 8.35 - 10.25% at Harare, Glandel, Bindura and Gwebi, respectively. The range in non-QPM hybrids was 7.00 - 8.10%, 9.00 - 13.10%, 10.90 - 11.90% and 9.50 - 10.45% at Harare, Glandel, Bindura and

Gwebi locations, respectively (Table 5.2). Generally, higher protein means were observed in non-QPM compared to QPM hybrids in all locations (Figure 5.1).

5.4.1.2 Zein peak area

Beta zein peak area (%)

Highly significant differences (P≤0.001) were seen at Glandel and Bindura, Gwebi (P≤0.01) and Harare (P≤0.05) in genotypes for β zein peak area (Table 5.3). In general, non-QPM hybrids recorded lower average β zein peak areas than QPM hybrids in all locations, even though the difference between QPM and non-QPM averages were insignificant at Gwebi. The highest β zein accumulation (12.64%) was observed in QPM TH15889 at Gwebi which differed significantly from the values of TH151022, TH151141, TH151144 and Local check 1. Genotype contribution to total β zein peak area variation was 75.98%, 95.99%, 96.62% and 83.91% for Harare, Glandel, Bindura and Gwebi, respectively. Beta zein accumulation in QPM hybrids ranged from 4.65 - 10.62%, 3.77 - 10.09%, 3.86 - 11.36% and 4.30 - 12.64% at Harare, Glandel, Bindura and Gwebi, respectively, while in non-QPM hybrids the range was from 4.70 - 7.58%, 2.81 - 3.47%, 3.62 - 4.14% and 7.33 - 9.79% at Harare, Glandel, Bindura and Gwebi, respectively (Table 5.4). Generally, a narrow range of β zein was observed in non-QPM compared to QPM hybrids.

Table 5.1 Analysis of variance for grain protein content (%) in maize hybrids at four locations

		Harare (Low N)		Glandel (Opt N)		Bindura (Opt N)		Gwebi (Opt N)	
Source	**DF**	**MS**	**% Variation**	**MS**	**% Variation**	**MS**	**% Variation**	**MS**	**% Variation**
Genotype	11	0.96***	91.49	2.93***	97.67	1.15***	94.42	0.89***	91.47
Block	1	0.67	5.79	0.04	0.13	0.004	0.03	0.03	0.25
Residual	11	0.03	2.72	0.07	2.21	0.067	5.55	0.08	8.27
CV (%)		2.63		2.51		2.39		3.06	

***P≤0.001; DF = Degrees of freedom; MS = Mean squares; Low N = Low nitrogen; Opt N = Optimum nitrogen; CV = Coefficient of variation

Table 5.2 Mean values and rankings for total protein content (%) in maize hybrids at four locations

Genotype	**Harare (Low N)**		**Glandel (Opt N)**		**Bindura (Opt N)**		**Gwebi (Opt N)**	
	Mean	**Rank**	**Mean**	**Rank**	**Mean**	**Rank**	**Mean**	**Rank**
VH06760	6.00	9	10.75	4	11.55	3	9.15	6
TH15938	5.90	10	9.65	9	9.70	11	9.35	5
TH15889	5.45	12	9.85	8	10.65	9	8.85	10
TH15851	6.50	5	10.51	5	11.10	5	8.95	7
TH15895	5.80	11	8.75	12	9.55	12	8.35	12
TH151082	6.30	6	10.00	7	10.10	10	8.55	11
TH151022	6.25	7	9.00	11	10.90	6	10.00	3
TH15976	6.85	3	10.10	6	11.65	2	10.25	2
TH151141	6.65	4	10.95	3	10.70	8	8.85	9
TH151144	6.20	8	11.35	2	11.45	4	8.95	8
QPM hybrid mean	**6.19**		**10.09**		**10.74**		**9.14**	
SC 627	7.00	2	13.10	1	11.90	1	10.45	1
Local Check 1	8.10	1	9.00	10	10.90	7	9.50	4
Non-QPM hybrid mean	**7.55**		**11.05**		**11.40**		**9.97**	
Grand mean	**6.42**		**10.25**		**10.85**		**9.87**	
LSD $_{(0.05)}$	**0.30**		**0.46**		**0.47**		**0.49**	

LSD = Least significant difference; Low N = Low nitrogen; Opt N = Optimum nitrogen

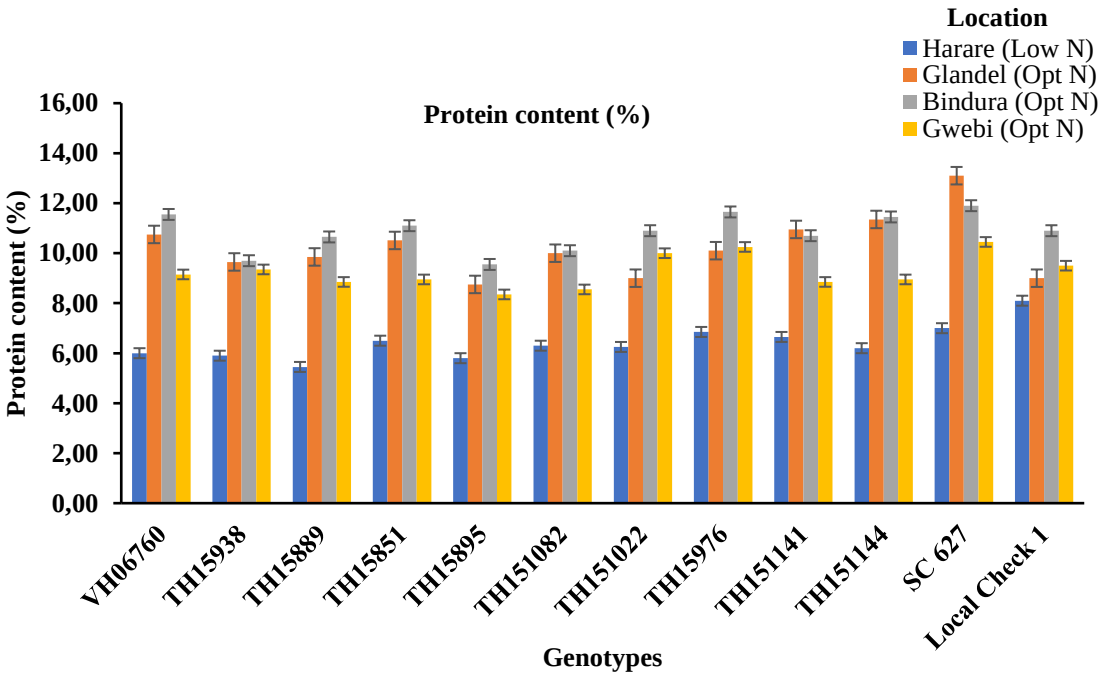

Figure 5.1 Protein content (%) in maize hybrids at four locations

Table 5.3 Analysis of variance for β zein peak area in maize hybrids at four locations

		Harare (Low N)		Glandel (Opt N)		Bindura (Opt N)		Gwebi (Opt N)	
Source	**DF**	**MS**	**% Variation**	**MS**	**% Variation**	**MS**	**% Variation**	**MS**	**% Variation**
Genotype	11	6.23*	75.98	13.32***	95.99	14.97***	96.62	13.59**	83.91
Block	1	2.77	3.07	0.25	0.17	3.16	1.85	0.21	0.12
Residual	11	1.72	20.95	0.53	3.84	0.24	1.73	2.59	15.97
CV (%)		19.04		12.27		7.03		17.53	

***P≤0.001; **P≤0.01; *P≤0.05; DF = Degrees of freedom; MS = Mean squares; Low N = Low nitrogen; Opt N = Optimum nitrogen; β = Beta zein; CV = Coefficient of variation

Table 5.4 Mean values and rankings for β zein peak area (%) in maize hybrids at four locations

Genotype	Harare (Low N)		Glandel (Opt N)		Bindura (Opt N)		Gwebi (Opt N)	
	Mean	**Rank**	**Mean**	**Rank**	**Mean**	**Rank**	**Mean**	**Rank**
VH06760	7.84	4	4.58	8	4.76	8	9.04	7
TH15938	6.28	8	3.77	10	9.46	3	10.92	4
TH15889	6.47	6	8.89	4	10.31	2	12.64	1
TH15851	6.10	9	5.09	6	8.37	5	8.48	8
TH15895	10.62	1	4.79	7	11.36	1	10.41	5
TH151082	6.40	7	10.09	1	7.02	6	12.37	2
TH151022	5.15	10	8.90	3	8.70	4	8.28	9
TH15976	8.46	2	9.16	2	6.96	7	11.24	3
TH151141	4.65	12	5.90	5	4.31	9	4.30	12
TH151144	8.40	3	4.00	9	3.86	11	5.32	11
QPM hybrid mean	**7.03**		**6.52**		**7.51**		**9.30**	
SC 627	4.70	11	3.47	11	3.62	12	9.79	6
Local Check 1	7.58	5	2.81	12	4.14	10	7.33	10
Non-QPM hybrid mean	**6.14**		**3.14**		**3.88**		**8.56**	
Grand mean	**6.88**		**5.93**		**6.91**		**9.17**	
LSD $_{(0.05)}$	**3.56**		**1.98**		**1.32**		**4.37**	

LSD=Least significant difference; Low N = Low nitrogen; Opt N = Optimum nitrogen; β = Beta zein

Gamma zein peak area (%)

The genotype effect on γ zein concentration was highly significant (P≤0.001) in all locations (Table 5.5). The contribution of genotype to γ zein variation ranged from 98.07 - 99.27% with the highest observed at Glandel and the lowest at Gwebi site. Gamma zein accumulation in different QPM hybrids ranged from 37.82 - 83.43%, 26.43 - 64.57%, 33.82 - 70.50% and 34.47 - 68.30% at Harare, Glandel, Bindura and Gwebi, respectively. For non-QPM hybrids, the range was from 36.26 - 38.75%, 20.22 - 22.29%, 25.72 - 31.27% and 24.77 - 28.33% at Harare, Glandel, Bindura and Gwebi, respectively (Table 5.6). Higher average concentration of γ zein was observed in QPM hybrids compared to non-QPM hybrids in all locations except at Harare and the differences between QPM and non-QPM averages were significant. Low N conditions recorded higher γ zein accumulation in QPM and non-QPM compared to optimum N conditions except for hybrid VH06760. The highest amount of γ zein was seen in TH151022 at Harare, TH15976 at Bindura, TH15895 at Glandel and TH15851 at Gwebi.

Table 5.5 Analysis of variance of γ zein peak area in maize hybrids at four locations

		Harare (Low N)		Glandel (Opt N)		Bindura (Opt N)		Gwebi (Opt N)	
Source	**DF**	**MS**	**% Variation**	**MS**	**% Variation**	**MS**	**% Variation**	**MS**	**% Variation**
Genotype	11	570.98***	99.26	661.23***	99.27	401.52***	98.74	362.41***	98.07
Block	1	16.55	0.26	4.29	0.06	4.08	0.09	3.97	0.09
Residual	11	2.75	0.48	4.54	0.68	4.75	1.17	6.79	1.84
CV (%)		2.77		4.48		4.38		5.48	

***P≤0.001; DF = Degrees of freedom; MS = Mean squares; Low N = Low nitrogen; Opt N = Optimum nitrogen; γ = Gamma zein; CV = Coefficient of variation

Table 5.6 Mean values and rankings for γ peak area (%) in maize hybrids at four locations

Genotype	**Harare (Low N)**		**Glandel (Opt N)**		**Bindura (Opt N)**		**Gwebi (Opt N)**	
	Mean	**Rank**	**Mean**	**Rank**	**Mean**	**Rank**	**Mean**	**Rank**
VH06760	44.47	9	55.50	8	56.39	6	59.30	2
TH15938	71.32	4	62.90	3	51.64	8	50.39	8
TH15889	70.43	6	59.31	5	61.67	2	55.74	3
TH15851	73.59	2	57.96	6	58.88	3	68.30	1
TH15895	67.58	7	64.57	1	58.04	5	55.64	4
TH151082	72.48	3	57.96	7	58.31	4	51.88	6
TH151022	83.43	1	63.03	2	54.62	7	54.38	5
TH15976	70.91	5	60.41	4	70.50	1	51.45	7
TH151141	37.82	11	53.58	9	36.85	9	35.54	9
TH151144	50.51	8	26.43	10	33.82	10	34.47	10
QPM hybrid mean	**64.25**		**56.17**		**54.07**		**51.71**	
SC 627	38.75	10	20.22	12	25.72	12	24.77	12
Local Check 1	36.26	12	22.29	11	31.27	11	28.33	11
Non-QPM hybrid mean	**37.51**		**21.26**		**28.49**		**26.55**	
Grand mean	**59.79**		**47.56**		**49.81**		**47.51**	
LSD $_{(0.05)}$	**4.51**		**5.79**		**5.92**		**7.08**	

LSD = Least significant difference; Low N = Low nitrogen; Opt N = Optimum nitrogen; γ = Gamma zein

Alpha zein peak area (%)

Genotype effect on α zein peak area was highly significant (P≤0.001) in all locations (Table 5.7). Genotype contribution to variation in α zein peak area was 96.44%, 99.18%, 98.75% and 98.51% at Harare, Glandel, Bindura and Gwebi, respectively. Alpha zein content ranged from 10.46 - 57.54%, 28.08 - 71.46%, 22.56 - 62.41% and 23.25 - 60.21% for QPM hybrids at Harare, Glandel, Bindura and Gwebi, respectively. In non-QPM hybrids, the range was from 55.58 - 56.56%, 71.88 - 76.33%, 64.60 - 70.69% and 64.34 - 65.45% at Harare, Glandel, Bindura and Gwebi, respectively (Table 5.8). Alpha zein peak area average was higher in non-QPM hybrids compared to QPM hybrids in all locations, and QPM hybrids and non-QPM hybrids averages differences were statistically significant in all trials.

5.4.2 Combined analyses of variance for total protein, β, γ and α zein peak area (%) in maize hybrids across optimum N environments

Genotypic effects were highly significant (P≤0.001) for all the characteristics investigated. Genotype contribution to variation ranged from 40.65%, 47.88%, 90.37% and 93.00% for total protein content, β, γ and α zein peak area, respectively (Table 5.9). Grain protein content ranged from 8.88 - 10.67% in QPM hybrids with an average of 9.98%, while in non-QPM the range was from 9.80 - 11.82% and the highest content (11.82%) was from SC 627 (check) and the protein content in SC 627 was significantly higher than that of all other hybrids. The effect of location was highly significant (P≤0.001) for all the traits except for total protein. Contribution of location to variation was 34.87%, 20.77%, 0.52% and 0.72% for total protein, β, γ and α zein peak areas, respectively. GEI effect was highly significant (P≤0.001) under the optimum N environments for all the characteristics, which shows that genotypes responded differently in different locations (Table 5.10). Contribution of GEI to variation was 21.71%, 24.90%, 7.93% and 5.16% for total protein content, β, γ and α zein peak area, respectively. Beta zein concentration ranged from 4.39 - 10.61% in QPM hybrids and 4.76 - 5.63% in non-QPM hybrids. For γ zein peak area, the range was from 30.95 - 61.71% for QPM hybrids and for non-QPM hybrids the range was from 23.57 - 27.30%. Alpha zein accumulation in maize endosperm in QPM hybrids ranged from 30.09 - 64.69% and in non-QPM the range was from 66.94 - 70.82%.

Table 5.7 Analysis of variance for α zein peak area (%) in maize hybrids at four locations

		Harare (Low N)		Glandel (Opt N)		Bindura (Opt N)		Gwebi (Opt N)	
Source	**DF**	**MS**	**% Variation**	**MS**	**% Variation**	**MS**	**% Variation**	**MS**	**% Variation**
Genotype	11	653.04***	96.44	746.29***	99.18	525.95***	98.75	439.19***	98.51
Block	1	12.18	0.16	7.95	0.09	13.95	0.24	2.42	0.05
Residual	11	22.99	33.95	5.47	0.73	5.44	1.02	6.42	1.44
CV (%)		14.91		5.06		5.39		5.85	

***P≤0.001; DF = Degrees of freedom; MS = Mean squares; Low N = Low nitrogen; Opt N = Optimum nitrogen; α = Alpha zein; CV = Coefficient of variation

Table 5.8 Mean values and rankings of α zein peak area (%) in maize hybrids at four locations

Genotype	**Harare (Low N)**		**Glandel (Opt N)**		**Bindura (Opt N)**		**Gwebi (Opt N)**	
	Mean	**Rank**	**Mean**	**Rank**	**Mean**	**Rank**	**Mean**	**Rank**
VH06760	47.69	4	41.83	5	38.81	6	31.67	10
TH15938	22.40	6	33.31	8	38.90	5	38.69	5
TH15889	21.16	7	31.80	9	28.02	11	31.62	11
TH15851	20.31	10	36.95	6	32.55	9	23.25	12
TH15895	10.46	12	30.64	10	30.61	10	33.96	9
TH151082	21.12	8	34.42	7	34.68	8	35.76	8
TH151022	11.93	11	28.08	12	36.68	7	37.34	6
TH15976	20.64	9	30.44	11	22.56	12	37.29	7
TH151141	57.54	1	67.74	4	58.87	4	60.21	3
TH151144	40.61	5	71.46	3	62.41	3	60.20	4
QPM hybrid mean	**27.39**		**40.67**		**38.41**		**39.00**	
SC 627	56.56	2	76.33	1	70.69	1	65.45	1
Local Check 1	55.58	3	71.88	2	64.60	2	64.34	2
Non-QPM hybrid mean	**56.07**		**74.11**		**67.65**		**64.89**	
Grand mean	**32.16**		**46.24**		**43.28**		**43.31**	
LSD $_{(0.05)}$	**13.03**		**6.36**		**6.34**		**6.89**	

LSD = Least significant difference; Low N = Low nitrogen; Opt N = Optimum nitrogen; α = Alpha zein

Table 5.9 Combined analysis of variance for total protein content, β, γ and α zein peak area (%) across optimum N environments

		Total protein (%)		β zein peak area (%)		γ zein peak area (%)		α zein peak area (%)	
Source	**DF**	**MS**	**% Variation**	**MS**	**% Variation**	**MS**	**% Variation**	**MS**	**% Variation**
Genotype	11	3.24***	40.65	27.54***	47.88	1310.19***	90.37	1624.34***	93.00
Env	2	15.26	34.87	65.71***	20.77	41.27***	0.52	69.25***	0.72
Gen * Env	22	0.86***	21.71	7.17***	24.90	57.48***	7.93	45.05***	5.16
Residual	33	0.07	2.69	1.12	5.84	5.36	1.12	5.78	0.99
CV (%)		2.64		14.40		4.79		5.43	

***P≤0.001; DF = Degrees of freedom; MS=Mean squares; Env = Environment; Gen = Genotype; α = Alpha zein; β = Beta zein; γ = Gamma zein; CV=Coefficient of variation

Table 5.10 Mean values and rankings for total protein, β, γ and α zein peak area (%) across optimum N environments in maize hybrids

Genotype	**Type**	**Total protein (%)**		**β zein peak area (%)**		**γ zein peak area (%)**		**α zein peak area (%)**	
		Mean	**Rank**	**Mean**	**Rank**	**Mean**	**Rank**	**Mean**	**Rank**
VH06760	QPM	10.48	4	6.13	8	56.42	6	37.44	5
TH15938	QPM	9.57	10	8.05	6	54.98	8	36.97	6
TH15889	QPM	9.78	9	10.61	1	58.91	4	30.48	11
TH15851	QPM	10.18	5	7.30	7	61.71	1	30.91	10
TH15895	QPM	8.88	12	8.85	4	59.45	3	31.74	9
TH151082	QPM	9.55	11	9.82	2	55.23	7	34.95	7
TH151022	QPM	9.97	7	8.62	5	57.34	5	34.03	8
TH15976	QPM	10.67	2	9.12	3	60.78	2	30.09	12
TH151141	QPM	10.17	6	4.84	10	32.94	9	62.27	4
TH151144	QPM	10.58	3	4.39	12	30.95	10	64.69	3
QPM hybrid mean		**9.98**		**7.77**		**52.87**		**39.36**	
SC 627	Non-QPM	11.82	1	5.63	9	23.57	12	70.82	1
Local Check 1	Non-QPM	9.80	8	4.76	11	27.30	11	66.94	2
Non-QPM hybrid mean		**10.81**		**5.19**		**25.44**		**68.88**	
Grand mean		**10.12**		**7.34**		**48.29**		**44.28**	
LSD $_{(0.05)}$		**0.26**		**1.03**		**2.26**		**2.35**	

LSD = Least significant difference; α = Alpha zein; β = Beta zein; γ = Gamma zein

5.4.3 Comparison of maize hybrids for total protein content, β, γ and α zein peak area (%) under different environmental conditions

Genotype performance under low N conditions showed that total protein content, β and α zein proteins were reduced in both QPM and non-QPM hybrids compared to optimum N conditions (Table 5.11). Total protein under low N conditions ranged from 5.65 - 6.85% and 7.00 - 8.10% in QPM and non-QPM hybrids, respectively. Across optimal conditions, the total protein content ranged from 8.88 - 10.58% and 9.80 - 11.82% in QPM and non-QPM hybrids, respectively. For β zeins, under low N conditions, the range was from 4.65 - 10.62% and 4.70 - 7.58% in QPM and non-QPM hybrids, respectively. Across optimum N conditions, the range was from 4.39 - 10.61% and 4.76 - 5.63% in QPM and non-QPM hybrids, respectively, however, VH06760, TH15895, TH151144 and Local check 1 had higher amounts of β zein under low N than under optimum N conditions. For α zein protein, under low N conditions, the range was from 10.46 - 57.54% and 55.58 - 56.56% in QPM and non-QPM hybrids, respectively. Across optimum N conditions, the values ranged from 30.09 - 64.69% and 66.94 - 70.82% in QPM and non-QPM hybrids. In contrary, γ zein proteins accumulation was high in the hybrids under low N than optimum N conditions, except for VH06760. Under the low N environment, γ zein proteins ranged from 37.82 - 83.43% and 36.26 - 38.75% in QPM and non-QPM hybrids, respectively. Across optimum N trials, γ zein proteins ranged from 30.95 - 61.71% and 23.57 - 27.30% for QPM and non-QPM hybrids, respectively.

Table 5.4 Mean values for total protein, β, γ and α zein peak area (%) in maize hybrids under low and optimum N environments

Genotypes	Type	Protein content (%)		β zein peak area (%)		γ zein peak area (%)		α zein peak area (%)	
		Low N	Opt N	Low N	Opt N	Low N	Opt N	Low N	Opt N
VH06760	QPM	6.00	10.48	7.84	6.13	44.47	56.42	47.69	37.44
TH15938	QPM	5.90	9.57	6.28	8.05	71.32	54.98	22.40	36.97
TH15889	QPM	5.45	9.78	6.47	10.61	70.43	58.91	21.16	30.48
TH15851	QPM	6.50	10.18	6.10	7.30	73.59	61.71	20.31	30.91
TH15895	QPM	5.80	8.88	10.62	8.85	67.58	59.45	10.46	31.74
TH151082	QPM	6.30	9.55	6.40	9.82	72.48	55.23	21.12	34.95
TH151022	QPM	6.25	9.97	5.15	8.62	83.43	57.34	11.93	34.03
TH15976	QPM	6.85	10.67	8.46	9.12	70.91	60.78	20.64	30.09
TH151141	QPM	6.65	10.17	4.65	4.84	37.82	32.94	57.54	62.27
TH151144	QPM	6.20	10.58	8.40	4.39	50.51	30.95	40.61	64.69
QPM hybrid mean		**6.19**	**9.98**	**7.03**	**7.77**	**64.25**	**52.87**	**27.39**	**39.36**
SC 627	Non-QPM	7.00	11.82	4.70	5.63	38.75	23.57	56.56	70.82
Local Check 1	Non-QPM	8.10	9.80	7.58	4.76	36.26	27.30	55.58	66.94
Non-QPM hybrid mean		**7.55**	**10.81**	**6.14**	**5.19**	**37.51**	**25.44**	**56.07**	**68.88**
Grand mean		**6.42**	**10.12**	**6.88**	**7.34**	**59.79**	**48.29**	**32.16**	**44.28**
LSD $_{(0.05)}$		**0.30**	**0.26**	**3.56**	**1.03**	**4.51**	**2.26**	**13.03**	**2.35**

LSD = Least significant difference; Low N = Low nitrogen; Opt N = Optimum nitrogen; α = Alpha zein; β = Beta zein; γ = Gamma zein

The results from the HPLC, the zein profile peaks shows that espression of storage proteins is similar regardless of he location in some genotypes as in Figure 5.2. The peaks also depict that accumulation of zein proteins in maize endosperm is high in optimum N compared to low N environment as shown in Figure 5.3, suggesting that maize produced in low N have high nutritional content then optimum N.

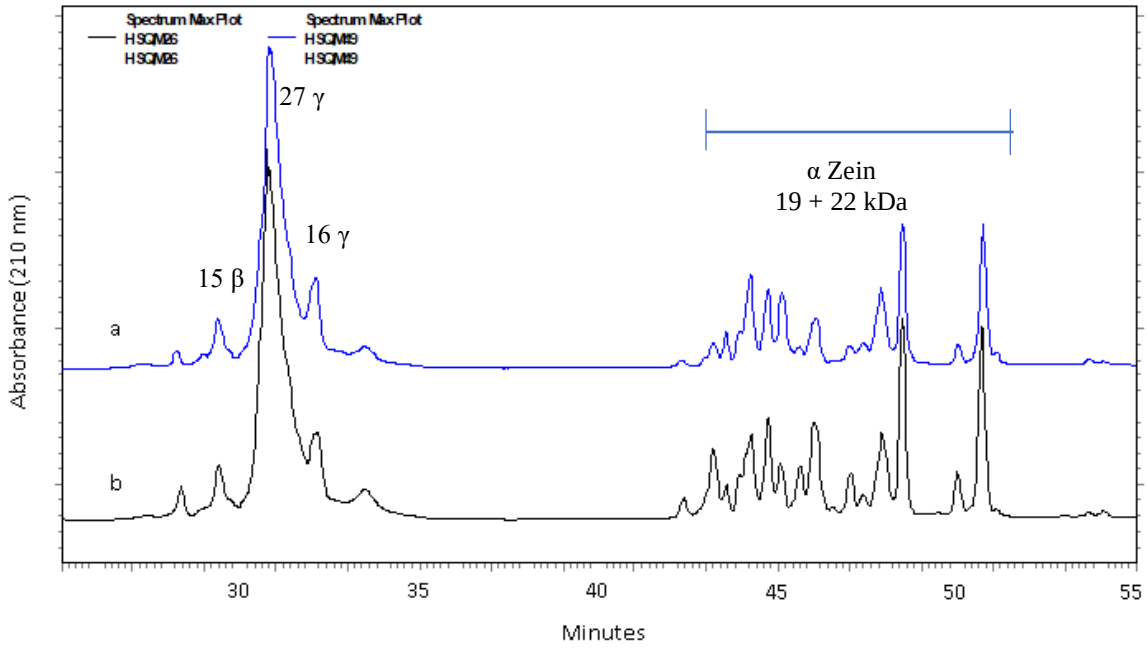

Figure 5.2 Zein peak area profile for hybrid VH0670 (a) at Glandel and (b) at Bindura

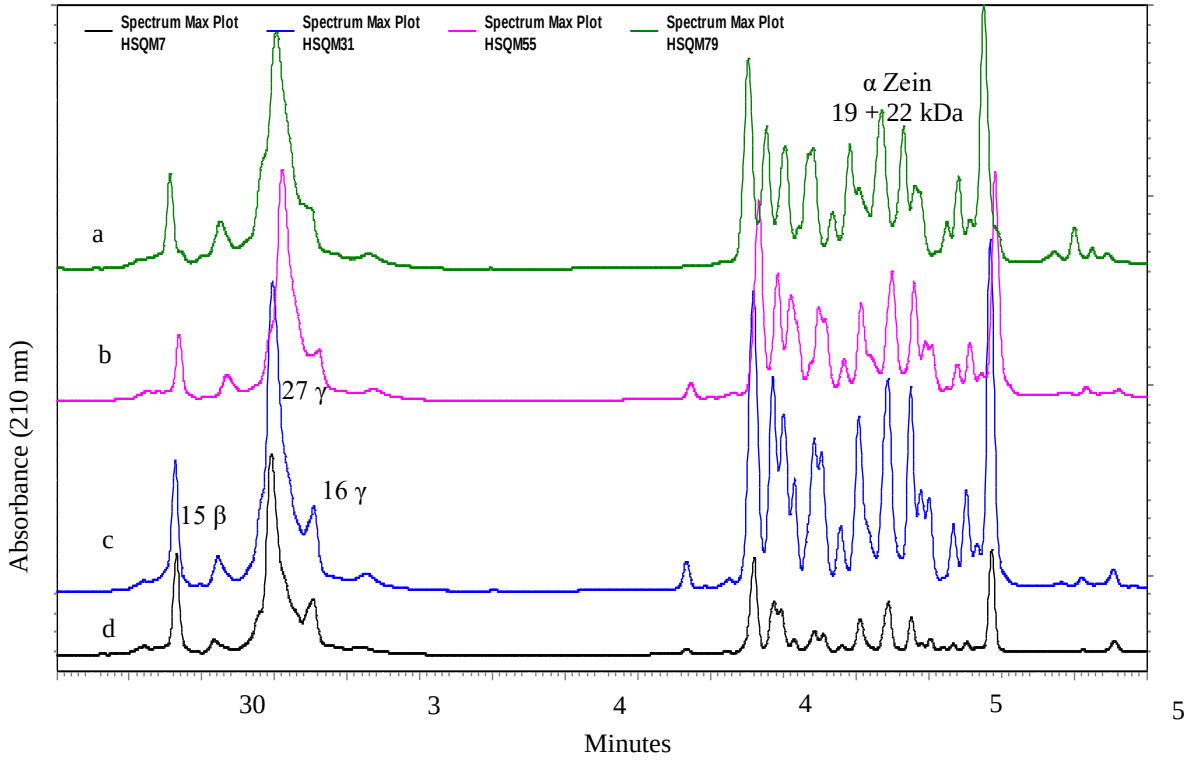

Figure 5.3 QPM TH151144 showing high α zein under optimum N conditions (a, b and c) at Glandel, Bindura and Gwebi, respectively, compared to low N conditions (d) at Harare

5.5 Discussion

There were significant differences for total protein content between maize hybrids at all locations, and total protein recorded under low N was lower than under optimum N environments. This was similar to results of Ngaboyisonga *et al*. (2012) and Ngaboyisonga and Njoroge (2014) who reported that low N growing environment reduces grain protein content in both QPM and normal maize. An increase in N supply leads to increase in total grain protein (Bruns and Elbelhar 2006; Cheetham *et al*., 2006; Seebauer *et al*., 2010). Ngaboyisonga *et al*. (2008) noted high grain protein and tryptophan content in maize kernel produced under low N and irrigation environmental conditions. Research by Prasanna *et al*. (2001), Zhai and Zhang (2007) and Bello *et al*. (2014) found that higher protein contents were observed in QPM hybrids than local checks. Bello *et al*. (2014) reported protein content ranging from 8.90 - 10.67% and 7.31 - 7.33% in QPM and non-QPM, respectively. Protein content in the current study ranged from 5.45 - 6.85% for QPM hybrids and 7.00 - 8.10% in non-QPM hybrids under low N conditions. Local check 1 hybrid (check) outperformed all other genotypes and among QPM

hybrids, TH15976 was the best, but the difference in protein content between Local check 1 and TH15976 was statistically significant. The high protein content in non-QPM than in QPM hybrids suggests that the characters is more controlled by genetics than the environment.

Significant differences in genotypes, environments and GEI was reported for protein content across low N, optimum N and drought conditions (Ngaboyisonga *et al*., 2012). Wegary *et al*. (2011) found significant environmental effects and GEI for protein content across low and optimal N conditions, which were similar to findings in the present study where genotype and GEI effects for grain protein content were significant, but differs in that environmental effects were not significant in the current study. Studies by Duarte *et al.* (2004) and Phalafala (2013) found significant differences in QPM hybrids for total protein content, and this agrees with the current study. Total protein ranged from 8.88 - 10.67% in QPM hybrids and the range was from 9.80 - 11.82% in non-QPM hybrids. A similar protein content range of 6 - 12% was reported by Watson (2003) in different maize genotypes. The present study indicated that the protein content was relatively lower compared to the optimum level of 13.50% and 13.40% reported by Gibbon and Larkins (2005) and Phalafala (2013), respectively. However, protein contents obtained in this study were higher compared to average protein of 10.01% reported by Prasanna *et al*. (2001). Non-QPM hybrids had higher amounts of protein than QPM and this contradicts findings by Prasanna *et al*. (2001), Zhai and Zhang (2007) and Bello *et al*. (2014) who reported higher protein content in QPM compared to local varieties. Overall, genotype SC 627 had the highest protein content while QPM hybrid TH15976 had the highest protein content amongst QPM hybrids under optimum N conditions.

Significant variations were observed in hybrids for β, γ and α zeins in all locations and combined ANOVA showed highly significant effects for genotype, location and GEI for all the zein types investigated. The zein profile showed that the peaks eluted in the following order; β, γ and γ (the first three peaks), then several other peaks emerged last as α zein proteins, and similar zein profile pattern were found by Dombrick-Kurtzman and Beitz (1993); Eyherabide *et al.* (1996); O'Kennedy (2011). According to Dombrick-Kurtzman and Beitz (1993) and O'Kennedy (2011), these zeins have molecular masses of 15 kDa (β), 27 kDa and 16 kDa (γ), and 19 kDa and 22 kDa (α) zeins. Variations in total number of peaks were observed in tested hybrids, and this is similar to findings by Flint-Garcia *et al*. (2009). Total number of peaks ranging from 11 - 30 for γ, β and α zeins were seen in QPM TH151141 and Local check 1, respectively and different zeins had different area percentages that were highly significant. This

agrees with findings of Flint-Garcia *et al*. (2009) who reported different peak area % and numbers of peaks in inbred lines and other maize groups. O'Kennedy (2011) noted variations in number of peaks where 11 peaks were observed in Zein 1 (α and δ) and 14 peaks in Zein 2 (γ and β) giving a total of 25 peaks. In present study, 14 peaks were observed in α zeins and maximum of 7 peaks for β and γ zeins, which proves the existence of genetic variations for zein proteins in QPM hybrids. According to Flint-Garcia *et al.* (2009), different retention times were observed among and between maize groups, which is consistent with the present study.

Different elution times were observed among tested hybrids for different zein categories which ranged from 10 - 54 minutes. The longest retention time was observed in α zeins, while the lowest was seen in β zeins. These results are similar to those reported by Dombrick-Kurtzman and Beitz (1993), Flint-Garcia *et al*. (2009) and O'Kennedy (2011). Retention time and peak area % differed within the β, γ and α zeins, which shows that genotypes differed. These findings are consistent with results reported by Wilson (1991) who observed variations in all zeins, including δ zeins, while O'Kennedy (2011) found variations in retention time within β zein proteins. However, in present sudy, similar zein profile patterns were observed in β, γ and α for hybrid VH06760 that was planted at Glandel and Bindura sites (Figure 5.2 a and b), respectively. This shows that some proteins expressed in similar way in the same hybrids, despite being grown in different locations.

Low N conditions were found to reduce α zein accumulation in both QPM hybrids and non-QPM. Similarly, Tsai *et al*. (1980) found reduction in zein amount under low N conditions in *o*2 hybrids and local checks, although the difference within *o*2 hybrids were insignificant. In cereal seeds, storage proteins tend to increase with increased N supply (Mùller *et al*., 1997). Significant variations were evident in QPM hybrids and checks for β, γ and α zeins in all locations in the current study. This indicates that hybrids were different from one another.

Genotype, location and GEI effects were significant for β, γ and α zein proteins across optimum N environments. Paiva *et al*. (1991) reported highly significant differences in zein proteins. In their findings, larger peak area of γ zeins were found in QPM compared to local checks, with similar accumulation of β and α zeins. Similar findings were observed in the present study where on average, larger γ zein peak area of 64.26% in QPM compared to non-QPM that had 37.50% peak area. In the present study, high amounts of γ zeins accumulated in QPM hybrids and low amount of α zeins were seen. This contradicts findings of Paiva *et al*. (1991) who found the same accumulation of β and α zeins in QPM and local cultivars.

Gerde *et al*. (2016) noted significant differences in genotypes, locations and GEI for Zein 1 (α and δ) and Zein 2 (β and γ). In their study, they found that increasing N supply to maize increases α zein accumulation in the maize endosperm, while β and γ zein accumulation depends on genetic makeup of the varieties. Tsai *et al*. (1992) found that α and γ zein protein content accumulation was higher in maize hybrids when N supply was increased while low peak area was reported for β zein. This agrees with the present research findings since higher α zein concentration was found in almost all the hybrids under optimum N compared to low N conditions. Hybrid TH15895 recorded the lowest α zein accumulation, which means total zeins were reduced and the non-zein proportion increased, hence increasing levels of lysine and tryptophan, although the value was statistically not different from that of hybrids TH15938, TH15889, TH15976, TH15851, TH151082 and TH151022. Khan *et al*. (2014) stated that a significant decrease in α zein in maize genotypes result in increasing amounts of lysine in grain kernel. Under optimum N conditions, hybrid TH15976 recorded the lowest α zein concentration, but it was not statistically different from values of hybrids TH15889, TH15851 and TH15895.

Decreased α zein accumulation in *o*2 maize genotypes compared to common maize varieties has been reported previously (Tsai *et al*., 1978; Schmidt *et al*., 1992; Harvey, 2007) and this agrees with the current study where lower α zein accumulation was noted in QPM compared to non-QPM hybrids. According to Tsai *et al*. (1978), *o*2 is capable of reducing the zeins by more than 50%, hence increasing the essential amino acids content in the maize endosperm. The *o*2 gene decreases the 22 kDa zein gene transcriptional factors responsible for encoding the polypeptides and makes the kernel opaque in appearance. The 22 kDa zeins have a specific sequence in the promotor that allows *o*2 to bind and this is missing in other zein classes, hence *o*2 has a large effect on 22 kDa zein storage proteins (Schmidt *et al*., 1992). According to Mùller *et al*. (1997), Schmidt *et al*. (1992) and Tripathy *et al*. (2017), the *o*2 mutant gene has a larger reduction effect on the 22 kDa than the 19 kDa protein, hence an increase in non-zein globulin, albumin and glutelin accumulation that have a balanced amino acid content, improving maize nutritional quality. The alteration of storage protein that is caused by the *o*2 gene increases β, γ and δ zeins that increases sulfur concentration in the endosperm improving its quality compared to α zein accumulation. Maize transgenic lines were reported to accumulate less α zein that resulted in balanced amino acids content in the endosperm (Frizzi *et al*., 2010).

In the current study, higher γ zein content was observed in QPM compared to non-QPM in low and optimum N conditions. This was similar to findings of Eyherabide *et al*. (1996), who reported higher accumulation of γ zeins in QPM than in local varieties. According to Wallace *et al*. (1990) and O'Kennedy (2011), γ zein protein in maize endosperm is responsible for endosperm hardness. O'Kennedy (2011) reported three times more γ zein content in hard textured endosperm compared to soft endosperm. Hard maize endosperm texture is preferred most by small-scale farmerswho have no access to pesticides, as it is less susceptible to pests such as weevils compared to soft textured endosperms.

5.6 Conclusions

Environmental conditions had significant effects on the concentration of total protein and accumulation of zein storage proteins in both QPM and non-QPM hybrids. Overall, protein and zein content was reduced under low N conditions, except for γ zein. The non-QPM hybrids Local check 1 and SC 627 had the highest protein content under low and optimum N conditions. Of the QPM hybrids, TH15976 had the highest protein content at all locations. Alpha zein was the lowest in QPM TH15895 and TH15976 under low and optimum N conditions, respectively. The highest β zein content was observed in QPM TH15895 and TH15889 under low and optimum N conditions, respectively. Gamma zein was the highest in TH151022 and TH15851 under low and optimum N conditions, respectively. Reduction of α zein under low N is of great importance, since maize production under low N simulates production conditions of small-scale farmers, a condition common to African countries. This will result in decreased total zein concentration and an increased proportion of non-zein proteins, improving nutritional quality of maize by increasing the concentration of essential amino acids; lysine and tryptophan. Variation observed for total protein and different zein storage proteins is important, as it will allow selection for improved nutritional quality.

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CHAPTER SIX

THE RELATIONSHIP BETWEEN GRAIN YIELD AND NUTRITIONAL CHARACTERISTICS UNDER LOW AND OPTIMUM NITROGEN CONDITIONS

6.1 Abstract

Grain yield and nutritional quality traits are important aspects to consider in addressing food and nutrition insecurity. Correlation and principal component analyses (PCA) were done to determine the relationship between grain yield and nutritional traits in QPM hybrids under low and optimum N conditions. Under optimum N conditions, highly significant (P≤0.01) positive relationships were observed between Fe and Zn content, tryptophan content and γ zein, β and γ zeins, while oil and tryptophan content, and γ zein and tryptophan content correlations were highly significant (P≤0.05). There were highly significant (P≤0.001) negative associations between α and γ zeins, and α and β zeins. Under low N conditions, highly significant(P≤0.05) positive correlations were observed between protein content and α zein, while highly significant (P≤0.001) negative relationships were observed between γ and α zeins. The PCA under both soil conditions showed that genotypes TH151082 and TH15938 maintained high tryptophan, oil, γ zein, and phytic acid content. Significantly positive correlations are important for simultaneous traits improvement.

6.2 Introduction

Maize is amongst most important staple food crops in the world. The FAO (2018) reported that in 2017/2018 season, about 1045 million metric tons of maize were produced in the world, of which 206.8 million metric tons were produced in African countries, SSA inclusive. One of the major challenges to maize production is yield losses, caused by different factors. According to Langa (2005) poor soil fertility and low soil moisture content due to drought and long dry spells are some of the major production constraints farmers face that reduce grain quantity and quality.

Maize yield is critical as a factor of total production, which consequently affects food availability and prices. Besides yield, nutritional value of maize grain, which is defined in terms of nutrient density and balance, is also crucial. Current research therefore focuses on enhancing nutritional value of genotypes that are high yielding and disease tolerant. Different approaches have been employed, such as conventional breeding and transgenic biofortification

(Bouis *et al*., 2011), Nazir *et al*. (2016) reported that agronomic biofortification can also enhance mineral content in staple crops.

Despite the research effort to enhance grain yield and nutritional quality, fertilizer use in Africa is relatively low (Singh *et al*., 2014) and this negatively affects agricultural productivity. According to Liverpool-Tasie *et al*. (2016), most farmers have limited access to fertilizers, and according to Odhiambo and Magandini (2008) the high cost of fertilizers places it out of the reach of most small-scale farmers who do not have access to resources. Therefore, breeding for high grain yield and nutritional quality for N stress conditions is appropriate for the smallholder farmers.

Conventional breeding methods have been used to improve yield and nutritional traits in crops. It has been reported in crops such as wheat, that when breeding for high yield, some characters such as protein content are compromised (Liu *et al*., 2014). Negative association was observed in maize for grain yield and mineral concentration (Bänziger and Long, 2000), and this makes simultaneous improvement of the traits a difficult task. According to Agrama (1996), breeding programme proficiency depends on the magnitude of the association that exists between grain yield and other components. It is therefore important that relationships between yield and quality parameters and amongst quality parameters themselves, are established. This is necessary for designing the breeding programme as well as establishing breeding objectives. Statistical analysis such as correlation, regression and PCA are approaches that can be used to establish and quantify relationships between characteristics.

In view of high malnutrition, interventions such as biofortification, for example, development of QPM, have been employed to improve nutritional quality of maize. In recognition of poor soil status in SSA, which is a common phenomenon, it is necessary to assess the performance of the QPM hybrids in terms of grain yield and nutritional quality properties and determine relationships that exist amongst them as affected by N levels and determine the best genotypes based on the synchrony of the best parameters.

6.3 Materials and methods

6.3.1 Planting locations

As described in Chapter 3 (3.3.1)

6.3.2 Planting materials

As described in Chapter 3 (3.3.2)

6.3.3 Experimental design and field management

As described in Chapter 3 (3.3.3)

6.3.4 Data analysis

Grain yield, oil content and nutritional quality traits data collected were subjected to PCA and correlation analysis by Pearson's Product Moment Correlation (PPMC) coefficient determination using GenStat (2014).

6.4 Results

6.4.1 Correlation analysis

6.4.1.1 Grain yield and nutritional quality for QPM across optimum N locations

Significant positive correlations were observed between grain yield and MRFe ($P \leq 0.05$); MRFe and MRZn ($P \leq 0.001$); MRFe and oil content ($P \leq 0.05$); oil content and tryptophan content ($P \leq 0.05$); γ zein and tryptophan content ($P \leq 0.01$); β and γ zeins ($P \leq 0.01$); Fe and Zn content ($P \leq 0.01$); phytic acid and oil content ($P \leq 0.05$); and tryptophan content and α zein ($P \leq 0.05$). Significant ($P \leq 0.001$) negative correlations were observed between α and β zeins; and α and γ zeins (Table 6.1).

Table 6.1 Pearson's correlation coefficients for 12 measured characteristics of maize hybrids across optimum N environments

Characteristics	Yield	Fe	Zn	Phytic acid	MRFe	MRZn	Oil	Tryptophan	Protein	Alpha zein	Beta zein
Fe	-0.42										
Zn	0.00	0.72**									
Phytic acid	0.08	0.24	0.41								
MRFe	0.60*	-0.25	0.08	0.53							
MRZn	0.42	-0.25	0.07	0.44	0.86***						
Oil	0.21	-0.21	0.05	0.57*	0.57*	0.55					
Tryptophan	0.09	-0.05	0.01	0.45	0.13	0.04	0.65*				
Protein	-0.18	0.21	-0.03	0.32	0.26	0.38	-0.07	-0.44			
Alpha zein	-0.06	-0.17	-0.16	-0.30	0.09	0.20	-0.27	0.65*	0.51		
Beta zein	0.11	0.02	0.07	0.11	0.07	0.11	0.29	0.55	-0.45	-0.86***	
Gamma zein	0.05	0.18	0.17	0.33	-0.11	-0.23	0.18	0.77**	-0.50	-0.99***	0.81**

***P≤0.001; **P≤0.01; *P≤0.05; MRFe = Phytic acid to iron molar ratio; MRZn = Phytic acid to zinc molar ratio

6.4.2 Principal component analysis

6.4.2.1 Performance of maize hybrids across optimum N environments

PCA results show that four principal components (PC) were significant, having eigenvalues greater than 1 (Yong and Pearce, 2013) and explained 84.60% of the variation in the genotypes. PC1 and PC2 explained 58.72% of the variation as shown in Figure 6.1. Beta zein, γ zein and tryptophan content contributed significantly to PC1 (their eigenvectors were more 0.3) (Richman, 1988), and explained 34.22% of the variation in the genotypes. MRFe, MRZn, phytic acid and oil content contributed significantly to PC2, which explained 24.50% of the variation in the genotypes. The PC3 and PC4 contributed 17.44% and 8.44% of variation, respectively. The main characteristics that significantly contributed to PC3 was yield while yield and Zn content contributed most to PC4.

PCA, which is also visual presentation of relationships amongst parameters, is shown in Figure 6.1. Strong positive relationships as exhibited by small angles of less than 60^{o} were observed between MRFe and MRZn; oil and tryptophan content; tryptophan and Zn content; phytic acid and oil content; γ zein and tryptophan content; β and γ zeins; and tryptophan content and β zein. Strong negative relationship was observed between α and γ zeins; α and β zeins; α zein and tryptophan content; protein and Zn content; and protein and tryptophan content as exhibited by more than 90^{o} angle difference between characteristics.

Table 6.2 Principal component analyses showing eigenvectors and eigenvalues for the 12 measured characteristics in maize hybrids across optimum N environments

Characteristics	**Eigenvectors**			
	PC 1	**PC 2**	**PC 3**	**PC 4**
Yield	0.07	0.29	0.30	0.67
Fe	0.04	-0.16	-0.63	0.11
Zn	0.11	0.00	-0.54	0.50
Phytic acid	0.21	0.36	-0.35	-0.17
MRFe	0.03	0.55	0.03	0.15
MRZn	-0.09	0.47	-0.03	0.04
Oil	0.25	0.39	0.06	-0.26
Tryptophan	0.43	-0.01	0.07	-0.27
Protein	-0.27	0.26	-0.30	-0.32
Alpha zein	-0.47	0.12	0.02	0.01
Beta zein	0.40	-0.03	0.08	0.07
Gamma zein	0.47	-0.12	-0.03	-0.02
Eigenvalues	**4.11**	**2.94**	**2.09**	**1.01**
Individual %	**34.22**	**24.50**	**17.44**	**8.44**
Cumulative %	**34.22**	**58.72**	**76.16**	**84.60**

MRFe = Phytic acid to iron molar ratio; MRZn = Phytic acid to zinc molar ratio; PC = Principal component

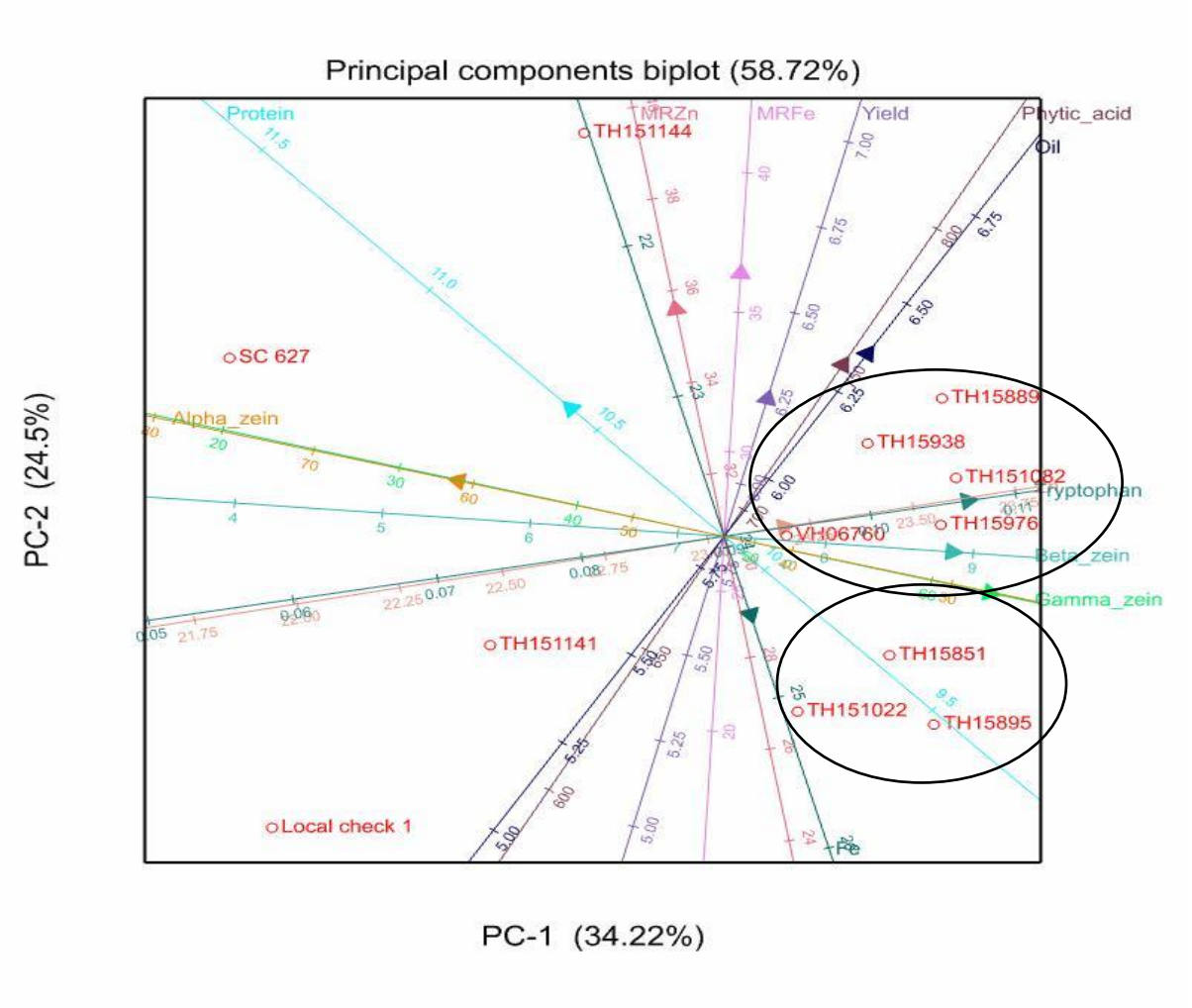

Figure 6.1 Principal component biplot showing the distribution of 12 maize hybrids for 12 measured traits across optimum N environments

6.4.1.2 Grain yield and nutritional quality for maize hybrids under low N condition

Significant positive correlations were observed between phytic acid and MRFe ($P \leq 0.05$); MRFe and β zein ($P \leq 0.01$); phytic acid and Zn content ($P \leq 0.01$); MRFe and β zein ($P \leq 0.01$); MRZn and γ zein ($P \leq 0.05$); protein content and α zein ($P \leq 0.05$); and MRZn and oil content ($P \leq 0.05$). There were significant negative associations between α and γ zeins ($P \leq 0.001$); Fe content and MRFe ($P \leq 0.01$); oil and protein content ($P \leq 0.05$); and MRZn and α zein ($P \leq 0.05$). Positive correlations were observed between Fe content and β zein; phytic acid and tryptophan content; and oil content and γ zein content, but were insignificant. There were negative correlations between protein content and γ zein; oil content and α zein; and phytic acid and α zein; but they were not significant (Table 6.3).

Table 6.3 Pearson's correlation coefficients for 12 measured characteristics of maize hybrids under low N environment

Characteristics	Yield	Fe	Zn	Phytic acid	MRFe	MRZn	Oil	Tryptophan	Protein	Alpha zein	Beta zein
Fe	-0.49										
Zn	0.12	-0.04									
Phytic acid	0.09	0.04	0.71**								
MRFe	0.42	-0.73**	0.47	0.58*							
MRZn	0.08	0.08	0.08	0.75**	0.39						
Oil	-0.18	0.28	-0.03	0.43	-0.01	0.64*					
Tryptophan	0.15	0.12	0.47	0.55	0.31	0.29	0.33				
Protein	0.25	-0.32	0.31	-0.07	0.15	-0.37	-0.64*	-0.22			
Alpha zein	0.04	0.00	-0.19	-0.53	-0.42	-0.59*	-0.52	-0.24	0.57*		
Beta zein	0.04	0.56	0.23	0.23	0.72**	0.12	0.10	0.18	-0.15	-0.30	
Gamma zein	-0.06	0.09	0.14	0.49	0.29	0.59*	0.51	0.14	-0.54	-0.99***	0.08

***P≤0.001; **P≤0.01; *P≤0.05; MRFe = Phytic acid to iron molar ratio; MRZn = Phytic acid to zinc molar ratio

6.4.2.2 Performance of maize hybrids under low N environment

Based on total eigenvalue of more than 1, four principle components were identified and they explained 81.82% of the variation in the dataset (Table 6.4). However, PC1 and PC2 explained 59.92% of the variation and hence were illustrated on the biplot (Figure 6.2). PC1 explained 36.35% of the variation in the dataset where γ zein, oil content, MRZn and phytic acid content contributed most to variation. PC2 explained 23.56% of the total variation and yield, MRFe, Zn and protein content significantly contributed to this PC. PC3 and PC4 explained 13.31% and 8.60% of the variation in the dataset, respectively and Zn, Fe and tryptophan, and yield, respectively, contributed the most to these PC's.

The biplot showed that genotypes TH15851, TH151022, TH15082, TH15938 and TH15985 varied in performance but had high tryptophan, MRZn, γ zein, oil and phytic acid content. The angles between the characteristics were less than 90^{o} indicates that they were correlated. TH15976 had high grain yield, β zein content, MRFe and Zn content and had acute angles between the traits and were highly correlated, while Local check 1 had high protein content.

Table 6.4 Principal component analyses showing eigenvectors and eigenvalues for 12 measured characteristics in maize hybrids under low N environment

Characteristics	**Eigenvectors**			
	PC 1	**PC 2**	**PC 3**	**PC 4**
Yield	0.01	0.35	-0.17	0.62
Fe	0.01	-0.43	0.47	0.04
Zn	0.17	0.35	0.49	-0.06
Phytic acid	0.36	0.21	0.04	0.06
MRFe	0.23	0.46	-0.12	-0.01
MRZn	0.40	-0.12	-0.18	0.29
Oil	0.33	-0.25	0.04	-0.09
Tryptophan	0.23	0.09	0.43	-0.08
Protein	-0.28	0.39	0.18	0.18
Alpha zein	-0.43	0.06	0.16	0.01
Beta zein	0.16	0.30	-0.27	-0.67
Gamma zein	0.41	-0.12	-0.14	0.17
Eigenvalues	**4.36**	**2.83**	**1.59**	**1.03**
Individual %	**36.35**	**23.56**	**13.31**	**8.60**
Cumulative %	**36.35**	**59.92**	**73.22**	**81.82**

MRFe = Phytic acid to iron molar ratio; MRZn = Phytic acid to zinc molar ratio; PC = Principal component

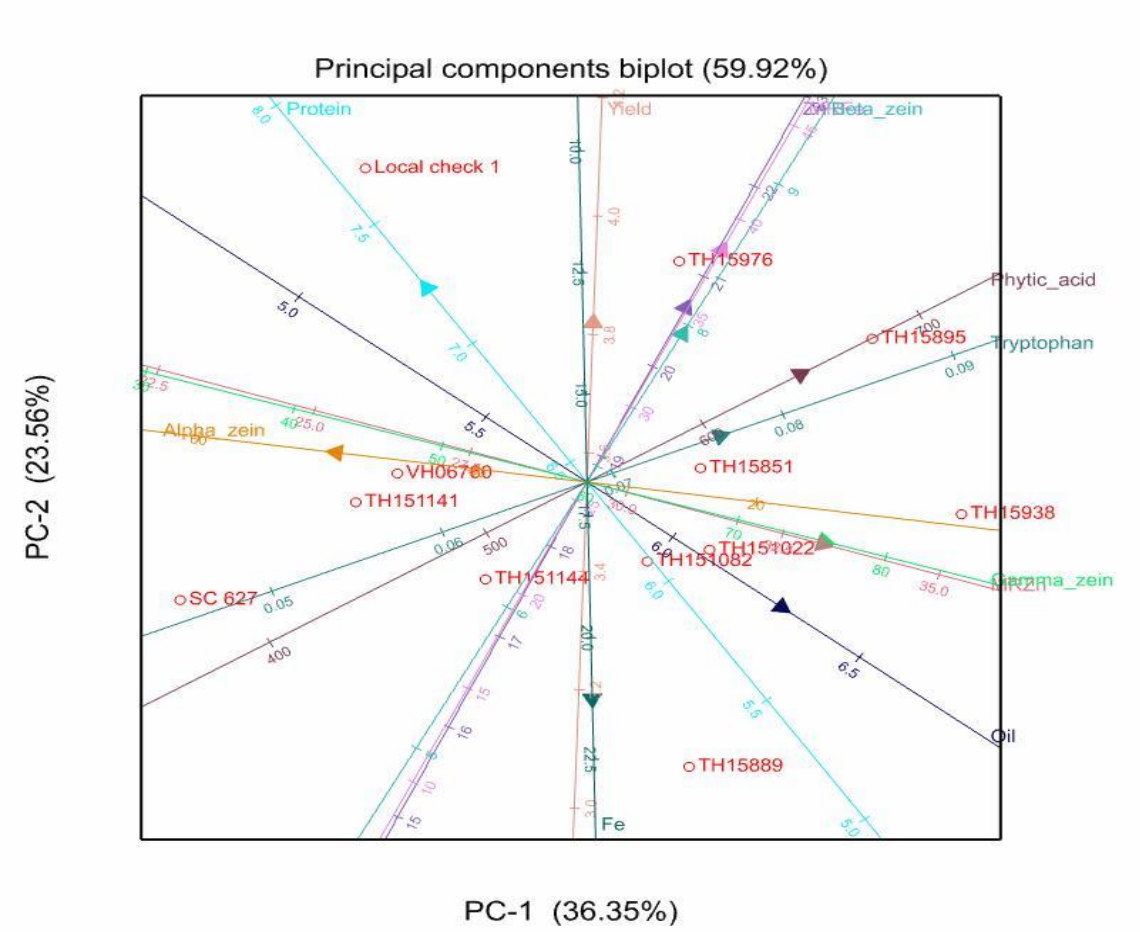

Figure 6.2 Principal component biplot showing the distribution of 12 maize hybrids for 12 measured traits under low N conditions

6.5 Discussion

6.5.1 Correlation analysis

Grain yield remains the primary and most important breeding objective in view of increasing production in relation to production resources (Zdunić *et al*., 2012). According to Tandzi *et al.* (2017), lower grain yield is the major factor contributing to low production and this is contributing to the low adoption of QPM genotypes by smallholder farmers. In addition, stress conditions such as drought and low N, which are the prevailing conditions in SSA (Ngaboyisonga *et al*., 2012), have also contributed to lower grain yields. A concentration of efforts on QPM with improved yield, nutritional attributes and adaptation to stressful conditions, is necessary to increase the adoption of QPM and hence its sustainability to combat several forms of malnutrition in developing countries. Unfortunately, grain yield and nutritional quality follow different metabolic pathways (Liu *et al*., 2014).

According to Băşa *et al*. (2016) and Marković *et al*. (2017) maize grain yield is a function of many components, including number and size of cobs, number of grains per cob and grain size. Grain nutritional quality, on the other hand, is defined in terms of nutrient density and proportion of nutrients. Simultaneous breeding for improved grain yield and quality has proved to be a challenge in crops such as wheat (Liu *et al*., 2014) due to having different metabolic and catabolic pathways. Positive correlation between yield and other nutritional factors are important for breeding purposes. Correlation analysis in the current study showed that selecting for improving MRFe and MRZn would not compromise grain yield in both low and optimum N conditions. However, increasing grain yield, in other studies, has been noted to compromise Fe and protein content (Barcutcular *et al*., 2016), making it difficult to combine the two breeding objectives in a breeding programme. In fact, they found a cause-effect relationship between grain yield and protein, and concluded that the increase in total protein decreases starch accumulation and hence results in low yield.

Bänziger and Long (2000) and Brkić *et al*. (2003) observed negative association between grain yield and Fe and Zn content, which is similar to the present study for Fe, but not for Zn, as no association existed between grain yield and Zn content under optimum conditions, and a weak positive correlation of 0.12 was observed under low N conditions, which suggests the possibility of enhancing the traits together without negative related effects. According to Murphy *et al*. (2008), grain yield positively correlated with Fe and Zn in 63 wheat cultivars, which is similar to present study under optimum N conditions, however this is contrary to the findings under low N where negative relationship was observed. They further suggested that the relationship observed is usually based on the genotypes used in the specific study. Lasztity *et al*. (1992) on the other hand, reported that increase in N increased both grain yield and total protein but decreased the essential to non-essential amino acid ratio.

In the present study, yield positively correlated with phytic acid, even though the relationship was not significant, in contrast to Pilu *et al*. (2005) and Mladenović-Drinić *et al*., (2009) who reported negative correlation between grain yield and phytic acid content. Mladenović-Drinić *et al*. (2009) found positive association between grain protein and phytic acid in their studies and similar results were observed in the present study across optimum N conditions, however the relationship was insignificant, and this contradicts the findings under low N conditions, where a weak negative correlation was seen. According to O'Kennedy (2011), protein content was significant and negatively correlated with β and γ zein and similarly, negative relationship

was observed between protein content with β and γ zein under low and optimum N conditions, although the correlation was not significant.

Tryptophan is one of the essential amino acids that the human and animal body does not synthesize but obtains from food. Its low concentration in cereals, such as common maize, makes it one of the limiting amino acids (Vivek *et al*., 2008). QPM has double the amount of tryptophan of non-QPM and hence is nutritionally superior to common maize. According to Ngaboyisonga *et al*. (2012), a decrease in maize grain yield is associated with an increase in tryptophan content, so much so that low N increases tryptophan content. In this study, a weak positive relationship between grain yield and tryptophan content, which was not significant under both low and optimum N conditions, was seen, suggesting that tryptophan is insensitive to N levels, which agrees with results reported by Kostadinović *et al*. (2016). The differences in the results could be attributed to varying weather conditions such as precipitation, which may also play a role in the modification of the *o*2 endosperm modifier, which affects lysine and tryptophan content. This was observed by Wegary *et al*. (2011) where more endosperm modification was observed at Harare that received less rain than Bako.

Tryptophan content was weakly and insignificantly associated with both Zn and Fe content, under both low and optimum N conditions. The strength of the relationship, however, increased with reduced N levels. Alpha, β and γ zeins increased with an increase in tryptophan, though the relationship weakened with reduced N levels. According to Khan *et al*. (2014) negative correlation exists between α zein and tryptophan content and a similar scenario was observed in the current study under low N conditions, although the relationship was insignificant. However significant positive association was seen between α zein and tryptophan content across optimum N conditions.

In addition to tryptophan, other relationships are worth mentioning as they affect protein quality and hence grain nutritional quality. Alpha, γ and β zeins are important in defining grain nutritional quality based on storage protein content (Sofi *et al*., 2009). An increase in α zein was associated with a significant decrease in both β and γ zeins, where these two fractions were significantly positively correlated under both low and optimum N. The results were similar to that of O'Kennedy (2011) who reported a positive relationship between γ and β zein proteins and that an increase in γ content has an effect on kernel hardness.

Mineral such as Zn and Fe are amongst two of the most important minerals whose its absorption is affected by phytic acid in cereal grains such as maize, which is the staple crop to the majority of people in developing and under-developed countries. It is not surprising therefore that over 2 billion people are deficient in either Fe or Zn, or both (Chakraborti *et al*., 2011). Breeding for high Zn and Fe content in maize is therefore crucial. Fortunately, Zn and Fe content are often positively correlated as reported by Menkir (2008), Chakraborti *et al*. (2011), Liu *et al*. (2014) and, Akinwale and Adewopo (2016). The same relation was observed in this study, where Zn content was highly significantly associated with Fe content ($r = 0.72$). Unfortunately, high Fe content was associated with low grain yield, although this was not the case for Zn content under low and optimum N conditions, even though low N weakened the relationship between Zn content and grain yield as was also reported by Chakraborti *et al*. (2009). Insignificant negative relationships were also reported between grain yield and micronutrients by Bänziger and Long (2000), and Akinwale and Adewopo (2016).

It has therefore, been noted that despite having different pathways, yield performance positively correlated with most of the characters studied, except for Fe, oil and γ zeins under low N and Fe, protein and α under optimum N conditions. Most of the relationships were statistically insignificant, hence are pseudo relationships, especially between grain yield and oil, γ zein, protein and α zein, which also changed with changing N levels. The relationship could be attributed to chance, except for grain yield and Fe where the relationship was maintained under both N levels.

Tryptophan, one of the key elements in QPM, positively correlated with all characters in the study except with Fe and protein under optimum N, even though the relationships were insignificant, except for oil and tryptophan content. This contradicts findings of Pixley and Bjarnason (2002) and Ngaboyisonga *et al*. (2012) who reported a significant positive relationship between tryptophan and protein content. Tryptophan was positively correlated with phytic acid, the micronutrient inhibitor. Hambidge *et al*. (2004) found an increase in mineral bioavailability in diets with reduced phytic acid content, especially in *lpa* mutant maize. Unfortunately, altering N levels did not affect the relationship. Phytic acid content was associated with low Fe content, which could worsen malnutrition due to Fe deficiency.

6.5.2 Principal component analysis

PCA was used to identify best performing genotypes based on the common characteristics under investigations. Genotypes VH06760, TH15976, TH151082, TH15938 and TH15889 were grouped based on high tryptophan, β and γ zeins and the traits were highly correlated. Genotypes TH15851, TH151022 and TH15895 were grouped together based on high Fe, γ and β zein values, but were low in protein content under optimum N conditions. Molar ratios were closely interrelated and related with grain yield, which was also seen in correlations. Local check 1 and SC 627 were totally different from the other hybrids and SC 627 had high accumulation of α zein. High content of α zein in SC 627 is not surprising, since non-QPM varieties has high levels of zeins and low proportion of non-zeins affecting its nutritional quality (Pachon *et al*., 2009). Under low N, TH151022, TH151082 and TH15938 had high levels of γ zein, oil content and MRZn and these traits were highly correlated, and an inverse relationship was observed between these traits and α zein. High phytic acid and tryptophan content was observed in TH15851 and TH15895, and the traits were highly correlated. Genotypes TH151141 and V06760 had high amount of α zein protein and low β zein and MRZn. This is contrary to results reported by O'Kennedy (2011) who observed high α and δ zein proteins that were highly correlated in different hybrids.

Genotypes behaved differently under different N environments. This agrees with Bänziger and Long (2000) who reported that genotype responses are based on environmental conditions. However, TH151082, TH15938 maintained high levels of tryptophan, phytic acid, γ zein and oil content under all tested environments. Tryptophan, oil, γ zein and phytic acid content explained most of the variations in the genotypes in both low and optimum N environments. The traits that are close to the origin of the biplot are not significant and usually do not contribute to variations observed in the dataset.

6.6 Conclusions

PCA showed that genotypes TH151082 and TH15938 maintained high values of nutritional characteristics like tryptophan, γ zein, phytic acid and oil content and showed the same relationship under low and optimum N conditions. Relationships observed between grain yield and nutritional traits in this study are of great importance, because it indicates the possibility of improving the specific nutritional traits and yield simultaneously in breeding efforts. However, these results are for a single season of experiments, hence the need to repeat trials over locations and seasons to confirm the conclusions. Again, sample size used in the data

collection have an impact on the correlation results, hence it is necessary to increase sample size and see the outcome of the correlation analysis which would help in results confirmation.

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CHAPTER SEVEN

GENERAL CONCLUSIONS AND RECOMMENDATIONS

In SA and SSA, maize is the major source of calorie, protein and minerals to rural and urban communities and is a raw material for the production of different products such as vegetable oil and starch. Despite its importance, maize is low in mineral content and contains high phytic acid that hinders mineral absorption besides lacking essential amino acids lysine and tryptophan. Consequently, there is high levels of malnutrition illnesses and malnutrition related deaths. Nutritional quality improvement in maize is therefore as important as improving grain yield in addressing food insecurity and hidden hunger that affect billions of people globally. In view of this, CIMMYT developed QPM genotypes which have increased level of lysine and tryptophan. Unfortunately, most small-scale farmers in Africa produce maize in low N conditions. Therefore, this research evaluated 10 QPM and two non-QPM hybrids obtained from CIMMYT-Zimbabwe with the following objectives: to determine the influence of different production environments on tryptophan and oil content, and grain yield in QPM hybrids; to determine the effect of different environments on Fe, Zn and phytic acid content in QPM hybrids and their bioavailability; to determine the influence of environmental conditions on protein quality and quantity of QPM hybrids and to determine the relationship between grain yield and nutritional characteristics under low and optimum N conditions.

Generally, environmental conditions affected grain yield and nutritional characteristics. The concentration of tryptophan, oil and grain yield were low at Harare (low N) compared to optimum N conditions. The averages were 0.076%, 5.76% and 3.55 ton ha^{-1} in low N conditions for tryptophan, oil and grain yield, respectively whereas optimum N conditions, the averages were 0.089%, 5.83% and 5.85 ton ha^{-1}, respectively. Overall, tryptophan content, oil content and grain yield were reduced in low N conditions and non-QPM compared to optimum N and QPM hybrids. Although there were some genotypes performing well under low N compared to optimum N conditions such as TH15938, TH151141 and Local check 1 for tryptophan, TH15938, TH15889, TH15895, TH151022 and Local check 1 for oil content and TH15976 for grain yield.

Likewise, mineral content was reduced under low N compared to optimum N conditions. The averages for Fe, Zn, phytic acid, MRFe and MRZn under low N were 16.80 mg kg^{-1}, 18.72 mg kg^{-1}, 546.50 mg 100 g^{-1}, 25.99 and 29.36, respectively. Across optimum N conditions, the averages for Fe, Zn, phytic acid, MRFe and MRZn were 23.93 mg kg^{-1}, 23.03 mg kg^{-1}, 698.80

mg 100 g^{-1}, 26.98 and 30.64, respectively. Even though some hybrids had high mineral content under low N conditions such as TH15889 (Fe), Local check 1 and TH15851 (Zn). Overall performance shows that QPM hybrids had high contents of minerals Fe and Zn under low and optimum N conditions than in non-QPM hybrids. However, this do not necessarily mean that QPM hybrids are superior over non-QPM hybrids as grain mineral content is influenced by different factors such as mineral amount existing in the soil, growing environmental conditions and type of fertilizer applied as reported in different studies. The low molar ratios in minerals were observed in QPM and non-QPM genotypes under both soil conditions suggesting high minerals absorption.

Grain total proteins, β and α zeins were reduced under low N than optimum N conditions except for γ zeins. The decrease content of α zeins in maize endosperm under low N conditions is necessary as the non-zeins content would increase resulting in high level of lysine and tryptophan, improving maize nutritional quality. At Harare (low N) site, total protein, β, γ and α zeins averages were 4.42%, 6.88%, 59.79% and 32.16%, respectively. Across optimum N conditions, total protein, β, γ and α zeins averages were 10.12%, 7.34%, 48.29% and 44.28%, respectively. However, highest total protein content was observed in non-QPM Local check 1 and SC 627 in low and optimum N conditions, respectively.

Correlations on the traits were observed that allows simultaneous selection and breeding for such traits. The Fe content was highly significant and positively associated with Zn, oil and tryptophan content, β and γ zeins, and tryptophan and γ zein under optimum N conditions. Under low N conditions, positive correlations were observed however, most of them were weak compared to optimum N conditions. Significant positive association were observed for total protein with α zein which is not a healthy situation considering that high α zein is associated with deficit in lysine and tryptophan content in maize kernels. Zinc and phytic acid content positive relationship would enhance micronutrient deficiency since phytic acid inhibit mineral absorption. Negative correlations between α and β zeins, α and γ zeins, and grain yield and Fe content under low and optimum N conditions were observed. PCA revealed that some traits were highly correlated such as phytic acid, tryptophan, oil and γ zein in low and optimum N conditions in hybrids TH15938 and TH151082, and this suggests these traits can be concurrently improved in these genotypes.

The results indicated that grain yield and grain nutritional quality were negatively affected by low N soil conditions compared to optimum N and this was confirmed in other studies. This is

possible because N is one of essential elements required in large quantities for crop growth and development as reported in different studies. Since N is a major component for amino acids and protein, a decrease in content for these traits under low N conditions is expected and this is confirmed in different studies. However, response of different maize hybrids under low N conditions differs, hence some specific hybrids-maintained grain yield and nutritional characteristics under these conditions.

This study has revealed that QPM hybrids generally were superior in terms of grain yield and nutritional quality traits in both soil conditions except in few cases where non-QPM outperformed QPM hybrids. It has also been noted that some QPM hybrids gave low grain yield and grain nutritional parameters under optimum N conditions which shows that genotypic effect had a role to play rather than environment alone. Therefore, results of the study revealed the importance of evaluating different maize hybrids in contrasting soil conditions in order to identify and recommend specific hybrids for specific soil environment to the benefit of the end users.

Genetic variation has been observed in all the traits under investigation in the study for single and combined ANOVA in tested maize hybrids. Large genetic variations observed in most of the traits under low and optimum N conditions signify the possibility of selecting and/or breeding QPM hybrids that are tolerant in such environmental conditions. Significant location indicates that sites were different from each other and significant GEI shows that hybrids performance was changing in different locations, hence they ranked differently in the environments. This depicts the importance of evaluating genotypes in different environments to determine superior hybrids with the associated traits.

In conclusion, the results of the study shows that most QPM hybrids outperformed the non-QPM in most of the traits investigated. Some genotypes have been identified that can be grown in low N conditions without significant reduction in grain yield and nutritional traits and this is appropriate for small-scale farmers that produce their crops under low N environment as they will eventually reduce micronutrient deficiency since they depend on maize as staple diet. The trials were for one year and they have to be repeated for more seasons to confirm the results. Again, planting experiment in different agro-ecological zones to assess the stability of the genotypes for all measured traits. Balancing the sites for low and optimum N conditions is crucial to determine superior genotype for the traits investigated across the environments, which has not been possible in this study.