Abiotic stress tolerance and nutritional traits of newly developed quality protein maize hybrids in sub-Saharan Africa

by

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Submitted in accordance with the academic requirements for the degree of

Philosophiae Doctor (PhD)

in the Department of Plant Sciences (Plant Breeding)

in the Faculty of Natural and Agricultural Sciences at the

University of the Free State, Bloemfontein,

South Africa

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July 2022

SUMMARY

Drought and poor soil fertility are some of the most serious maize production challenges in sub-Saharan Africa (SSA). Identification and development of quality protein maize (QPM) cultivars that have high yield potential and tolerance to these stresses is a reliable and affordable option to improve food security and malnutrition problems in the region, especially for small scale farming communities. Although several stress tolerant maize varieties have been released and disseminated for commercial production in SSA so far, limited development and release of stress tolerant and high yielding QPM varieties compared to normal maize varieties is evident. Limited attention has also been given to the development of nutritionally enriched varieties compared to grain yield improvement. Therefore, the main goal of this study was to study 40 newly developed QPM hybrids obtained from the International Maize and Wheat Improvement Center (CIMMYT) – Zimbabwe, under stressed and non-stressed environments to allow selection of QPM hybrids that could outperform the existing commercial QPM and normal maize cultivars with respect to grain yield and concentrations of tryptophan, iron (Fe), zinc (Zn) and molar ratios of Fe and Zn to phytic acid. The specific objectives were: (1) to determine variability and performance of QPM hybrids for grain yield and agronomic traits under stressed and non-stressed environments, (2) to determine tryptophan, Zn and Fe concentrations, and molar ratios of Zn and Fe to phytic acid in QPM hybrids grown under stressed and non-stressed environments, (3) to analyse genotype by environment interaction and grain yield stability of QPM hybrids and (4) to determine correlations among grain yield, agronomic and nutritional traits in QPM hybrids evaluated under stressed and non-stressed environments.

Significant variation was seen for grain yield, and almost all studied agronomic and nutritional traits under stressed and non-stressed environments. Phenotypic coefficient of variation (PCV) was higher than genotypic coefficient of variation (GCV) for grain yield and all other agronomic and nutritional traits under all conditions, indicating that environment effect was higher than genotype effect on the expression of the traits under stressed and non-stressed environments. Broad sense heritability of grain yield was higher than 0.6 across all environments, with the exception of managed drought conditions. Anthesis silking interval (ASI) had relatively high GCV estimates and genetic advance, as a percentage of the mean, across all conditions. This indicated that the presence of sufficient genetic variability among genotypes can improve synchronization under different management conditions through

selection. Grain yield was reduced by 47% under random stress, 68% under managed drought and 71% under low N conditions. Protein and tryptophan concentrations in the grain were decreased by 36.0% and 21% respectively under low N conditions and Fe and Zn concentration also decreased by 48% and 36% under low N stress and 63% and 9% under random stress, respectively. Some QPM hybrids showed better or comparable performance in terms of grain yield potential and nutritional quality traits compared with the best QPM and normal maize checks under different management conditions, indicating the genetic gain that has been made in the QPM breeding programme. Based on Additive Main effect and Multiplicative Interaction (AMMI) analysis and Genotype and Genotype by Environment interaction (GGE) biplot analysis entries 10 (CZH142238Q) and 14 (CZH15142Q) under optimum; 23 (CZH17192Q) under random stress; 19 (CZH17188Q) and 40 (CZH17209Q) under managed drought and 14 (CZH15142Q) under low N were the most stable and the highest yielding hybrids. Environments Kwekwe (KW), Bindura (BIN), Chokwe (CHO) and Bako (BK2) were identified as discriminating and representative sites for optimum conditions, random stress, managed drought and low N stress conditions, respectively, therefore these environments are promising for selecting well adapted genotypes in the respective management conditions. Grain yield was significant and positive correlated with number of ears per plant and negatively with days to anthesis and silking under low N stress. This confirmed the importance of these secondary traits in developing high yielding and early maturing genotypes. Grain yield was not significantly correlated with most of the nutritional quality traits under all management conditions, indicating a lack of common genes for simultaneous improvement of grain yield and these nutritional traits. Significant and positive correlations were observed between Fe and Zn under low N and random stress conditions.

Keywords: Bioavailability, drought, grain yield, G × E interaction, low N, malnutrition, minerals, QPM, stability

DECLARATION

I, Bitew Tilahun Engida, declare that this thesis for the degree of Philosophiae Doctor (PhD) in Plant Breeding in the Department of Plant Sciences, Faculty of Natural and Agricultural Sciences, at the University of the Free State (UFS) is my own work and has never been submitted by myself at any other university for the award of another degree qualification. The sources of materials and financial support have been acknowledged. In addition, I also agree that the UFS has the sole right to the publication of this thesis.

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1 July 2022

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Signature Date

DEDICATION

This work is dedicated to my family for their devotion and efforts in the success of my life

ACKNOWLEDGEMENTS

I would like to express my respectful and special thanks to my major promoter Prof. Maryke Labuschagne for her kindness, close supervision, constructive comments, and suggestions that leading to the success of this thesis. I would also like to express my sincere gratitude to co-promoters Dr. Amsal Tarekegn and Dr. Dagne Wegary for their advice and encouragement. They have helped me in different ways including arranging of research materials used in this study and sharing all necessary information and data of the trials conducted in different locations in eastern and southern Africa.

My special thanks go to co-promotor Dr. Angeline van Biljon for her close supervision of the laboratory work and valuable comments and suggestions that contributed to the success of this thesis. A heartfelt appreciation to Boingotlo Tshabang for his unreserved assistance during Fe and Zn analysis at the soil science laboratory, University of the Free State.

My sincere thanks to the UFS for hosting the study and financial support of my studies through the NRF SARChI chair of Prof. Maryke Labuschagne. I am also thankful to the Ethiopian Institute of Agricultural Research (EIAR) and Bako National Maize Research Center (BNMRC) for providing me study leave to continue my PhD study and support in different ways during the period of conducting the field experiments in Ethiopia.

All staff members of BNMRC and Ambo Agricultural Research Center in the maize breeding programme is highly acknowledged for their assistance in the conducting, monitoring and data collection of the trials. I extend my special thanks to Mr. Demoz Negera, CIMMYT-Ethiopia for his support in data collection and preparing seed samples needed for laboratory work. Melkasa and Hawasa Agricultural Center maize researchers and technical assistance are also acknowledged, although the data of the trials taken from these Centers were not used in this study.

I would like to thank Dr. Berhanu Tadesse and Mr. Beyene Abebe, the former and current Center Directors of Bako National Maize Research Center, respectively and Endale Tadesse, human resource management representative for facilitating administrative and other services required for my study. I also want to say thank you to Tolera Keno for his advice in data analysis and other technical aspects of this work.

My appreciation and heartfelt thanks go to all my friends and colleagues who provided me consistent moral support and motivation during my study. I express special thanks to Dr. Tesfaye Walle, post-doctoral student at the UFS, he made my life happy during when we stayed together at the campus. Prof. Edlegnaw Wale Zegeye and Dr. Achamyeleh Girma and his wife deserve thanks for making me feel at home during my stay at the UFS. The UFS Plant Sciences Department students Dr. Neila, Drikus and Augustine and others, I want to say thank you for your cooperation and assistance, academically and socially.

My gratitude goes to all the lecturers and staff members in the Department of Plant Sciences, particularly, Orpah Taylor for her kindness and help in managing all my administrative matters throughout my study period at the UFS.

My greatest thanks extended to my wife, Desta Wendimu for her patience and responsibility in taking care of our son, Leul during my study period.

Finally, I thank the Almighty God for standing by my side all the way and giving me strength and allowing me to reach this level.

ABBREVIATIONS

AEC	Average environment coordinate
AM1	Ambo
AMMI	Additive main effect and genotype by environment interaction
ANOVA	Analysis of variance
ASI	Anthesis silking interval
ASV	AMMI stability value
AW	Atomic weight
BC	Back cross
BIN	Bindura
BK2	Bako
BNMRC	Bako National Maize Research Center
CAAS	Chinese Academy of Agricultural Science
CHI	Chinhoyi
CHO	Chokwe
CHS	Chisumbanje
CHT	Chitala
CIMMYT	International Maize and Wheat Improvement Center
cm	Centimeter
CM	Conventional maize
Cu	Copper
CV	Coefficient of variation
DA	Days of anthesis
dH_2O	Distilled water
ddH_2O	Double distilled water
Df	Degrees of freedom
DNA	Deoxyribonucleic acid
DS	Days to silking
DW	Dry weight
EH	Ear height
EIAR	Ethiopian Institute of Agricultural Research
EPO	Ear position
EPP	Number of ears per plant
ESA	Eastern and Southern Africa
FAOSTAT	Food and Agriculture Organization Corporate Statistical Database
Fe	Iron
G × E	Genotype by environment interaction
G × Y	Genotype by year interaction
g	Gram
GA	Genetic advance
GAM	Genetic advance in percent
GCV	Genotypic coefficient of variation
GGE	Genotype main effect and genotype by environment interaction

GLS	Gray leaf spot
GS	Genomic selection
GWAS	Genomic wide association
GY	Grain yield
H^2	Broad sense heritability
H_2SO_4	Sulphuric acid
ha	Hectare
HAR	Harare
HCl	Hydrochloric acid
HNO_3	Nitric acid
IFPRI	International Food Policy Research Institute
IITA	International Institute of Tropical Agriculture
IPCA	Interaction principal component analysis
kg	Kilogram
KW	Kwekwe
LIC	Lichinga
LIO	Lionsden
LSD	Least significant difference
LUS	Lusaka
m	meter
MAG	Magobo
MAK	Makoholi
MAS	Marker assisted selection
MET	Multi-environment trials
Mg	Microgram
mg	Milligram
MKE	Msekera
Mm	Millimeter
Mn	Manganese
MPO	Mpongwe
MRFe	Phytic acid to iron molar ratio
MRZn	Phytic acid to zinc molar ratio
MSE	Managed stress environment
MWPA	Phytic acid molecular weight
N	Nitrogen
NARS	National Agricultural Research System
NIR	Near infrared transmission spectroscopy
Nm	Nanometer
NP	Number of plants
NRF	National Research Foundation
NUE	Nitrogen use efficiency
O2	*Opaque 2*
OD	Optical density
OPV	Open pollinated variety

P_2O_5	Diammonium phosphate
PA	Phytic acid
PC	Principal component
PCA	Principal component analysis
PCR	Polymerase chain reaction
PCV	Phenotypic coefficient of variation
PH	Plant height
PVA	Provitamin A
QI	Protein quality index
QPM	Quality protein maize
QTL	Quantitative trait loci
RAT	Rattray-Arnold
REML	Residual maximum likelihood
SARChI	South African Research Chairs Initiative
SAS	Statistical analysis software
Sen	Leaf senescence
SNP	Single nucleotide polymerase
SSA	Sub Saharan Africa
SSR	Simple sequence repeat
SVD	Singular value decompositions
t ha^{-1}	Ton per hectare
TCA	Trichloroacetic acid
TLB	Thurcicum leaf blight
UFS	University of the Free State
UMB	Umbuluzi
UN DESA	United Nations Department of Economics and Social Affairs
WHO	World Health Organization
ZAM	ZamSeed Farm
Zn	Zinc
σ^2A	Additive variance
σ^2D	Dominance variance
σ^2e	Error variance
σ^2g	Genotypic variance
σ^2I	Epistatic variance
σ^2p	Phenotypic variance
µg	Microgram
°C	Degree Celsius

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CHAPTER 1

Introduction

1.1 General background

Maize [*Zea mays* (L.) 2n = 20] belongs to the grass family (Graminae or Poaceae) and is a naturally cross-pollinating crop. Domestication of maize began 6,000 years ago and it has since rapidly increased its dominance all over the world (Pingali, 2001). It was introduced into Africa in the early 1500's by Portuguese traders (Dowswell et al., 1996) and became a popular food crop on the continent. Its popularity is largely associated with its high yield potential and diverse functionality as a food source for both humans and animals (Nuss and Tanumihardjo, 2010). It is also widely adapted and grown at varying altitudes and latitudes and cultivated in diverse ecological conditions ranging from cool to very hot temperatures, high rainfall to semi-arid areas, and in various types of soil (Shiferaw et al., 2011). Maize has become the second most cultivated cereal crop worldwide after wheat in terms of area coverage and the first in terms of production (FAOSTAT, 2020). The total maize production in the world in 2020 was around 1.16 billion ton from 202 million hectares (ha) (FAOSTAT, 2020). Of this, 90 million metric ton, or around 8% of world production from 21% of the area was in Africa (FAOSTAT, 2020). Among African regions, eastern and southern Africa (ESA) are the major maize production regions with 38% and 27% of production, respectively (FAOSTAT, 2020).

Maize is a primary staple food crop and a source of calories in the diets of more than 300 million people in sub-Saharan Africa (SSA) (Abate et al., 2017; Bankole et al., 2017; Wegary et al., 2018). In ESA regions, almost half of the calories and proteins are obtained from this crop. It also contributes a significant amount of livestock feed across the world (Tanumihardjo et al., 2020); especially in developed countries around 78% of its production serves as livestock feed (Sofi et al., 2009). Although maize is a source of essential macro and micro nutrients (Nuss and Tanumihardjo, 2010; 2011), the amount of some essential amino acids like lysine and tryptophan, and minerals such as Fe and Zn are inadequate (Prasanna et al., 2020). Thus, millions of African children and nursing mothers, who depend on maize for their daily diet, are vulnerable to malnutrition, including stunted growth, weakened immune system and impaired intellectual development (Prasanna et al., 2001; Rautiainen et al., 2016; Prasanna et al., 2020). Especially children who are lacking foods that have good levels of lysine and tryptophan,

causes kwashiorkor, a potentially fatal syndrome characterized by initial growth failure, irritability, skin lesions, oedema, and fatty liver (Vivek et al., 2008).

The discovery of the *opaque-2 (o2*) gene in the early 1960s by Purdue University researchers opened a new opportunity for developing nutritionally superior maize named quality protein maize (QPM) which has twice the amount of lysine and tryptophan of normal maize, as well as increased bioavailability (Nuss and Tanumihardjo, 2011; Maqbool et al., 2021). This mutant gene can alter the amino acid profile and composition of maize endosperm proteins which are responsible for reduction of zeins, which contain no lysine and tryptophan and increase the amount of non-zein proteins such as albumin, glutelin and globulin that contain essential amino acids (Krivanek et al., 2007; Sofi et al., 2009). However, for many years the *o2* gene had serious undesirable side effects on maize grain that made the grains taste unpleasant, and be chalky, lighter, and soft (starch granules are loosely packed) resulting in damaged kernels when harvesting, often making the kernels susceptible to cracking, ear rots, and weevils. In general, it was not acceptable for food processing and caused low yields due to low dry matter content of the grain (Maqbool et al., 2021). These were the major challenges for scientists for genetic improvement of grain yield with enhanced protein quality.

Through further research by CIMMYT and other scientists, the *o2* mutation was enhanced with modifier genes which, together, behave as a polygenic trait to produce hard kernel texture and vitreous phenotypes like that of normal (non-QPM) maize (Teklewold et al., 2015; Tripathy et al., 2017). After overcoming the problems associated with the *o2* gene, CIMMYT maize breeding programmes used the *o2* gene extensively and developed QPM donor stock with a normal looking endosperm (Vasal, 2000; Prasanna et al., 2001). Following the development of QPM donor stocks, several inbred lines and hybrids that have different genetic backgrounds (temperate, tropical and subtropical), maturity (early, medium and late maturity), grain colour (yellow and white) and texture (dent, flint, semi-dent and semi-flint) were developed (Vasal, 2000; Hossain et al., 2019; Maqbool, et al., 2021).

Many people, particularly in the developing countries, suffer from micronutrient malnutrition, caused by lack of one or more of the essential micronutrients in the diet (Menkir, 2008; Hodge, 2016). Among various essential micronutrient deficiencies, Fe and Zn are the most important. Fe deficiency can cause anemia, lack of cognitive development and growth, low reproductive performance, and diminished work capacity (Gupta et al., 2015). Zn deficiency also affects

people of all ages; however, newborn babies, young children, and pregnant and lactating women are more frequently affected, causing immune dysfunction, an increased risk of morbidity and mortality, complications during pregnancy and abnormal neuro behavioral development (Gibson, 2012; Gupta et al., 2015; Akhtar et al., 2018). Medical supply and food fortification have been attempted to solve these problems in many developing countries. However, the attempt was not successful as it was expensive and not affordable for the resource poor communities and remained a major problem in most parts of the world (Stein, 2010). Enhancing micronutrient contents through biofortification of staple food crops like maize is a sustainable and cost-effective strategy to alleviate these nutritional problems (Akhtar et al., 2018). Improving the nutritional quality of staple food like maize could, therefore, significantly impact provision of nutritionally balanced diets in developing countries, especially for disadvantaged women and children in ESA (Bänziger and Long, 2000).

In addition to improving protein quality, QPM genotypes can promote Fe and Zn absorption and increase bioavailability (Mallikarjuna et al., 2014; Prasanna et al., 2020). Chakraborti et al. (2011) reported that the concentration of Zn was higher in QPM genotypes than non-QPM genotypes. In addition, the CIMMYT biofortification breeding programme also identified a few QPM lines that can be used as source lines in pedigree-based selection to improve kernel Zn content in the tropical maize germplasm (Prasanna et al., 2020). This creates the opportunity for further investigation of QPM germplasm to identify QPM varieties that have high tryptophan content with increased levels of Fe and Zn and bioavailability of these minerals. This could improve the health status of people who consume mainly maize and thus do not have access to good sources of protein and micronutrients.

Like other types of maize, QPM production and productivity is also affected by abiotic and biotic stresses, such as low soil fertility, drought, shortage and on time unavailability of key inputs like quality seed and fertilizers, insect pests and diseases, and poor pre- and post-harvest management. Low agricultural inputs, low soil fertility and drought are the most important problems limiting maize production, food security and economic growth in developing countries (Shiferaw et al., 2011; Abate et al., 2015). Most SSA maize production areas are dependent on rain and exposed to unpredictable rainfall patterns that make maize growing environments vulnerable to drought stress (Fisher et al., 2015). Drought affects maize yield production at any stage of crop growth, but when it occurs at flowering and grain filling stages, the damage and yield reduction is significant (Magorokosho et al., 2003; Bänziger et al., 2006;

Wegary et al., 2014). Grain yield loss occurs because of premature death of plants, poor silking and anthesis synchronization during the flowering period, reduced number of ears per plant and seed size and weight, and decreased number of kernels per plant under stress conditions. Additional information on secondary traits such as anthesis silking interval (ASI), number of ears per plant, number of kernels per ear that have a strong association with grain yield under drought stress, have been used to select for drought tolerance in breeding programmes.

Low N level in the soil is the other major factor that contributes to low maize productivity in the tropics, especially in SSA where fertilizer use is very low and restorative use of crop residue relatively low and inefficient in many developing countries (Smale et al., 2011). When the plant is affected by severe N deficiency, high ASI, premature leaf yellowing followed by leaf senescence and reduced photosynthesis leaf area will occur. This causes stunted growth of the plant and results in low grain yield. It can cause up to 50% yield loss (Logrono and Lothrop, 1997). One of the most important strategies is the development of varieties with high N use efficiency (Meseka et al., 2013). CIMMYT and the National Agricultural Research System (NARS) have demonstrated and observed the existence of genetic variability among genotypes for grain yield under low N soil conditions (Worku et al., 2012; Ertiro et al., 2017; 2020). This variation appears to be the result of genotypic differences in many physiological processes and morphological features, including root architecture, N assimilation enzymes, maintenance of photosynthetic area after flowering, and remobilization of N from vegetative tissue to the grain (Worku et al., 2012; Prasanna, 2013).

Since some stresses are sporadic in nature, CIMMYT developed managed stress environments (MSEs), with both biotic and abiotic stresses, at its breeding hubs in Africa, Asia, and Latin America. Among MSEs, drought and low N managed stress screening sites are important in SSA countries to help evaluate maize genotypes (Ertiro et al., 2017). Drought stress evaluation is done by planting maize trials in the off-season and managing drought through irrigation, and omission of watering at seedling, flowering, and grain filling stages to assess drought tolerance of the varieties (Bolaños and Edmeades, 1996). Low N stress evaluations are done by using previously N-depleted plots by growing non-leguminous crops like maize with high population density (Bänziger et al., 1997). CIMMYT, in collaboration with NARS and other private partner companies, have made significant progress in the identification of drought and low N tolerant genotypes in both non-QPM and QPM genotypes (Ertiro et al., 2017; Mebratu et al., 2019). Some tolerant maize varieties have been released and disseminated in partnership with

various public and private organizations (Prasanna, 2013; Fisher et al., 2015). However, inadequate results have been published concerning the range of genetic variation, environmental effects and their interaction on grain yield potential, concentrations of tryptophan and mineral elements like Fe and Zn, and bioavailability of Fe and Zn in QPM genotypes under stressed (mainly drought and low N) and non-stressed environments. Therefore, evaluation of maize genotypes under low N and drought stress, and optimum management conditions was done to identify well performing QPM genotypes that are high yielding, with high nutritional quality and tolerance to low N and drought stress.

1.2 Aims, objectives and hypothesis

The aims of this study were to: i) develop high yielding and multi nutrient enriched QPM varieties to improve the livelihood of smallholder maize farmers suffered in food insecurity and malnutrition problems and ii) explore the effects of location and different management conditions on gain yield, agronomic and nutritional traits, and bioavailability.

The specific objectives of this study were to:

1) Determine hybrid performance and genotypic variability of QPM hybrids for grain yield and agronomic traits under stressed and non-stressed environments
2) Determine tryptophan, Zn and Fe concentrations, and the bioavailability of Zn and Fe in QPM hybrids grown under stressed and non-stressed environments
3) Analyse genotype by environmental interaction and grain yield stability of QPM hybrids
4) Determine correlations between grain yield, yield related traits, tryptophan content and bioavailability of Zn and Fe in QPM hybrids evaluated under stressed and non-stressed environments.

Hypothesis

- Significant genetic variability for grain yield and agronomic trait are available among QMP maize genotypes under stressed and non-stressed environments
- The performance of QPM hybrids for macro and micronutrients, and bioavailability is different under stressed and non-stressed environments.
- Grain yield of QPM maize genotypes is affected by different environments.

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CHAPTER 2

Literature review

2.1 Maize production and economic importance

Maize is one of the top three most important cereal crops grown throughout the world and is used for food, feed, biofuel, and raw material for many industrial products (Zhou et al., 2009; Wegary et al., 2019). It accounts for 40% of the cereal production in SSA, where more than 80% is used as food, providing at least 30% of the total calorie intake of the people, with consumption varying from 52 to 450 g per person per day (Ekpa et al., 2019; Prasanna et al., 2021). In SSA, the highest consumption rate is found in southern Africa, at 93 kg per capita followed by 56 kg in east Africa, 36 kg in central Africa and 31 kg in western Africa (FAOSTAT, 2020a). Countries like Lesotho, Malawi, South Africa, Zambia and Zimbabwe reported the highest consumption in these regions (Shiferaw et al., 2011).

The world maize production in 2020 was around 1.16 billion ton harvested from 202 million ha and it exceeded the production of wheat and rice by 50 and 52%, respectively (FAOSTAT, 2020b). In this year, Africa produced 90 million ton of maize that was harvested from 43.1 million ha. This was higher than wheat and rice, each contributing 26.9 and 38.7 million ton, respectively (FAOSTAT, 2020b). In Ethiopia, the annual maize production reached approximately 9.6 million ton annually with the national productivity of 4.2 ton ha^{-1} (FAOSTAT, 2020b). It is the second most popular staple food crop after tef (*Eragrostis tef* (Zucc.) Trotter) (Abate et al., 2015) with significant potential to feed more than 100 million people in the country (Wegary et al., 2019).

The demand for maize in the world continues to grow to feed the expanding world population (Shiferaw et al., 2011; Palacios-Rojas et al., 2020). The world population is growing at an exponential rate and reached 7.7 billion in 2019, having added one billion people since 2007 and two billion since 1994. It is expected to reach 8.5 billion in 2030 and 9.7 billion in 2050 (United Nations, Department of Economic and Social Affairs, Population Division (UN DESA), 2019). SSA will account for the highest population growth percentage in the world in the coming decades. It will increase by 52% from the current population of 1.066 billion between 2019 and 2050 (UN DESA, 2019). The demand for maize production will be triple that of the current production by the middle of the 21st century, to feed the world's growing

population (Ekpa et al., 2018). Development of maize varieties that have high yield potential and are adapted to abiotic stress conditions is a more reliable and affordable option to satisfy the demand of a growing population.

Maize contributes a significant amount of livestock feed resources. Especially in developed countries, more than 78% of maize production goes into livestock feed (Sofi et al., 2009), hence is consumed as indirect cycle products in the form of meat, eggs and dairy products (Shawa, 2019; Palacios-Rojas et al., 2020). The demand for maize for animal feed in the developing countries has also increased (Shiferaw et al., 2011). This has largely been driven by rapid economic growth in highly populated regions in Asia and Latin America, leading to increased demand due to a shift in the lifestyle of people and in feeding habits towards livestock and poultry products (Shiferaw et al., 2011; Chaudhary et al., 2012; Palacios-Rojas et al., 2020). For instance, in India maize production is increasing, with the animal feed industry consuming 13-14 million ton of maize, or over half of the country's maize production. Maize stalks are also used as fodder crop in the form of silage at the green stage and stover through direct feeding. In countries which predominantly follow a smallholder mixed farming system and where maize is predominantly used for human consumption, its residues are an important byproduct for livestock feed (Berhanu et al., 2012; Ertiro et al., 2013; Hellin et al., 2013). No targeted breeding was done to exploit the existence of varietal difference for further improvement of stover yields and stover feed quality traits; although previous research has indicated the existence of genetic variation among genotypes for feed traits (Adugna et al., 1998; 1999; Diriba et al., 2002; Ertiro et al., 2013). This indicated that a systematic genetic study and breeding effort for simultaneous improvement of food and feed traits should be incorporated as major traits during new variety development projects (Berhanu et al., 2012).

Most staple cereal crops in SSA are characterized by high carbohydrate content but are low in other nutrients like essential amino acids (such as lysine and tryptophan), minerals like Fe and Zn and vitamins like provitamin A, which are vital for human health and wellbeing. So far relatively little attention has been paid to improving the nutritional quality of these staple crops (Ranum et al., 2014; Bouis and Saltzman, 2017; Ekpa et al., 2018). Accessing affordable and highly nutritious food is very difficult in poor societies (Bouis et al., 2011). As a result, millions of African children and nursing mothers are vulnerable to malnutrition causing stunted growth, weakened immune system and impaired intellectual development, especially where maize is a staple crop and diet diversification is very limited (Prasanna et al., 2001). Improving the

nutritional quality of maize has, therefore, been a goal of maize breeding programmes of CIMMYT, the International Institute of Tropical Agriculture (IITA), and several national programmes (Prasanna et al., 2020). Significant progress has been made in the development, testing, and deployment of QPM, and provitamin A (PVA) and Zn enriched maize in SSA, Asia, and Latin America through biofortification (Maqbool and Beshir, 2019; Prasanna et al., 2020).

2.2 Quality protein maize

2.2.1 Overview of QPM development

Structurally, maize grain constitutes of 82% endosperm and the remaining 12% and 6% are the germ and pericarp, respectively (Prasanna et al., 2001). Though endosperm is the major source of carbohydrate, 80% of protein content is found in the endosperm while the rest is in the germ. The average proportion of endosperm protein fractions are 3% albumins, 3% globulins, 60% prolamins or zeins, and 34% glutelins (Vasal, 2000). All fractions other than zein are balanced and quite rich in lysine and tryptophan (Vasal, 2000). Zein is deficient in lysine and tryptophan. The poor nutritional quality of conventional maize in storage protein is related to a higher proportion of zein, and therefore, maize should be eaten with supplementary quality protein sources such as crops or animal products to alleviate the deficiency (Prasanna et al., 2001).

After the poor nutritional quality of maize became known (Osborne and Mendel, 1914), breeders made a serious effort to improve it through conventional breeding methods. A large number of elite maize germplasm and accessions were screened to identify superior cultivars for this trait. However, because of the lack of a simple genetic system, it is impossible to use direct backcross breeding to improve lysine and tryptophan content of maize (Sofi et al., 2009). A few years later, a naturally existing mutant gene *opaque* 2 (*o2)* was discovered, with improved protein content, which had double the lysine and tryptophan content in the endosperm as compared with conventional maize genotypes. This created an exciting opportunity for researchers to improve maize protein composition of the endosperm, that could help to improve the nutritional status of maize consumers, especially in the developing countries (Vasal, 2000; Vivek et al., 2008; Wegary et al., 2011). Through extensive manipulation of the *o2* gene, QPM germplasm was developed that have homozygous recessive *(o2o2)* genes, different from the homozygous dominant *(O2O2)* genes in conventional maize. QPM development includes manipulation of enhancers of the *o2* containing endosperm, increasing lysine and tryptophan levels, and manipulation of genes that modify the *o2* to confer

either soft endosperm or hard endosperm (Vivek et al., 2008). CIMMYT remained the major source of global QPM germplasm by developing QPM germplasm with the *o2* gene, incorporated along with modifiers (Twumasi-Afriyie et al., 2011). Several QPM populations and pools adapted to different ecological conditions, having different maturity groups, grain colour, and texture were developed (Prasanna et al., 2001). A few advanced maize populations in CIMMYT's maize breeding programme were successfully converted to QPM and a few varieties were released and deployed in collaboration with CIMMYT and NARS in SSA and Asian countries (Prasanna et al., 2001; Vivek et al., 2008; Teklewold et al., 2015).

2.2.2 Nutritional benefits of QPM

Malnutrition is a persistent problem in Africa, especially in rural areas where maize is used as the main source of carbohydrate and protein and people have limited access to a diverse diet (Prasanna et al., 2001; Vivek et al., 2008; Tandzi et al., 2017). Biofortified crops like QPM has significant nutritional benefits in combating malnutrition if it is produced and consumed in sufficient quantities (Krivanek et al., 2007; De Groote et al., 2010; Tandzi et al., 2017). It can provide a more balanced protein for humans and other monogastric animals. The quality and biological value of its protein are much higher than that of conventional maize. For example, the biological value of common maize protein is equal to about 40% of the biological value of milk protein, whereas the biological value of QPM protein is about 90% of that of milk protein (Nuss and Tanumihardjo, 2011). Thus, the nutritional benefits of QPM approach those of milk protein, a common standard of nutritional excellence.

Nutritional benefits of QMP germplasm have been demonstrated in livestock and children feeding experiments. Infant feeding experiments with QPM have repeatedly shown that QPM used as a weaning food in different SSA countries reduces stunting, increases weight and height, thus improving child health (Vivek et al., 2008; Teklewold et al., 2015). Studies conducted in western Ethiopia, where maize is a dominant crop, showed the positive effects of QPM on both height and weight of the children (Akalu et al., 2010; Teklewold et al., 2015). QPM feed trials conducted on pigs showed a significant growth rate as compared with pigs fed conventional maize, and they grew twice as fast as those fed conventional maize (Vivek et al., 2008). Nutritional experiments conducted on poultry and pigs at the Chinese Academy of Agricultural Science (CAAS) also confirmed the superiority of QPM over conventional maize in improving the growth and performance of animals (Qi et al., 2004). QPM can be used as supplementary food for humans that are more vulnerable to malnutrition, like pregnant women,

lactating mothers, and young children (Prasanna et al., 2001; Vivek et al., 2008). QPM flour can also be very useful for refugees and other people facing nutritional problems around the world (Tandzi et al., 2017).

In addition, different studies also reported that QPM genotypes with *o2* alleles are indicated as a good source of essential micronutrients like Fe and Zn, low phytate (for increased bioavailability of nutrients) compared to conventional maize genotypes (Bänziger and Long, 2000; Chakraborti et al., 2009; 2011a; Prasanna, 2013; Prasanna et al., 2020). The CIMMYT biofortification breeding programme also identified three QPM lines, CML176, CML491, and CML492 to be used as lines in pedigree-based selection to improve kernel Zn content in tropical maize germplasm (Prasanna et al., 2020). This indicates the possibility to develop maize genotypes that have multiple nutritional quality traits.

2.2.3 The *opaque-2* gene

The *o2* gene is the central component of the genetic system that controls storage protein genetic transcription in maize kernels, and the allele is inherited and presented in a simple recessive homozygous form *(o2o2)* (Teklewold et al., 2015). It causes a reduction in zein storage protein content in the endosperm and has an important role in increasing the non-storage protein fraction (non-zein), which is nutritionally more balanced. Zein storage protein comprises of alpha (α), beta (β), gamma (γ) and delta (δ) zeins aggregated in a distinctive spatial pattern within the protein body (Sofi et al., 2009). Each of these vary in molecular mass, solubility in alcohol and other aqueous solutions and amino acid sequences. The α-zeins are the largest fraction and are rich in glutamine, leucine and proline, but are deficient in essential amino acids lysine and tryptophan (Tripathy et al., 2017). On the other hand, the δ-zeins are the smallest fraction, however, they are rich in lysine and tryptophan. The *o2* gene doubles the lysine and tryptophan content in the endosperm by decreasing the synthesis of zein proteins (Harvey, 2007; Tripathy et al., 2017).

2.2.4 Negative effects of the *opaque-2* gene

Despite *o2* maize being nutritional superior, initially, it was not popular with farmers or consumers, mainly due to the pleiotropic effects of this mutated gene that brought negative effects on the physical properties of the endosperm and other important agronomic traits (Duarte et al., 2004; Lealem, 2017). It adversely affected several traits, such as causing kernel damage, making it more prone to insect pests and diseases such as weevil and ear rot, and

generally reducing grain yield (Prasanna et al., 2001). In addition, the taste and appearance of the kernels were unacceptable to consumers, who ultimately rejected the enhanced-protein varieties in the market (Krishna et al., 2017). Scientists at CIMMYT identified various endosperm modifier loci called *o2* modifiers (Mo2s) that could favourably change the kernel characteristics, thereby overcoming negative features of the opaque kernel phenotype while maintaining lysine and tryptophan levels (Vasal, 2000; Krivanek et al., 2007). These modifier genes do not have any effect on yield but interact to convert the softness of endosperm to a vitreous phenotype like conventional maize, increasing kernel weight and density (Sofi et al., 2013; Teklewold et al., 2015), thereby increasing grain yield production. Finally, selection for hard and vitreous endosperm modification was rapidly incorporated into *o*2 breeding schemes and initial QPM breeding efforts at CIMMYT focused on conversion of subtropical and tropical lowland adapted normal maize populations to *o2* versions through backcross-recurrent selection procedures, with a focus on accumulating the hard endosperm phenotype, maintaining protein quality and increasing yield and resistance to ear rot (Villegas et al., 1992; Krivanek et al., 2007).

2.2.5 QPM genetics and breeding strategy

Research on QPM initially emphasized the development of donor stocks by selection of modified grain texture in QPM backgrounds using various selection schemes (Vasal, 2000; Babu and Prasanna, 2014). This was followed by large scale conversion of normal maize with a wide array of genetic backgrounds from different agro-climatic zones into QPM using these donor stocks (Krivanek et al., 2007; Sofi et al., 2013; Babu and Prasanna, 2014). Since maize is a cross-pollinated crop, development of hybrids using parental lines from diverse heterotic groups, resistant or tolerant to biotic and abiotic stress and nutritionally enriched, is a major breeding approach to increase food and nutrition security. The success of this method depends on development and identification of suitable inbred lines using an appropriate breeding scheme (Tripathy et al., 2017). Currently QPM breeding strategies at CIMMYT, IITA and NARS mainly focus on pedigree breeding to develop new inbred lines from QPM x QPM and QPM x CM (conventional maize) crosses, whereby the best performing inbred lines, complementary in different traits, are crossed to establish new segregating families. In addition to this, sometimes new inbred lines are developed from segregating families in the same process from the broad-based populations (Krivanek et al., 2007; Babu and Prasanna, 2014).

QPM breeding is a complex process as it needs the simultaneous manipulation of three genetic systems: the first is to ensure the presence of the recessive mutant allele of the *o2* gene, the second is to confer the modifier/enhancer of *o2* containing high lysine and tryptophan, and the third is to ensure the *o2* modifier gene converts soft endosperm to hard endosperm (Prasanna et al., 2001; Duarte et al., 2004). QPM breeding also requires a large amount of labour, time and financial resources as the *o2* allele must be in a homozygous recessive state along with the polygenic endosperm modifiers as compared with conventional breeding procedures. Moreover, it requires self-pollination, visualization of kernels on a light table and rigorous biochemical tests in each backcross generation (Babu et al., 2013).

Molecular marker assisted selection (MAS) methods are useful in enhancing selection efficiency and accelerating the process of development of new QPM varieties having high yield potential with high nutritional quality (Ribaut and Hoisington, 1998; Prasanna et al., 2020). For instance, a rapid line conversion strategy has been developed, combining high protein quality and kernel modification in normal maize inbred lines through a two-generation backcross programme that employs foreground selection for *o2* in both backcross 1 (BC1) and BC2 generations combined with background selection for the recipient genome at the BC2 generation and phenotypic selection for kernel modification and other desirable agronomic traits in two subsequent selfed generations (Babu et al., 2005). While marker assisted foreground selection (Melchinger, 1990) helps in identifying the gene of interest without extensive phenotypic assays, marker-assisted background selection (Frisch et al., 1999) significantly speeds up the rate of genetic gain in backcross breeding. There are a few studies of MAS for maize improvement using *o2* specific molecular markers reported by Babu et al. (2005), Gupta et al. (2009) and Prasanna et al. (2010).

Among polymerase chain reaction (PCR) based allele-specific markers, simple sequence repeats (SSRs) and single nucleotide polymorphisms (SNPs) are becoming an efficient and attractive option, particularly for oligogenic traits like QPM (Babu et al., 2004). Multiple genes have been identified in controlling amino acid content (Tandzi et al., 2017). The cloning and characterization of the *o2* gene, followed by detection of three SSR markers (phi057, phi112 and umc1066) within the gene led to effective differentiation of the *O2* and *o2* alleles (Bantte and Prasanna, 2003). A combination of bulked segregant analysis and genome-wide SNP scan (using Illumina's Golden Gate assay) in phenotypically contrasting progenies have identified several genomic regions putatively associated with kernel hardness and high tryptophan

concentration which, if validated using targeted SSRs, and segregating populations, will greatly aid in designing a comprehensive MAS system for cost-effective QPM hybrid cultivar development (Babu et al, 2009). Molecular markers have also been used to investigate genetic diversity and suggesting heterotic patterns of QPM inbred lines (Lealem, 2017; Njeri et al., 2017; Ababulgu et al., 2018; Wegary et al., 2018; Terefe et al., 2019). This could help to increase diversity levels of QPM germplasm by introduction of maize germplasm from different tropical maize breeding programmes (Njeri et al., 2017).

2.3 Micronutrients

Many populations, particularly in developing countries, are affected by micronutrient malnutrition. This is known as "hidden hunger", caused by a lack of essential vitamins and minerals that are required by the human body in small quantities (Menkir, 2008; Tulchinsky, 2010; Nguyen et al., 2014). More than two billion people across the world are affected by micronutrient malnutrition (Muthayya et al., 2013; Hodge, 2016; Garg et al., 2018). So far agriculture researchers have given more attention to increasing of grain yield to improve food security rather than increasing nutritional value (Garg et al., 2018). This approach has resulted in a rapid rise of micronutrient deficiencies in humans, imposing many short- and long-term adversities in terms of ill health, mortality and mental problems in children (Tulchinsky, 2010; Shetty, 2011). Among micronutrient deficiencies, Fe and Zn are the most important risk factors. Around 17.3% of the world's population is at risk of inadequate Zn intake (Wessells et al., 2012) while almost 30% are anemic, due to Fe deficiency (WHO, 2013). Besides improving food security, agriculture should follow new strategies to develop nutrient rich crops in sufficient quantities to combat micronutrient related diseases, especially for poor people in developing countries where their diet is dominated by micronutrient poor crops like maize (Khush et al., 2012).

One empowering tool for fighting micronutrient deficiencies is the development of nutritious staple food crops using biofortification. Biofortification is the application of conventional breeding and biotechnology to develop staple food crops with increased concentrations of bioavailable micronutrients in the edible portion of crops, without detrimental effects (Nestel et al., 2006). Most essential minerals are not synthesized by humans and monogastric animals and they have to get these minerals from their diet (Hindu et al., 2018). So far micronutrient enrichment of diets through supplementation and commercial food fortification has been implemented (Garg et al., 2018). However, due to its unavailability on a large scale for long

times, and low buying capacity of resource-poor people in developing countries, the problem is still not solved (Bouis and Welch, 2010). Improvement of micronutrient concentration with improved bioavailability in staple crops like maize, through breeding, is a stable and cost-effective strategy (Garg et al., 2018).

2.3.1 Importance of iron and zinc

Fe and Zn are the most important essential minerals involved in different biological processes in human bodies like production of enzymes, hormones, and other substances that help to regulate growth and the development and functioning of the immune and reproductive system. Fe is present in red blood cells as a part of hemoglobin and is used as an oxygen transporter from the lungs to tissues, assisting the oxidative metabolism. Most of the time Fe is stored in the liver as ferritin and hemosiderin, and there is no physical system for its excretion (Hurrell and Egli, 2010; Abbaspour et al., 2014). Fe is involved in several metabolic reactions like energy production, immune defence, control function of hormones, and it is the main component of various heme and non-heme Fe enzymes, cytochromes, and ferredoxin electron carriers (Frossard et al., 2000). Fe also plays vital roles in enzymes which form bile acids and steroid hormones in the liver and controlling of signals in some neurotransmitters, such as serotonin and dopamine systems in the brain (Kumar et al., 2015).

Zn is the other most important micronutrient for human health which is present in biological organisms where it plays catalytic, structural, and regulatory roles (Gibson, 2012). It is required in more than 300 enzymes and has an important function in metabolic pathways, including the central nervous system, and as an integral component of Zn finger proteins that regulate deoxyribonucleic acid (DNA) transcription (Levenson and Morris, 2011). It plays a significant role in cell division and growth, immune response, and reproductive functions (Thakur et al., 2015). In general, Zn is necessary for a wide range of biochemical, immunological, and clinical functions (Gibson, 2012).

2.3.2 Iron and zinc deficiency

Deficiency in Fe and Zn ranks 5th and 6th among the top 10 most risk factors accountable for illnesses and causing disease in developing countries (Šimić et al., 2009; Akhtar et al., 2018). Insufficient amounts of Fe in the diet can cause anemia, poor cognitive development and growth, and low reproductive performance and work productivity (Bouis, 2002; Ghandilyan et al., 2006). Zn deficiency also affects people of all ages; however, newborn babies, young

children, and pregnant and lactating women are more frequently affected (Gibson, 2012; Akhtar et al., 2018). In severe Zn deficiency, multiple disturbances occur, including impaired growth, defects in the immune system, dermatitis, diarrhea, delayed sexual and bone maturation, impaired taste acuity, adverse pregnancy outcomes, and neurobehavioral changes (Solomons, 2003; Gibson, 2012).

2.3.3 Genetic variation and breeding strategies for iron and zinc contents

The development of an efficient breeding programme to enhance mineral contents in maize depends on the genetic variability among genotypes (Menkir, 2008). Several studies have reported the existence of significant genetic variability for Fe and Zn concentration in maize (Bänziger and Long, 2000; Oikeh et al., 2003; Menkir, 2008; Chakraborti et al., 2011a, b; Prasanna et al., 2011; Hindu et al., 2018). The range of Fe and Zn concentration variation also indicates the possibility to meet the target level of Fe (52 µg g^{-1}) and Zn (33 µg g^{-1}) in maize that has been set by the HarvestPlus programme (Bouis and Welch, 2010). For example, Bänziger and Long (2000) reported Fe concentration range values from 9.6 to 63.2 mg kg^{-1} and Zn from 12.9 to 57.6 mg kg^{-1} in tropical maize germplasm, including CIMMYT's white-grained germplasm bank core accessions, all of CIMMYT's white and yellow maize germplasm pools and populations, and numerous elite and advanced southern African breeding lines. Even if some researchers evaluated only a few genotypes, they also reported the presence of high kernel Fe and Zn concentration variation among genotypes. For example, Prasanna et al. (2011) observed kernel Fe and Zn concentration variation from 11.28 to 60.11 mg kg^{-1} and 15.14 to 52.95 mg kg^{-1}, respectively among 30 diverse maize genotypes. Queiroz et al. (2011) reported a Zn concentration range from 17.5 - 42 mg kg^{-1} and Fe from 12.2 - 36.7 mg kg^{-1}, among 22 tropical maize inbred lines. On the other hand, Oikeh et al. (2003) reported a lower range for Fe (15.5 -19.1) mg kg^{-1} and for Zn (16.5 - 20.50) mg kg^{-1} among 20 early maturing (75-90 days) tropical maize varieties.

To do efficient breeding for Fe and Zn content, information about environment and G × E interaction effects is important. So far, significant environment and G × E interaction effects have been reported for both kernel Fe and Zn concentrations in maize. Oikeh et al. (2004) evaluated 20 early maturing elite tropical maize genotypes over two years at three locations and they reported significant environment and G × E interaction effects for both Fe and Zn. Chakraborti et al. (2011b) evaluated 31 diverse maize inbred lines over two years at two locations and found significant environment and G × E interaction effects in the expression of

both the micronutrients. Prasanna et al. (2011) also evaluated 30 diverse maize genotypes over three years and observed significant environment and G × E interaction effects in the levels of kernel Fe and Zn content. Generally, all the above studies showed that the concentration of Fe and Zn differ from location to location and year to year. That means the level of Fe and Zn content of a genotype in one environment may not necessarily be the same in other environments or years, which complicates breeding for Fe and Zn content improvement in staple food crops like maize (Akhtar et al., 2018).

CIMMYT and IITA breeding programmes identified a large number of maize inbred lines which have high Zn and Fe contents, higher than the target level of Zn and Fe which was established as 33 and 52 µg g^{-1}, respectively, in maize kernels (Maqbool and Beshir, 2019). According to Hindu et al. (2018) and Maqbool and Beshir (2019) some of the inbred lines were also characterized for tolerance against different stresses; therefore, manipulation of these characterized lines in breeding programmes will also facilitate the introgression of stress tolerance along with high Fe and Zn contents in promising maize genotypes. The other interesting thing is that genotypes that have the *o2* gene background can promote Fe and Zn absorption and increase bioavailability compared to non-QPM genotypes (Bänziger and Long, 2000; Hindu et al., 2018; Prasanna et al., 2020). In QPM genotypes, the *o2* gene partially inhibits zein synthesis, while it increases the other protein fractions (glutelins, albumins and globulins), proteins also known to bind Zn in the endosperm of maize (Hindu et al., 2018). Zn also plays an important role in tryptophan biosynthesis, which is increased in QPM (Hindu et al., 2018). Chakraborti et al. (2011a) reported that the concentration of Zn in QPM hybrids was higher than in non-QPM genotypes. However, not all QPM germplasm have high kernel Zn concentration, and it is possible to find non-QPM inbred lines, hybrids and open pollinated varieties (OPVs) that have high Zn content (Prasanna et al., 2020). Out of the 923 lines used to conduct genome wide association studies (GWAS) for Zn, Hindu et al. (2018) reported that only 31 were QPM or had a QPM background and 33.3% had Zn values higher than 30 µg g^{-1} dry weight (DW). In contrast, of the 892 non-QPM used in the panel, 19.9% had values higher than 30 µg g^{-1} DW, and about 6% of them had values higher than the breeding target (33 µg g^{-1} DW). These results indicate significant potential to develop high Zn along with better quality protein through biofortification (Hindu et al., 2018; Prasanna et al., 2020).

Breeding approaches like mutation breeding, quantitative trait loci (QTL) mapping, marker assisted selection (MAS) and genomic selection (GS) are effective to accelerate genetic gain

in maize breeding programmes for improvement of Fe and Zn contents (Qin et al., 2012; Hindu et al., 2018; Maqbool and Beshir, 2019; Prasanna et al., 2020). Identification of QTL for mineral accumulation in maize is a useful approach to be exploited in MAS for the development of mineral enriched maize genotypes (Qin et al., 2012; Maqbool and Beshir, 2019). In QTL mapping studies for detecting Fe and Zn contents, a few genomic regions containing several QTL were identified on different chromosomes (Qin et al., 2012; Jin et al., 2013). Qin et al. (2012) identified three stable QTL for kernel Zn and Fe concentrations in combined analysis across two environments. Jin et al. (2013) identified five significant QTL controlling grain Zn and Fe content in a $F_{2:3}$ mapping population for QTL mapping and meta-analysis studies. Zhang et al. (2017) also studied the QTL for Zn, Fe, copper (Cu) and manganese (Mn) concentrations in maize kernels in a single environment and across multiple environments. In this study they detected a total of 64 and 67 QTL for single and multiple environment analyses, respectively. GWAS of 923 tropical/sub-tropical CIMMYT maize inbred lines identified a total of 20 and 26 SNPs, which were significantly associated with kernel Zn and Fe contents, respectively (Hindu et al., 2018). This could create another opportunity for improving Fe and Zn contents through biofortification.

3.3.4 Bioavailability of Fe and Zn and phytic acid content in maize

Bioavailability is defined as the proportion of the total amount of a mineral element that is digested, absorbed, and used for metabolic processes (Welch and Graham, 2004; Kumar et al., 2015; Akhtar et al., 2018). The total content of Fe and Zn in maize genotypes is not always guaranteed to be bioavailable for humans and monogastric animals. High levels of phytate (myo-inositol hexaphosphate) in maize is the major factor that inhibits the bioavailability of Fe and Zn. Phytate, being negatively charged, has a strong tendency to chelate positively charged metal ions such as Fe and Zn, thereby resulting in highly insoluble salts. This reduces their bioavailability for monogastric animals, including humans, because they lack the enzyme phytase in their digestive tract (Šimić et al., 2009; Gupta et al., 2015). Therefore, the development of Fe and Zn rich maize genotypes should be combined with the enhanced bioavailability of nutrients for improving human health (Akhtar et al., 2018).

Reducing the phytic acid concentration in maize and raising the concentration of promoters of Fe and Zn absorption, such as sulfur-containing amino acids, could be an important strategy for Fe and Zn biofortification (Welch and Graham, 2004). Several methods have been developed to reduce the phytic acid concentration to improve the bioavailability of Zn and Fe,

including genetic improvement as well as several pre-treatment methods such as fermentation, soaking, germination and enzymatic treatment of grains with phytase enzyme (Gupta et al., 2015). It is also important to integrate a low phytate programme with breeding for high Fe and Zn content to increase bioavailability of Fe and Zn. A few research findings have shown the presence of significant genetic variability for phytic acid content and phytic acid (PA)/Fe and PA/Zn molar ratios in maize germplasm. For example, Šimić et al. (2009) observed significant genetic variations for PA/Fe and PA/Zn molar ratios in 294 F_4 inbred lines of a maize population. Queiroz et al. (2011) reported the presence of high genetic variability for PA/Zn molar ratio (18.0 - 43.5) and PA/Fe molar ratio (16.3 - 45.5) among 22 tropical maize inbred lines. Shawa (2019) also observed significant differences among QPM genotypes for phytic acid content and the molar ratios of phytic acid to minerals under optimum and low N conditions. All these studies indicated the possibility of breeding for bioavailability improvement of Fe and Zn content in maize germplasm.

2.4 Variability, heritability and correlation

2.4.1 Phenotypic and genotypic variability

Genetic variability among germplasm collections is important for improved variety development, particularly genetic variability for a given trait is important for its improvement through the design of a systematic breeding strategy (Engida et al., 2007). Variability is the occurrence of differences among individuals within a population due to genetic makeup and/or environment effects (Allard, 1999; Falconer and Mackay, 1996). Phenotypic (σ^2p) and environmental (σ^2e) variability is the observable variation present in the traits in a population and is the result of both genetic and environmental effects. Its magnitude can differ from location to location and between different management levels. On the other hand, variation due to genetic differences among individuals within a population is called genetic variability (σ^2g), and it is the main target of plant breeding (Falconer and Mackay, 1996; Singh, 2005). Its magnitude is of utmost importance as it provides the basis for effective selection and is a source of useful traits (Singh, 2005). Welsh (1990) also said genetic variability is of prime interest to the plant breeder because effective management of this variation can produce permanent gain in the performance of the plant. Genetic variability is also further partitioned into additive genetic variance (σ^2A), dominance genetic variance (σ^2D) and epistatic genetic variance (σ^2I). The additive genetic variance, which is the variance of breeding values, is the most important component. It determines the inherited properties of the population and the response of the population to selection. Knowing the nature and magnitude of genetic variation in a population

also helps to select the type of breeding methods to follow for the genetic improvement of a crop.

Estimation of the genotypic and phenotypic coefficient of variation is useful in detecting the amount of variability present in each character within a population (Kalloo, 1988). Ababulgu et al. (2018) reported on genetic variability of highland QPM inbred lines, where the highest GCV was recorded for plant aspect followed by thousand kernel weight, while traits such as ear aspect, ear height and grain yield, had moderate values and lower GCV values were recorded for anthesis date and ASI. On the other hand, they recorded the highest PCV for ASI, plant aspect, grain yield, ear aspect and thousand kernel weight. Conversely, low PCV values were observed for anthesis date and ear diameter, while moderate values were recorded for ear height. For a breeding programme of any crop, information on the nature and magnitude of genetic variation within the species for grain yield, agronomic and nutritional quality traits are important to breed and identify improved genotypes.

The development of QPM germplasm in the SSA region has been led by CIMMYT, IITA and NARS. In these institutes a few advanced maize populations were converted to QPM using modified backcross and recurrent selection methods (Prasanna et al., 2001; Vivek et al., 2008). During the conversion process they focused on grain yield, kernel modification and appearance, ear rot resistance, plant aspect, major foliar disease resistance. More consideration was also given to broadening of the genetic base of well adapted QPM germplasm to suit different stresses (Krivanek et al., 2007). Many genotypes adapted to the SSA region have been developed in different breeding programmes. Knowing the genetic variability of QPM populations for grain yield, and agronomic and nutritional quality traits is helpful in initiating QPM improvement programmes as well as adopting appropriate selection techniques.

2.4.2 Heritability and genetic advance

Broad sense heritability is defined as the proportion of genotypic variance to phenotypic variance (σ^2g/σ^2p). It gives a useful indication of the relative selection value in the individual genotypes selected based on their phenotypes. If heritability is low, some environmental factors are considered responsible for phenotypic expression of the trait (Singh, 2005). Broad sense heritability is the portion of phenotypically expressed variation within a given environment and it measures the degree to which a trait can be modified by selection. Since broad sense

heritability does not give a clear picture of transmissibility of variation from generation to generation (because the genetic variation includes the fixable and non-fixable dominance and epistatic variation), its utilisation is limited in crop improvement.

According to Falconer and Mackay (1996), heritability in the narrow sense is defined as the ratio of additive genetic variance to phenotypic variance (σ^2A/σ^2P). Narrow sense heritability expresses the extent to which phenotypes of a population are determined by the genes transmitted from the parents. The magnitude of narrow sense heritability in the population is mainly responsible for changing the genetic composition of a population through selection (Holland et al., 2003). If the proportion of phenotypic variability is high, it will be difficult to select for inherited differences. On the other hand, if environmental variability is small in relation to genotypic differences, selection will be efficient.

Heritability can also help to determine stability of a trait across different environments (Hamdi, 1992). The opportunity to exploit crop adaptation to moisture stress and low N environments may become important at any stage in a breeding programme targeted at addressing environments prone to such stresses. Ideally, success in such endeavors would be most likely if the crop traits are expressed and highly heritable in the target environment (Ceccarelli et al., 1998). Selection of quantitative traits like grain yield based on yield per se is not efficient. To make it efficient, it is better observing heritability of grain yield and other traits which are closely associated with it.

Heritability estimates can be used to predict genetic advance under selection so that breeders can anticipate improvement from different types and intensities of selection. Genetic advance is the genetic progress of selected plants over the original population. The estimates of genetic advance help to understand the type of gene action involved in the expression of various polygenic traits (Kassaye, 2006). High heritability coupled with high genetic advance would be a perfect indication of genotypic variation among genotypes for that specific trait, and that selection would be efficient (Bänziger and Cooper, 2001). Especially grain yield is highly influenced by environmental conditions; it has a complex mode of inheritance and low heritability under stressed environments (Ertiro et al., 2017; Tapera, 2017). Because of this, during selection of varieties for grain yield, it is important to give more attention to heritability and genetic advance of both grain yield and secondary traits highly correlated with yield under

drought and low N environmental conditions, such as number of ears per plant, ASI, leaf senescence, leaf chlorophyll content and several other morpho-physiological traits.

Jilo et al. (2018) reported genetic advance as percent of mean at 5% of selection intensity. A high genetic advance was obtained for plant height, ear length and 100 kernel weight, indicating the potential for effective selection and improvement of the population. Ababulgu et al. (2018) also reported high heritability (≥ 0.70), expressing high genetic advance as a percentage over the mean at 5% selection intensity for ear height, tassel size and thousand kernel weight, whereas high heritability and moderate genetic advance were exhibited for plant height, leaf width, leaf number, and ear length. Therefore, the estimation of genetic parameters like heritability and genetic advance for QPM hybrids and elite inbred lines adapted to the SSA region will help to predict the genetic gain under selection.

2.4.3 Correlation

Knowledge of correlations that exist among characters is useful for plant breeders, to provide the basis for planning more efficient breeding programmes (Erago, 2015), for developing improved genotypes that are high yielding, with good nutritional value and resistance to biotic and abiotic stress. Correlation coefficients are a measure of a linear association among traits (Kozak et al., 2012). Mainly, two types of correlations exist in quantitative genetics. These are phenotypic and genotypic correlations, which are caused by genetic or environmental effects (Sodini et al., 2018). The association between two traits that can be directly observed is called phenotypic correlations. It involves both genetic and environmental effects (Sodini et al., 2018) and is determined from direct measurements of traits. Genotypic correlation is the association of breeding values of the two traits (Falconer, 1989). Genetic correlation measures the result of pleiotropic gene action or the extent of the same genes or closely linked genes cause co-variation in two different traits. If genetic correlation exists between two traits, selection for one trait will cause changes on the other trait (Hallauer and Miranda, 1988).

Therefore, phenotypic and genotypic correlations between traits of maize genotypes are helpful in increasing breeding efficiency. So far, phenotypic and genotypic associations among grain yield, yield related and nutritional quality traits have been studied extensively by different researchers. Mandefro (1998) reported positive and significant correlation of grain yield and other agronomic traits, both at genotypic and phenotypic level, and suggested that improving one of these traits can result in the improvement of grain yield, but path analysis was suggested

to determine the direct and indirect effects of each of these traits on grain yield. Habtamu and Hadji (2010) also found positive and significant correlation between grain yield and ear height, plant height, ear length, ear diameter, number of kernels per row, days to maturity and thousand kernel weight, while negative and significant association of yield was showed with days to silking. Plant height and ear height were also positively and highly correlated in their study. They also found positive and highly significant correlation of grain yield with plant height, ear length and number of kernels per row at genotypic level. Nzuve et al. (2014) reported that grain yield was positively and strongly correlated with ear height and plant height and found that tall plants with high ear placement gave better yields, which could be attributed to the high dry matter accumulation from the high number of leaves.

In the hybrids from a diallel cross of 10 inbred lines, Hadji (2004) reported positive and significant correlation of grain yield with ear height, plant height, number of ears per plant, ear length, ear diameter, number of kernels and 1000 kernel weight. Genotypic correlation of grain yield with these traits was larger than the phenotypic correlation. Tilahun et al. (2017) also reported positive and significant correlation between grain yield and number of ears per plant, plant height, ear height, and ear position, while negative correlation was observed with gray leaf spot (GLS), days to anthesis and silking, plant aspect, and ear aspect in hybrids from a diallel cross with 10 QPM inbreds. Ngaboyisonga et al. (2012) who reported significant and positive correlation between total protein and tryptophan concentrations in the grain.

Kernel Fe and Zn concentration is often correlated positively and significantly in tropical maize genotypes (Menkir, 2008). This correlation may be attributed to pleiotropic effects or linkage of the genes governing the kernel Fe and Zn concentration, which indicates the possibility of simultaneous improvement of Fe and Zn. Chakraborti et al. (2011a) also reported positive and significate correlation between kernel Fe and Zn concentration, while grain yield showed positive and non-significant correlation with both Fe and Zn which suggested the possibility of simultaneous improvement of both the kernel micronutrients without a negative impact on grain yield in the QPM hybrids. However, there are also some contrasting reports which suggest that there is weak or no correlation between kernel Zn and Fe concentration (Prasanna et al., 2011; 2020).

Understanding the relationship among grain yield and yield related traits recorded from different management conditions would indicate the possibility of improvement of grain yield

performance of genotypes in all conditions using appropriate selection methods. Ertiro et al. (2017) reported positive correlation of grain yield under optimum conditions with grain yield under both drought stress and low N conditions while no correlation was seen between drought stress and low N conditions, from 49 testcross hybrids with six commercial checks evaluated across 11 optimum, drought and low N conditions. Weber et al. (2012) also reported positive genetic correlation for grain yield between low N and optimum N environmental conditions, but the correlation decreased as stress intensity increased. This suggested that selection in optimum conditions would not be effective in identifying superior genotypes for stressed environments. Monneveux et al. (2005) reported negatively significant correlation between grain yield and number of kernels per ear in genotypes evaluated under low N conditions, which showed the existence of nutrition shortage during grain filling stages. On the other hand, significantly positive correlations were observed between plant and ear height, and days to anthesis, ASI and days to anthesis under low N conditions. This indicated the potential value of these traits in a low N selection programme.

The association between grain yield and major maize foliar diseases were also reported in different studies. In diallel cross hybrids generated from eight inbred lines selected based on GLS reaction, Wegary et al. (2008) reported that grain yield was poorly correlated with GLS disease parameters. This indicated the existence of low disease pressure among hybrids. Daniel et al. (2008) reported negative correlation between grain yield and Thurcicum leaf blight (TLB) in commercial hybrids and OPVs adapted to the mid-altitude sub-humid agroecology of Ethiopia. On the other hand, Abera et al. (2016) indicated that grain yield had weak and non-significant positive correlations with TLB disease parameters in inbred lines adapted to the mid-altitude sub-humid agroecology of Ethiopia. This indicates that all disease parameters placed lower pressure on yield in that specific season.

2.5 Genotype × environment interaction and stability

Genotype performance is the result of genotype, environment and G × E effects (Crossa et al., 1990; Yan and Kang, 2003). G × E interaction is important only when it is significant and causes significant change in genotype ranking in different environments, for example, when crossover interaction occurs in evaluation of trials, and different genotypes are superior in different environments (Yan and Kang, 2003). G × E interaction has a negative impact on heritability. The lower the heritability of a trait, the greater the difficulty in improving that trait via selection. Information on the structure and nature of G × E interaction is important in plant

breeding programmes because it can help determine if cultivar development should be done for all environments of interest or if cultivars should be developed for specific target environments (Gissa, 2008).

Various causes have been described as sources of G × E interaction such as fluctuation of rainfall during the cropping season, emerging and remerging disease, insects, poor soil fertility, drought and other stresses (Bänziger et al., 2006). Consequently, a variety which performs well in one environment during one season may not perform well in different testing sites and seasons. Therefor it is difficult to allocate a variety that is successfully adapted to a certain location or across locations, and to identify desirable genotypes and ideal testing environments that can be used for the selection of superior genotypes (Wolde et al., 2018; Mebratu et al., 2019). Therefore, developing resistant or tolerant genotypes to different stresses may decrease G × E interaction effects and it is vital to include G × E interaction studies in breeding programmes for drought and low N stress conditions. Mebratu et al. (2019) reported significant G × E interaction effects on grain yield under stressed and non-stressed environmental conditions and depicted the possibility of developing high-yielding and stable QPM hybrids for low N and drought stress. Abakemal et al. (2016) also reported the existence of significant differences in G × E interaction effects on grain yield in 68 QPM maize hybrids evaluated in seven environments, and they identified ideal hybrids that could be suitable for transitional highland agro-ecologies of Ethiopia. Mengesha et al. (2019) reported the existence of G × E interaction variation both for grain yield and provitamin A content in 21 maize synthetics evaluated in nine locations and four years. They identified stable and high provitamin A content genotypes recommended for production in the low-land tropics of western and central Africa (WCA), with similar environments. In addition to grain yield, Prasanna et al. (2011) also identified high and stable maize genotypes for kernel Fe and Zn concentration.

A variety, to be commercially successful, should perform well across a range of environments where it will be grown (Wolde et al., 2018). Thus, multi-environment trials (METs) over sites and years are important to select superior as well as stable maize hybrids and identify representative sites for the target environment (Yan et al., 2000; Mebratu et al., 2019). A variety that has high yielding potential in most testing environments and is stable across all testing environments, is the ideal in maize breeding programmes (Abera, 2013). Stable cultivars have little or no interaction with environments (Tollenaar and Lee, 2002). Grain yield stability is defined as the ability of a cultivar to avoid significant variation over a range of environmental

conditions (Kang, 1998). Statistical analysis of G × E interaction is important to analyse multi-environment trails. Several biometrical data analysis methods have been reported to analyses patterns of G × E interaction and stability.

Additive main effect and genotype by environment interaction (AMMI) and genotypic main effects plus genotype × environment interaction (GGE) biplots are the most common statistical tools used to analyse METs data to reveal patterns of G × E interaction (Gauch and Zobel, 1997; Yan et al, 2000). AMMI increases the precision of yield estimation and allows selection of higher yielding genotypes (Crossa et al., 1990). The results can be represented graphically in an easily interpretable way in informative biplots which detect both main effects and G × E interaction effects (Purchase et al., 2000) and can be used to determine stability of genotypes across locations. GGE biplot analysis is a graphical tool and the variation due to genotypes and G × E interaction for trait of interest is examined based on the principal component analysis (PCA) of environment centered data and it is also suggested as a powerful analysis method for delineating mega-environments, and for genotype and test environment evaluation (Yan et al., 2000; 2007). Moreover, GGE biplot analysis is efficient to identify the best performing genotypes, based on mean yield and stability of cultivars (Yan and Tinker, 2006). Therefore, using both AMMI and GGE biplot analysis to study G × E interaction effects is useful for increasing breeding efficiency.

2.6 Effects of drought and low N stress

Maize production and productivity in SSA are affected by biotic and abiotic factors such as drought, low soil fertility, high pressure of weeds, insect pests, diseases, low input availability (especially quality seed and fertilizer), high cost of agricultural inputs and limited use of inputs (Meseka et al., 2013; Shiferaw et al., 2011). Among these factors, drought and low N availability in the soil are the most frequently occurring stresses which largely contribute to limiting maize production, food security, and economic growth in SSA (Bänziger et al., 2006; Makumbi et al., 2011; Mebratu et al., 2019). Most SSA maize production areas are dependent on rainfed conditions and are exposed to unpredictable rainfall patterns. This has caused many maize growing environments to be vulnerable to drought stress (Fisher et al., 2015). Bänziger and Diallo (2004) reported a close relationship between maize production and amount of rainfall across ESA. Drought can occur at any growth stage of maize, but the crop is more sensitive at flowering and grain-filling periods (Menkir and Akintunde, 2001; Magorokosho et al., 2003; Bänziger et al., 2006; Wegary et al., 2014). Grain yield loss occurs because of

premature death of plants, low silking and anthesis synchronization during the flowering period, reduced number of ears per plant, seed size and weight, and decreased number of kernels per plant under stress conditions. Every year around 40% and 25% of Africa's maize production areas are affected by occasional and frequent droughts with up to 25% and 50% yield losses, respectively. Total crop failure is common when drought become severe (Fisher et al., 2015).

Nitrogen (N) is one of the most essential elements that plays a significant role in plant growth and development. Maize is highly sensitive to the levels of N in the soil. Since most African farmers are poor, fertilizer application in their fields is far below the recommended rate or not commonly used (Bänziger and Lafitte, 1997; Makumbi, 2005; Meseka et al., 2013). Severe N deficiency causes high ASI, premature leaf yellowing followed by leaf senescence, and reduces photosynthesis leaf area. This causes stunted growth and development of the plant, resulting in low grain yield production. More than 50% yield reduction was reported by Bänziger and Lafitte (1997) in maize genotypes under low N stress conditions. Betrán et al. (2003) compared maize hybrid yield under low and high N conditions and reported that grain yield of hybrids in the low N environment was 33% of grain yield in the high N environment. Ertiro et al. (2017) also compared grain yield of hybrids evaluated under drought and low N conditions and reported a 50% and 69% grain yield reduction, respectively. This indicated that further efforts are needed to identify genotypes that have good yield potential under both drought and low N conditions. Smalberger and du Toit (2004) observed the existence of N sensitive cultivars. They reported that the genotype that had high yield potential (6.93 t ha^{-1}) under optimum N had a low yield potential (0.98 t ha^{-1}) under low N conditions. A number of studies have also been conducted to identify the best performing genotypes under both optimum and low N environments in QPM (Setimela et al., 2017; Mebratu et al., 2019), and in normal maize (Worku et al., 2008; Meseka et al., 2013; Ertiro et al., 2017).

Development of maize genotypes that can tolerate drought and low N levels will minimise farmers' risk, reduce the effects of lack of fertilizer, and help them to attain food self-sufficiency on a small area, free land and labour to grow cash crops, and reduce expansion into new land (Shiferaw et al., 2011). Scientists have identified maize genotypes that can tolerate drought and low N conditions. Bänziger et al. (1999) reported some tropical maize genotypes with good performance under drought and low N conditions. Zambezi and Mwambula (1997) also reported improvement of drought tolerance in a maize population, Tuxpino Sequia, which

resulted in improved performance under low N conditions. Genotype improvement under drought stress was strongly associated with selection gains under low N conditions (Bänziger et al., 2002). Meseka et al. (2013) reported that crosses with at least one tolerant parent produced tolerant hybrids, whereas most crosses between susceptible lines resulted in susceptible hybrids. From line by tester mating of newly developed lines and testers generated through conventional pedigree breeding, double haploid technology and molecular breeding, Ertiro et al. (2017) identified hybrids having high and stable grain yield under drought stress, low N and optimum conditions that can be commercialized in small-scale farmers' fields in SSA. From a 15 QPM inbred line diallel cross, Wegary et al. (2014) also reported that the performance of hybrids was not consistent across optimal, drought and low N environments for all tested traits and they suggested that an appropriate breeding programme for such materials should allow for the development of stable cultivars or cultivars adapted to specific environments. Mebratu et al. (2019) identified desirable hybrids that have higher grain yield and stability under optimum, low N, and drought stress environments from 106 F_1 line by tester QPM hybrids. Understanding such relationships and their genetic basis would aid the development of stress tolerant QPM varieties, which are likely to improve food security and nutritional status of farmers. These studies showed the possibility of identification of new QPM hybrids that have good grain yield and nutritional quality potential in all management conditions and may also help to focus maize breeding programmes in developing multiple stress tolerant varieties without a grain yield penalty under optimum conditions.

Since some stresses are sporadic in nature, CIMMYT developed MSEs for both biotic and abiotic stresses at its breeding sites in Africa, Asia, and Latin America. Among MSEs, drought and low N managed stress screening sites are important in SSA countries for evaluation of maize genotypes (Ertiro et al., 2017). Drought stress evaluations are done by planting maize trials in the off-season and managing drought through irrigation, and water is withheld at the seedling, flowering, and grain filling stages to assess drought tolerance of the varieties (Bolaños and Edmeades, 1996). For low N stress evaluations, N depleted plots were created by growing high feeder crops like maize with high population density in the summer season, and irrigated wheat during the winter season (Bänziger et al., 1997). CIMMYT in collaboration with NARS and other private partner companies, have screened many genotypes and significant progress has been made in the identification of drought and low N tolerant genotypes in both conventional and QPM maize genotypes (Ertiro et al., 2017; Mebratu et al., 2019). Some

tolerant maize varieties have been released and disseminated in partnership with various public and private organizations (Prasanna, 2013; Fisher et al., 2015).

2.7 Conclusions

Maize is the most important staple food crop in SSA countries and serves as a main source of carbohydrates, proteins and some vitamins and minerals. However, conventional maize, which is commonly used, lacks essential amino acids (lysine and tryptophan) and has poor bioavailability of minerals like Fe and Zn. Consequently, many people suffer from malnutrition related diseases which may, in severe cases, lead to death. Besides increasing production and productivity of maize to ensure food security, nutritional quality improvement is an important issue in SSA to address malnutrition problems and to bring economic growth. In this region, most maize production areas are affected by low soil fertility and drought stress. Many studies also reported the existence of genetic variation among QPM genotypes for nutritional quality and quantity, as well as low N and drought stress tolerance. Development of maize varieties that have high nutritional quality and tolerance to drought and low N stress, can minimise farmers' risks, reduce the effects of lack of fertilizer, and help to attain food self-sufficiency.

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CHAPTER 3

Genetic variability and hybrid performance of newly developed QPM hybrids for grain yield and agronomic traits evaluated under stressed and non-stressed environments

Abstract

Drought and low N stresses are the most important abiotic production constraints contributing to low yields in SSA. Identifying and developing of drought and low N tolerant varieties can make a significant contribution towards minimizing yield losses due to these abiotic stresses. The objectives of this study were 1) to determine genotypic variability of grain yield and other traits of QPM genotypes under stressed and non-stressed environments and, 2) to identify new QPM hybrids that have good grain yield and agronomic performance under optimum and stressed environments. In this study, 45 hybrids, including five checks, were evaluated under 14 optimum, nine random stress, three managed drought and eight low N stress environments in Ethiopia, Zimbabwe, Zambia, Mozambique, and Malawi in 2018 to 2020. Highly significant variation was observed among environments and genotypes for all measured traits. G × E interaction was also significant for grain yield and most other agronomic traits in all management conditions. The highest yielding entries were 14 (CZH15142Q), and 9 (CZH142237Q) across environments; 44 (QS7646) and 12 (CZH15099Q) under optimum conditions; 14 (CZH15142Q), 10 (CZH142238Q), 34 (CZH17203Q), 9 (CZH142237Q14) under random stress; 9 (CZH142237Q) and 14 (CZH15142Q) under managed drought and 14 (CZH15142Q) under low N stress conditions. These selected QPM hybrids were significantly better than the best commercial QPM check (MamaMQ623) and had high or comparable yields with the best non-QPM commercial check (PAN53). Grain yield was reduced by 48% under random drought, 68% under managed drought and 71% under low N environments and ASI increased by 80% under random stress, 218% under managed drought and 203% under low N conditions. Broad sense heritability for grain yield was high under optimum conditions, random stress, low N and across combined environments, while very low heritability was observed under managed drought conditions. In general, maize yield production and malnutrition in SSA could be improved by using QPM genotypes that are tolerant to drought and low N stress.

Keywords: Drought, grain yield, genetic variability, heritability, low N, QPM

3.1 Introduction

Maize is a popular and multipurpose cereal crop grown world-wide and significantly contributes to meet the growing world demand for food, feed and raw materials for different industries (Shiferaw et al., 2011). Over 300 million people depend on maize to fulfill food and nutritional needs and for economic growth in SSA (Bankole et al., 2017). Maize accounts for half of the calories and protein consumed in ESA and one-fifth of the calories and protein consumed in West Africa. Maize occupies over 50% of land devoted to cereal production in over 50% of countries in SSA (Masuka et al., 2017). Even though the cultivated area and grain yield production of maize have shown significant progress in a few countries in SSA, the average productivity has stagnated at less than 2 t ha^{-1} (Cairns et al., 2013; Ertiro et al., 2020). It is also far below the average world productivity (5.75 t ha^{-1}) (FAOSTAT, 2020) and the potential that can be achieved with improved cultivars and efficient crop management (Abate et al., 2017). Low productivity is largely associated with abiotic, biotic and socio-economic constraints, mainly attributed to inadequate farmer access to improved maize hybrid varieties, slow variety turnover, low soil fertility, erratic rainfall distribution, shortage and unavailability of key inputs like fertilizers on time, and emergence of new insect pests and diseases (Shiferaw et al., 2011; Meseka et al., 2013; Beyene et al., 2015; Worku et al., 2016; Abate et al., 2017; Ertiro et al., 2017).

Drought and low soil N levels are major challenges in smallholder farmers' fields (Bänziger et al., 2006; Makumbi et al., 2011; Wegary et al., 2014; Ertiro et al., 2017; Mebratu et al., 2019a). Combined effects of these stress factors can be devastating (Badu-Apraku et al., 2016). Global warming aggravates the frequency and intensity of droughts (Betrán et al., 2003; Ertiro et al., 2017). Drought affects maize at all stages of development, but the flowering and grain-filling periods are the most sensitive with yield losses ranging from 10 – 50% (Bänziger et al., 2006; Fisher et al., 2015). Nitrogen plays a significant role in plant growth and development (Makumbi, 2005). However, N deficiency is common in farmers' fields in the tropics where fertilizer application is far below the optimal rate, or not used at all (Bänziger and Lafitte, 1997; Makumbi, 2005; Meseka et al., 2013). Sustainable maize production and productivity under stressed environments could be improved through both crop management and genotype improvement (Gazal et al., 2020).

Development of maize varieties that can tolerate abiotic stress conditions, is a more reliable and affordable option for poor farmers that grow maize in drought prone and unfertile soil and

is also an economically feasible approach in terms of cost reduction of artificial fertilizer and irrigation infrastructure development (Campos et al., 2004; Bänziger et al., 2006; Meseka et al., 2013; Wegary et al., 2014; Beyene et al., 2015; Ertiro et al., 2017). CIMMYT, in collaboration with the NARS and other private partners, have made significant progress in the development of QPM with high tryptophan and lysine levels (Vasal, 2000; Prasanna, et al., 2001; Teklewold et al., 2015; Twumasi-Afriyie et al., 2016), but still malnutrition problems remain high in SSA (Krivanek et al., 2007; Vivek et al., 2008; Setimela et al., 2017a). Identifying and development of high yielding, well adapted and stress tolerant QPM cultivars is a possible solution to improve food security and malnutrition problems in the region (Badu-Apraku et al., 2016; Setimela et al., 2017a). The main goal of this study was to evaluate 40 newly developed three-way QPM hybrids of CIMMYT under stressed and non-stressed environments to identify hybrids that could outperform the existing commercial QPM and non-QPM cultivars with respect to grain yield and some other agronomic traits.

Since most economically important traits are complex and controlled by quantitative genes, knowing the relative amount of phenotypic and genotypic variance, heritability and genetic advance for different traits could contribute to more effective selection (Ali et al., 2017; Josia, 2019). Phenotypic variability is the observable variation present in the traits in a population and is the result of both genetic and environmental effects. Its magnitude can differ from location to location and between different management levels. On the other hand, variation due to genetic differences among individuals within a population is called genotypic variability, and it is the main target of plant breeding (Singh, 2005). Its magnitude is of utmost importance as it provides the basis for effective selection and is a source of useful traits (Singh, 2005). Welsh (1990) also added that genetic variability is a prime interest to the plant breeder because effective management of this variation can produce permanent gain in the performance of the plant. Knowledge on genetic variability of QPM genotypes under different environmental conditions will be helpful for future QPM improvement breeding programmes as well as adopting appropriate selection strategies (Setimela et al., 2017a; Mebratu et al., 2019b).

Heritability shows the value of selection for a particular trait in various types of progenies (Lule et al., 2012). A high heritability implies that the phenotypic performance would be a perfect indication of genotypic value and that selection will be efficient because the selected character will be transmitted to its progeny (Bänziger and Cooper, 2001). Low heritability indicates a masking effect of the environment on genotypic effect, which makes selection impractical

(Singh, 2005). High heritability coupled with high genetic advance for specific traits will improve selection efficiency (Bänziger and Cooper, 2001). Grain yield is highly influenced by environmental conditions, has a complex mode of inheritance, and has low heritability under stressed environments. It is therefore important to give more attention to variation and heritability of secondary traits highly correlated with grain yield under drought and low N conditions, such as number of ears per plant, ASI, leaf senescence, leaf chlorophyll content and several other morpho-physiological traits (Chapman and Edmeades, 1999; Bänziger et al., 2000; Wegary et al., 2014; Beyene et al., 2015). The objectives of this study were 1) to determine genotypic variability of grain yield and other traits of QPM genotypes under stressed and non-stressed environments and, 2) to identify new QPM hybrids that have good grain yield and agronomic performance under optimum and stressed environments.

3.2 Materials and methods

3.2.1 Experimental genotypes

Forty newly developed QPM hybrids were obtained from CIMMYT-Zimbabwe and partner seed companies for this study (Table 3.1). These hybrids were selected based on grain yield, disease resistance and agronomic performance from previous preliminary or early-stage trial evaluations at CIMMYT testing sites for further regional evaluation under different environmental conditions. Five commercial checks (two QPM, two normal maize and one local check), were included in the trials. In combined analysis, the local check was excluded from the analysis because different local checks were used at different locations. These experimental hybrids were evaluated across 34 environments in 14 optimum, nine random drought stress, three managed drought and eight low N environments in five countries: Ethiopia, Zimbabwe, Zambia, Mozambique, and Malawi in 2018 to 2019 (Table 3.2).

3.2.2 Experimental procedures

The trials were conducted using a 5 × 9 alpha lattice design with two replications. At all sites, each entry was planted in two rows per plot of 5 m row length with spacing of 0.75 m between rows and 0.25 m between plants except at Bako in 2018 where both optimum and low N sites were planted at 4 m row length and 0.3 m spacing between plants and one row per plot. Ambo (optimum site) in 2020 was planted at 5 m row length and 0.25 m spacing between plants and one row per plot. Two seeds were planted per hole and then thinned to one plant three weeks after emergence, to obtain a final plant population density of 53,333 plants per hectare (ha). Fertilizer was applied based on recommendations for each location. For low N trials, all

recommended rates of phosphorous, potassium and sulfur fertilizer were applied at planting, without further top dressing of urea. For optimum, random drought and managed drought trials, phosphorus, potassium and sulfur fertilizer sources were applied at planting, followed by a top dressing of urea fertilizer three weeks after planting. In Ethiopia, fertilizer was applied at a rate of 92 kg N and 69 kg diammonium phosphate (P_2O_5) ha^{-1}. Nitrogen was applied twice, at knee height and anthesis stage. All other agronomic practices such as weeding, hoeing, and pest management were done as needed. Optimum, random stress and low N trials were grown under rain fed conditions. Random drought stress trials were evaluated in sites that experiencing in random drought conditions. Low N trials were conducted on N depleted sites by growing high N feeder crops like sorghum, maize or wheat on it for a few years and removing all crop stover from the field after harvest (Bänziger et al., 2000; 2006). For managed drought stress, the trials were conducted during the dry/winter season and water was supplied through irrigation from planting to two weeks before expected anthesis (Bänziger et al., 2006).

Table 3.1 Name, source, and type of hybrid used in this study

Entry	Pedigree	Source	Hybrid type	Entry	Pedigree	Source	Hybrid type
1	ZS261	DR and SS	QPM check	24	CZH17193Q	CIMMYT	New
2	Mama MQ623	DR and SS	QPM check	25	CZH17194Q	CIMMYT	New
3	PAN53	Pannar	NM check	26	CZH17195Q	CIMMYT	New
4	SC533	Seed Co	NM check	27	CZH17196Q	CIMMYT	New
5	CZH04032	CIMMYT	New	28	CZH17197Q	CIMMYT	New
6	CZH132044Q	CIMMYT	New	29	CZH17198Q	CIMMYT	New
7	CZH132018Q	CIMMYT	New	30	CZH17199Q	CIMMYT	New
8	CZH142236Q	CIMMYT	New	31	CZH17200Q	CIMMYT	New
9	CZH142237Q	CIMMYT	New	32	CZH17201Q	CIMMYT	New
10	CZH142238Q	CIMMYT	New	33	CZH17202Q	CIMMYT	New
11	CZH15098Q	CIMMYT	New	34	CZH17203Q	CIMMYT	New
12	CZH15099Q	CIMMYT	New	35	CZH17204Q	CIMMYT	New
13	CZH15117Q	CIMMYT	New	36	CZH17205Q	CIMMYT	New
14	CZH15142Q	CIMMYT	New	37	CZH17206Q	CIMMYT	New
15	CZH16006Q	CIMMYT	New	38	CZH17207Q	CIMMYT	New
16	CZH16022Q	CIMMYT	New	39	CZH17208Q	CIMMYT	New
17	CZH16021Q	CIMMYT	New	40	CZH17209Q	CIMMYT	New
18	CZH17187Q	CIMMYT	New	41	CZH17210Q	CIMMYT	New
19	CZH17188Q	CIMMYT	New	42	MH1633	Mukushi	New
20	CZH17189Q	CIMMYT	New	43	MH1634	Mukushi	New
21	CZH17190Q	CIMMYT	New	44	QS7646	Mukushi	New
22	CZH17191Q	CIMMYT	New				
23	CZH17192Q	CIMMYT	New	45	Local check		

NM = normal maize; QPM = Quality protein maize

Table 3.2 Description of the study sites

Site#	Country	Location	Types of management	Latitude	Longitude	Altitude (masl)	Rainfall (mm)	Temperature(^{0}C) Min	Max
1		Ambo	Optimum	8°57'N	38°7'E	2225	1050	10.4	26.3
2		Ambo	Low N	8°57'N	38°7'E	2225	1050	10.4	26.3
3		Bako	Optimum	5°78'N	37°009'E	1650	1267	12.5	29.5
4	Ethiopia	Bako	Low N	5°78'N	37°009'E	1650	1267	12.5	29.5
5		CIMMYT Harare	Optimum	17°49'S	31°1'E	1489	820	5.5	23.8
6		CIMMYT Harare	Low N	17°49'S	31°1'E	1490	820	5.5	23.8
7		Gwebi	Random drought stress	17°13'S	31°E	1406	637	4.9	26.2
8		Gwebi	Low N	17°13'S	31°E	1406	637	4.9	26.2
9		Rattray-Arnold	Optimum	17°67'S	31°17'E	1452	865	5.3	23.6
10		Rattray-Arnold	Low N	17°67'S	31°17'E	1452	865	5.3	23.6
11		Bindura	Random drought stress	17°18'S	31°19'E	1118	976	4.8	27.8
12		Glendale	Optimum	17°08'S	31°3'E	1250	669	7.4	28.1
13		Chinhoyi University	Optimum	17° 21'S	30° 11'E	1168	NA	NA	NA
14		Lionsdale	Optimum	19°19'S	30°58'E	1438	620	6.1	29
15		Kwekwe	Optimum	19°00′S	29°45′E	1187	NA	NA	NA
16		AREX Harare	Low N	17°49'S	31°1'E	1489	NA	NA	NA
17		Gweru	Random drought stress	19°28'S	29°48'E	1457	NA	NA	NA
18		Makaholi	Managed drought	19°83'S	30°78'E	1111	561	22.22 (mean)	
19	Zimbabwe	Chisumbanje	Managed Drought	20°48'S	32°14'E	455	455	NA	NA
20		Mpongwe	Optimum	13°32'S	28°03'E	1300	1500	NA	NA
21		Lusaka	Optimum	15°19'S	28°0'E	1153	NA	NA	NA
22		Magobo	Random drought stress	14°3'S	32°7'E	1075	NA	NA	NA
23		Zam seed main farm	Random drought stress	15°19'S	28°18'E	NA	NA	NA	NA
24		ETG Farm	Random drought stress	15°42'S	28°26'E	1277	NA	NA	NA
25		Chokwe	Managed drought	24°53'S	33° E	NA	NA	NA	NA
26	Zambia	Msekera	Optimum	13°38'S	32°39'E	1267	909	NA	NA
27		Lichinga	Random drought stress	13°30'S	35°23'E	1305	1060	NA	20.52
28	Mozambique	Umbeluzi	Random drought stress	26°58'S	32°38'E	23	440	NA	24.54
29		Meru	Random drought stress	09°44'S	33°23'E	1231	NA	NA	NA
30	Malawi	Chitala	Optimum	13°13'S	340°7'E	733	1046	23.94 (mean)	

NB: In Ethiopia, the trial was evaluated in two seasons; NA = the data is not available, Min = minimum; Max = maximum

3.2.3 Data collection

Grain yield and agronomic data were collected from all sites for all management conditions. Grain yield was recorded in kg at harvesting by weighing the total ears per plot. This was adjusted to 12.5% moisture content to estimate grain yield expressed in ton per ha. The moisture content of grain from each plot was measured by moisture tester and used to calculate grain yield per ha. Days to anthesis was recorded from planting date to when 50% of the plants in a plot started pollen shedding. Days to silking was recorded from planting date to when 50% of the plants in the plot produced 2 – 3 cm long silks. ASI was calculated as the difference between the number of days to silking and days to anthesis. The mean plant and ear height was measured in centimetre from five random plants in a plot, plant height was measured from the ground level to the first tassel branch, and ear height was measured from the ground to the top useful ear insertion base of the node. Ear position was calculated as a ratio of the ear height to plant height. At harvesting, the total number of harvested plants and ears were counted in all plots at each site. Number of ears per plant was calculated as the ratio of total ears in a plot to total plants harvested in a plot. Leaf senescence (on a scale from 1 to 10) was scored on stress trials, where 1 indicates no senescence (green) and 10 indicates that leaves are yellow and dead.

3.3 Data analysis

3.3.1 Analysis of variance

Individual and combined environment analysis of variance (ANOVA) was done on all collected data using a PROC mixed effect model procedure of SAS statistical package (SAS, 2002). Hybrids were considered as fixed; while environment, replication and incomplete blocks within replications were considered random factors. Across environments combined analyses were carried out by using adjusted hybrid means from each environment analysis in accordance with the lattice design (Cochran and Cox, 1960). In the combined environment analysis ANOVA, genotype effects were tested for significance using the corresponding interaction with the environment as the error term, whereas the genotype × environment interaction was tested against the pooled error. Mean comparison among hybrid was done by the least significant difference (LSD) test at 5% probability levels of significance (Gomez and Gomez, 1984).

The combined ANOVA linear mixed model was followed:

$$Y_{ijk} = \mu + G_i + E_j + R_k(E_j) + B_o[R_k(E_j)] + GE_{ij} + \varepsilon_{ijko}$$

Where, Y_{ijk} is the observed mean of the i^{th} genotype (G_i) in the environment (E_j), in the k^{th} block (B_k), μ is the overall mean, G_i is the effect of i^{th} genotype, E_j is the effect of j^{th} environment, R_k is replication effect of the k^{th} replication in the j^{th} environment, $B_o\,[R_k(E_j)]$ is the random effect of incomplete block o within replicate k^{th} and environment j^{th}, GE_{ij} is the interaction effect of the i^{th} genotype as well as j^{th} environment and ε_{ijko} is the pooled error.

3.3.2 Estimated magnitude of variance

Estimates of genotypic (σ^2g), genotype × environment, ($\sigma^2g\times e$) and error (σ^2e) variances were calculated using META-R software by using the residual maximum likelihood (REML) method (Alvarado et al. 2020). The REML has been used to remove bias in the estimation of variance components that develops when fixed effects are vulnerable to mistake. It is mostly used to deal with imbalanced experimental designs. The estimators maximise only the portion of the likelihood that does not depend on fixed effects. Phenotypic variance (σ^2p) was calculated by the formula suggested by Hallauer et al. (2010).

Phenotypic variance (σ^2p) = $\sigma^2g + \frac{\sigma 2g\times e}{e} + \frac{\sigma 2e}{r\times e}$, where r = number of replications, e = number of environments.

Phenotypic and genotypic coefficients of variation, broad sense heritability and genetic advance expressed as percent of the mean were determined based on the method suggested by Johnson et al. (1955).

- Genotypic coefficient of variation in % (GCV) = $\frac{\sqrt{\sigma 2g}}{x}$ ×100, where σ^2g = genotypic variance and x = mean of the trait
- Phenotypic coefficient of variation in % (PCV) = $\frac{\sqrt{\sigma 2p}}{x}$ ×100, where σ^2p = phenotypic variance and x= mean of the trait
- Broad sense heritability (H^2) = $\frac{\sigma 2g}{\sigma 2p}$, where σ^2g = genotypic variance and σ^2p = phenotypic variance
- Genetic advance (GA) = $K\sigma pH^2$, where σp = phenotypic standard deviation of the trait, H^2 = broad sense heritability and K = selection differential where K= 2.056 at 5% selection intensity

- Genetic advances in percent (GA%) = $\frac{GA}{X}$ × 100, GA = genetic advance, and x = mean of the trait

3.4 Results

3.4.1 Analysis of variance

Across optimum management and random drought stress conditions, highly significant differences were exhibited among QPM hybrids and commercial checks for all measured traits (Tables 3.3a and 3.3b), indicating the existence of variation among hybrids for grain yield and agronomic traits. The effect of environments was also highly significant for all studied traits. Similarly, G × E interaction effects were significant for all studied traits except for ear height and ear position under both optimum and random drought stress conditions and plant height under random drought stress. The effects of genotype, environment and G × E interaction under managed drought and low N stress were also significant for all studied traits (Tables 3.3c and 3.3d). In combined environment analysis, hybrids varied significantly for grain yield, and all studied agronomic traits (Table 3.3e), indicating the existence of genetic variability among hybrids. Environment and G × E interaction effects were also highly significant for grain yield and all other measured traits. This suggested that the hybrid rankings differed across all testing sites.

3.4.2 Hybrid performance

Mean of grain yield was 5.4 t ha^{-1} overall environments, 8.3 t ha^{-1} under optimum, 4.3 t ha^{1} under random drought stress, 2.7 t ha^{-1} under managed drought stress and 2.4 t ha^{-1} under low N stress conditions (Table 3.4). Grain yield was reduced by 48% under random drought stress, 68% under managed drought and 71% under low N conditions compared to optimum environments. Days to anthesis and silking were also decreased by 7 and 6% under random drought stress, and 11 and 8% under managed drought stress conditions, and increased by 13% and 16% under low N conditions, respectively. ASI was increased by 80%, 218% and 203% under random drought stress, managed drought and low N compared to optimum conditions, respectively. Plant and ear height was reduced by 20% and 25% under random drought stress, 30% and 29% under managed drought and 23% and 27% under low N environments, respectively. Ear position was also reduced by 7% under random drought stress and 2% under managed drought and 6% under low N conditions. Number of ears per plant was reduced by 11% under random drought stress, 24% under managed drought and 20% under low N environments.

Table 3.3 Mean squares for grain yield and agronomic traits for 44 QPM hybrids and commercial checks evaluated under different environmental conditions in 2018 to 2020

a) Optimum conditions

Source variation	Df	GY t ha^{-1}	DA days	DS days	ASI days	PH cm	EH cm	EPO ratio	EPP #
E	13	48.88**	15379.77**	15059.59**	58.19**	54214.64**	13878.79**	0.08**	1.49**
Rep (E)	14	5.07**	12.07**	13.17**	2.08	2148.87**	1114.91**	0.01**	0.05*
Block (Rep × E)	224	1.89**	6.15**	6.63**	2.24**	540.27**	395.47**	0.00**	0.04**
Genotype	43	3.84**	28.38**	33.89**	4.37**	1348.01**	1246.41**	0.01**	0.26**
G × E	559	1.45**	3.63**	3.21**	1.71**	234.97*	182.16	0.00	0.03*
Residuals	378	0.74	2.19	2.05	1.25	189.56	159.70	0.00	0.02
CV (%)		10.37	2.07	1.97	101.85	5.44	9.77	8.50	13.34
mean		8.28	71.55	72.65	1.10	253.27	129.33	0.51	1.16

b) Random drought stress

Source variation	Df	GY t ha^{-1}	DA days	DS days	ASI days	PH cm	EH cm	EPO ratio	EPP #
E	8	80.94**	3484.04**	3920.19**	74.00**	101942.57**	73274.73**	0.53**	1.77**
Rep (E)	9	4.73**	9.09**	13.98**	1.68**	705.07*	285.53*	0.00	0.04
Block (Rep × E)	144	1.79**	7.52**	8.86**	1.10**	725.00**	279.32**	0.00	0.05**
Genotype	43	3.06**	14.76**	15.56**	1.32**	940.76**	426.83**	0.00**	0.08**
G × E	344	0.99**	3.01	3.46	1.03**	315.92	134.48	0.00	0.03**
Residuals	243	0.68	2.52	2.94	0.63	347.86	124.42	0.00	0.02
CV (%)		19.02	2.39	2.50	40.03	9.22	11.46	10.95	14.41
mean		4.32	66.54	68.52	1.98	202.35	97.34	0.47	1.03

c) Managed drought

Source	Df	GY t ha^{-1}	DA days	DS days	ASI days	PH cm	EH cm	EPO ratio	EPP #
E	2	17.78**	17486.41**	22467.66**	375.38**	216330.15**	121370.95**	0.61**	2.28**
Rep (E)	3	6.59**	12.22**	55.41**	19.34**	6808.82**	1824.47**	0.01**	0.02
Block (Rep × E)	48	1.32**	4.80**	13.84**	5.68**	538.29**	319.32**	0.00**	0.02**
Genotype	43	0.90**	9.47**	19.99**	5.54**	381.12**	259.12**	0.00**	0.03**
G × E	86	0.74**	2.85**	5.22**	2.68*	241.87**	99.89**	0.00**	0.03**
Residuals	81	0.30	1.08	2.28	1.63	115.64	39.30	0.00	0.01
CV (%)		20.58	1.64	2.25	36.46	6.06	6.86	6.02	12.47
mean		2.67	63.53	67.03	3.50	177.34	91.35	0.50	0.88

d) Low N stress

Source variation	Df	GY t ha^{-1}	DA days	DS days	ASI days	PH cm	EH cm	EPO ratio	EPP #	Df	Sen 0-10 scale
E	7	86.76**	14032.51**	15738.13**	190.99**	51332.55**	21802.58**	0.10**	0.92**	3	116.39**
Rep (E)	8	3.99**	89.38**	144.75**	10.01**	4011.45**	1627.38**	0.01**	0.13**	4	8.21**
Block (Rep × E)	128	0.91**	17.55**	28.77**	5.91**	591.30**	316.74**	0.00**	0.05**	64	0.94**
Genotype	43	1.18**	27.81**	45.48**	7.66**	657.48**	325.76**	0.01**	0.05**	43	1.10**
G × E	301	0.53**	6.19**	8.75**	4.78**	239.26*	130.32**	0.00*	0.03**	129	0.66**
Residuals	216	0.22	4.25	4.98	2.19	190.90	123.54	0.00	0.02	108	0.28
CV (%)		19.29	2.54	2.64	44.45	7.04	11.81	9.82	15.87		16.46
mean		2.40	81.08	84.41	3.33	196.16	94.14	0.48	0.92		3.22

e) Combined for all environments

Source	Df	GY t ha^{-1}	DA days	DS days	ASI days	PH cm	EH cm	EPO ratio	EPP #
E	33	647.02**	13990.74**	15070.01**	189.66**	145555.55**	61095.69**	0.24**	2.36**
Rep (E)	34	4.86**	29.48**	48.07**	5.36**	2616.11**	1078.56**	0.01**	0.06**
Block (Rep × E)	544	1.58**	9.07**	13.07**	3.10**	601.00**	339.48**	0.00**	0.04**
Genotype	43	5.42**	66.93**	91.14**	9.91**	2554.27**	1770.41**	0.01**	0.27**
G × E	1419	1.08**	4.05**	5.00**	2.37**	257.99*	152.84**	0.00**	0.03**
Residuals	918	0.56	2.66	3.00	1.34	225.26	131.23	0.00	0.02
CV (%)		13.97	2.27	2.34	55.99	6.83	10.49	9.28	14.13
mean		5.35	71.76	73.83	2.07	219.65	109.23	0.49	1.05

* P ≤0.05, ** P ≤ 0.01; E = environment; Rep = replication; G × E = genotype × environment interaction; CV = Coefficient of variation; Df = degree of freedom; GY= grain yield; DA = days of anthesis; DS = days to silking; ASI = anthesis silking interval; PH = plant height; EH = ear height; EPO = ear position; EPP = number of ears per plant

Table 3.4 Percentage of grain yield and agronomic performance of under random stress, managed drought and low N stress conditions compared to the performance under optimum management condition

Traits	OP	RS	% change	MD	% change	LN	% change
Grain yield (t ha^{-1})	8.3	4.3	48	2.7	68	2.40	71
Days to anthesis (days)	71.5	66.5	7	63.5	11	81.08	-13
Days to silking (days)	72.6	68.5	6	67.0	8	84.41	-16
Anthesis silking interval (days)	1.1	2.0	-80	3.5	-218	3.33	-203
Plant height (cm)	253.3	202.3	20	177.3	30	196.16	23
Ear height (cm)	129.3	97.3	25	91.3	29	94.14	27
Ear position (cm)	0.51	0.47	7	0.50	2	0.48	6
Number of ears per plant (#)	1.16	1.03	11	0.88	24	0.92	20

Opt = Optimum management; RS = random drought stress; MD = managed drought; LN = low nitrogen; negative value of traits indicates an increase under random drought stress, managed drought and low N stress conditions

3.4.2.1 Hybrid performance under optimum environments

Across optimum environments, grain yield ranged from 7.3 t ha^{-1} to 8.9 ha^{-1} with an overall mean of 8.3 t ha^{-1} (Table 3.5). The top ten QPM hybrids showed comparable yield performance with the best non-QPM commercial check (PAN53) and 10.9 and 11.9% yield advantage compared to the mean of checks and the best QPM check, respectively. The highest yielding QPM hybrid, entry 44 (QS764612) also showed a 13.7% grain yield advantage and comparable days to anthesis and silking over the best QPM check, and lower days to anthesis, plan height and ear height compared to the mean of the best normal maize check. However, this best hybrid showed 47.5% and 103% higher ASI compared to the best QPM and normal maize checks, respectively (Table 3.5).

Days to anthesis and silking of the trial ranged from 69.7 to 74.0 days and 70.5 to 75.5 days with an overall mean of 71.5 and 72.6 days, respectively (Table 3.5). The mean of the top ten hybrids showed comparable days to anthesis and silking with the mean of checks and the best QPM and normal maize checks. ASI ranged from 0.3 to 2.1 days with a mean of 1.1 days. The top ten hybrids showed 20.3, 35.5 and 10.7% lower ASI than the mean of checks and best QPM and normal maize checks, respectively. All top yielding hybrids had an ASI of less than two days except entry 14 (2.1 days). Among the top ten high yielding hybrids, 50% recorded lower plant and ear height than the grand mean. Minimum and maximum ear placement of the trial ranged from 0.48 and 0.56, respectively, with a mean of 0.51. Most of the top ten QPM hybrids showed good ear placement and had similar ear placement with the best QPM and normal maize checks. Minimum and maximum number of ears per plant was 1.0 and 1.3, respectively

with a grand mean of 1.2. Except for entry 17, all top yielding QPM hybrids showed similar or more than the mean number of ears per plant. The mean of the top ten hybrids had 19.3, 11.5 and 25.3% higher number of ears per plant compared to the mean, best QPM and normal maize checks, respectively. The top yielding hybrid, entry 44(QS7646) also showed 14.6 and 28.8% higher number of ears per plant compared to the best QPM and normal maize checks, respectively.

Table 3.5 Mean of grain yield and agronomic performance of 10 top QPM hybrids and commercial checks evaluated under optimum conditions

Entry	Genotype name	GY t ha^{-1}	DA days	DS days	ASI days	PH cm	EH cm	EPO ratio	EPP #
44	QS7646	8.9	70.2	72.3	2.1	253.1	129.2	0.51	1.3
12	CZH15099Q	8.9	70.5	71.1	0.7	246.0	125.2	0.51	1.2
9	CZH142237Q	8.8	71.7	72.0	0.4	244.0	121.0	0.50	1.3
17	CZH16021Q	8.8	71.8	73.1	1.4	257.2	129.7	0.50	1.0
6	CZH132044Q	8.7	72.4	73.1	0.8	258.9	134.6	0.52	1.2
38	CZH17207Q	8.7	74.0	74.7	0.7	259.3	144.0	0.56	1.3
10	CZH142238Q	8.7	71.0	71.8	0.8	243.9	118.7	0.49	1.2
20	CZH17189Q	8.7	71.0	71.7	0.7	264.7	140.1	0.53	1.3
14	CZH15142Q	8.7	70.6	71.6	1.0	250.2	120.1	0.48	1.2
11	CZH15098Q	8.6	69.8	70.5	0.8	245.1	122.9	0.50	1.3
1	ZS261 (QPM check one)	7.3	70.1	71.4	1.3	250.4	124.5	0.50	1.1
2	Mama MQ623 (QPM check two)	7.8	70.3	71.7	1.4	244.0	122.7	0.50	1.1
3	PAN53 (normal maize check)	8.8	72.1	73.1	1.0	256.4	129.3	0.51	1.0
4	SC533 (normal maize check)	7.6	70.1	71.0	0.9	247.9	121.6	0.49	1.0
LSD		0.5	0.8	0.8	0.6	7.2	6.6	0.02	0.1
Mean		8.3	71.5	72.6	1.1	253.3	129.3	0.51	1.2
Minimum		7.3	69.7	70.5	0.3	235.9	117.5	0.48	1.0
Maximum		8.9	74.0	75.5	2.1	268.8	145.1	0.56	1.3
Mean of top 10 hybrids		8.7	71.3	72.2	0.9	252.2	128.6	0.5	1.2
Mean of checks		7.9	70.6	71.8	1.2	249.7	124.5	0.5	1.0
Top 10 hybrids over checks (%)		10.9	0.91	0.6	-20.3	1.0	3.2	1.9	19.3
Top 10 hybrids over best QPM check (%)		11.9	1.42	0.7	-35.3	3.4	4.8	1.1	11.5
Top 10 hybrids over best normal maize check (%)		-1.25	-1.1	-1.3	-10.7	-1.6	-0.5	0.7	25.3
Best hybrid over best QPM check (%)		13.7	-0.2	0.8	47.5	3.7	5.3	1.5	14.6
Best hybrid over best normal maize check (%)		0.36	-2.7	-1.2	103	-1.3	-0.0	1.1	28.8

GY = grain yield, DA = days to anthesis, DS = days to silking, ASI = anthesis silking interval, PH = plant height, EH = ear height, EPO = ear position, EPP = number of ears per plant, LSD = least significant difference

3.4.2.2 Hybrid performance under random drought stress environments

Grain yield random drought stress ranged from 3.2 to 5.3 t ha^{-1}, with an overall mean of 4.3 t ha^{-1} (Table 3.6). The top ten QPM hybrids showed significantly higher grain yield than the mean of checks and the best QPM check. However, they showed slightly lower yields than the best commercial normal maize check. The highest yielding QPM hybrid, entry 14 recorded a 30.3 and 7.8% grain yield advantage over the best QPM and normal maize checks, respectively.

Minimum days to anthesis were 64.7 while the maximum was 69.0 with a mean of 64.7 days; and mean days to silking was 68.5 with a range of 66.4 to 70.9 days. The top ten QPM hybrids did not show a significant difference in days to anthesis and to silking compared to the mean of checks, best QPM and normal maize commercial checks. ASI ranged from 1.4 to 3.0 days with an overall mean of 2.0 days. The top ten QPM hybrids showed 11.5, 22.8 and 19.0% lower ASI days than the mean of the checks, best QPM and normal maize commercial checks, respectively. The best yielding hybrid, entry14 (CZH15142Q) had a slight decrease in days to anthesis and silking, and 25.6 and 22.0% lower ASI compared to the mean of the best QPM and normal maize checks, respectively. Plant height ranged between 182.7 and 215.5 cm with a mean of 202.3 cm. Ear height ranged between 85.9 and 109.4 cm with a mean of 97.3 cm. The respective mean, minimum and maximum values of ear position were 0.47, 0.44 and 0.52, respectively. The top ten QPM hybrids showed 2.7 and 10.1% 0.4% higher plant height, and 4.3 and 6.1% higher ear height compared to the mean of the checks and best QPM check, respectively. However, they showed lower plant and ear height, and ear position as compared to the best normal maize check. Mean number of ears per plant was 1.0, with a range of 0.9 to 1.2 ears. The top ten QPM hybrids showed an increase in number of ears of 15.4, 12.3 and 26.7% compared to the mean of the checks, best QPM and normal maize checks, respectively. The first top QPM hybrid also had 8.5 and 22.4% higher number of ears per plant compared to the best QPM and normal maize commercial checks, respectively.

Table 3.6 Mean of grain yield and agronomic performance of the top 10 QPM hybrids and checks evaluated under random drought stress conditions

Entry	Genotype name	GY t ha^{-1}	DA days	DS days	ASI days	PH cm	EH cm	EPO ratio	EPP #
14	CZH15142Q	5.3	65.2	67.0	1.8	202.5	93.1	0.45	1.1
44	QS7646	4.9	65.6	67.8	2.3	197.6	91.2	0.46	1.1
23	CZH17192Q	4.9	67.3	69.1	1.8	211.2	102.3	0.48	1.0
10	CZH142238Q	4.8	65.2	67.1	1.8	192.0	92.8	0.47	1.1
9	CZH142237Q	4.8	66.5	68.1	1.6	201.0	98.7	0.48	1.1
34	CZH17203Q	4.7	66.7	68.6	1.9	202.2	100.4	0.49	1.1
8	CZH142236Q	4.7	66.9	68.6	1.7	193.7	90.4	0.46	1.1
38	CZH17207Q	4.7	69.0	70.9	1.9	206.7	106.0	0.51	1.2
18	CZH17187Q	4.6	66.4	68.6	2.2	215.5	101.2	0.46	1.1
16	CZH16022Q	4.6	67.4	69.1	1.6	188.8	87.8	0.46	1.0
1	ZS261	3.2	65.4	67.4	1.9	191.7	85.9	0.44	1.0
2	Mama MQ623	4.1	65.3	67.7	2.4	182.7	90.9	0.49	1.0
3	PAN53	4.9	66.5	68.8	2.3	212.5	103.8	0.48	0.9
4	SC533	3.5	64.7	66.4	1.7	196.5	89.1	0.44	0.9
LSD		0.5	1.0	1.1	0.5	12.2	7.3	0.03	0.1
Mean		4.3	66.5	68.5	2.0	202.3	97.3	0.47	1.0
Minimum		3.2	64.7	66.4	1.4	182.7	85.9	0.44	0.9
Maximum		5.3	69.0	70.9	3.0	215.5	109.4	0.52	1.2
Mean of top 10 hybrids		4.8	66.6	68.5	1.8	201.1	96.4	0.5	1.1
Mean of checks		3.9	65.5	67.6	2.1	195.8	92.4	0.5	0.9
Top 10 hybrids over checks (%)		22.0	1.7	1.3	-11.5	2.7	4.3	1.6	15.4
Top 10 hybrids over best QPM check (%)		17.6	2.1	1.2	-22.8	10.1	6.1	-4.0	12.3
Top 10 hybrids over best normal maize check (%)		-2.7	0.2	-0.5	-19.0	-5.4	-7.1	-2.2	26.7
Best hybrid over best QPM check (%)		30.3	-0.1	-1.0	-25.6	10.8	2.4	-7.6	8.5
Best hybrid over best normal maize check (%)		7.8	-1.9	-2.6	-22.0	-4.7	-10.3	-5.9	22.4

GY= grain yield; DA = days of anthesis; DS = days to silking; ASI = anthesis silking interval; PH = plant height; EH = ear height; EPO = ear position; EPP = number of ears per plant, LSD = least significant difference

3.4.2.3 Hybrid performance under managed drought stress

Grain yield ranged between 1.8 t ha^{-1} to 3.6 t ha^{-1} with a mean of 2.7 t ha^{-1} (Table 3.7). The top ten QPM hybrids showed 23.7%, 15.9% and 6.8% higher grain yield compared to the mean of the checks, best QPM and normal maize checks, respectively. The top yielding hybrid, entry 9 (CZH142237Q) also showed 29.3 and 19.2% yield advantage over the best QPM and normal maize checks, respectively (Table 3.7). Days to anthesis ranged from 60.5 to 66.2, with an overall mean of 63.5 days while days to silking varied from 63.2 to 71.0 with a mean of 67 days. All top ten QPM hybrids recorded comparable days to anthesis and silking with the mean of checks and the best QPM and normal maize check hybrids. ASI ranged from 1.5 to 5.5 days with a mean of 3.5 days, which was higher than the optimum and random drought environments mean. The top ten hybrids showed 36.6, 31.6 and 13.3% lower ASI compared to the mean of checks and best QPM and normal maize checks, respectively. Among the top ten hybrids, the top yielding hybrid, entry 9 (CZH142237Q), showed 42.1 and 26.7% lower ASI than the best QPM and normal maize checks, respectively. Mean, minimum and maximum plant height of

the trials were 177.3 cm, 159.9 cm and 194.6 cm, respectively. Ear height varied from 78.5 cm to 108.9 cm, with a mean of 91.3 cm. The mean, minimum, and maximum value of ear position was 0.5, 0.46 and 0.56, respectively. Number of ears per plant ranged from 0.70 to 1.01, with a mean of 0.88. Most of the top ten QPM hybrids did not show significant differences in number of ears per plant with the overall mean and best commercial QPM and normal maize checks.

Table 3.7 Mean of grain yield agronomic performance of the top 10 QPM hybrids and commercial checks evaluated under managed drought stress

Entry	Genotype name	GY t ha^{-1}	DA days	DS days	ASI days	PH cm	EH cm	EPO ratio	EPP #
9	CZH142237Q	3.63	64.2	66.0	1.8	165.9	87.5	0.52	0.92
10	CZH142238Q	3.52	62.5	64.7	2.2	181.4	85.0	0.46	0.90
23	CZH17192Q	3.34	64.5	67.5	3.0	194.6	106.9	0.53	0.84
40	CZH17209Q	3.30	62.5	64.7	2.2	178.2	101.6	0.55	0.96
14	CZH15142Q	3.21	62.5	64.2	1.7	180.3	83.3	0.46	1.01
12	CZH15099Q	3.17	62.0	63.8	1.8	172.8	95.2	0.54	0.97
35	CZH17204Q	3.15	62.0	64.3	2.3	184.5	92.9	0.49	0.99
22	CZH17191Q	3.10	63.8	65.2	1.3	176.9	94.4	0.52	0.94
8	CZH142236Q	3.09	63.0	64.7	1.7	190.1	86.8	0.46	0.86
7	CZH132018Q	3.03	64.7	68.3	3.7	156.9	81.7	0.51	0.96
1	ZS261	2.6	61.0	65.5	4.5	186.4	88.5	0.47	0.93
2	Mama MQ623	2.8	62.3	65.5	3.2	172.7	88.0	0.49	0.87
3	PAN53	3.0	63.8	66.3	2.5	183.8	92.4	0.50	0.92
4	SC533	2.1	61.0	64.5	3.5	168.2	80.8	0.47	0.70
LSD		0.6	1.2	1.7	1.5	12.4	7.2	0.03	0.13
Mean		2.7	63.5	67.0	3.5	177.3	91.3	0.50	0.88
Minimum		1.8	60.5	63.2	1.3	156.9	78.5	0.46	0.70
Maximum		3.6	66.2	71.0	5.5	194.6	108.9	0.56	1.01
Mean of top 10 hybrids		3.3	63.2	65.3	2.2	178.2	91.5	0.5	0.9
Mean of checks		2.6	62.0	65.5	3.4	177.8	87.4	0.5	0.9
Top 10 hybrids over checks (%)		23.7	1.8	-0.2	-36.6	0.2	4.7	4.6	9.2
Top 10 hybrids over best QPM check (%)		15.9	1.3	-0.3	-31.6	3.1	4.0	2.8	7.9
Top 10 hybrids over best normal maize check (%)		6.8	-1.0	-1.5	-13.3	-3.1	-0.9	1.0	1.2
Best hybrid over best QPM check (%)		29.3	2.9	0.8	-42.1	-4.0	-0.6	6.5	6.6
Best hybrid over best normal maize check (%)		19.2	0.5	-0.5	-26.7	-9.8	-5.3	4.7	0.0

GY = grain yield; DA = days of anthesis; DS = days to silking; ASI = anthesis silking interval; PH = plant height; EH = ear height; EPO = ear position; EPP = number of ears per plant, LSD = least significant difference

3.4.2.4 Hybrid performance under low N environments

Mean grain yield of hybrids ranged from 1.9 to 3.2 t ha^{-1} with an average of 2.4 t ha^{-1} (Table 3.8). More than 50% of hybrids in the trial showed higher grain yield than the mean. Grain yield comparison between the highest yielding new QPM hybrid, entry14 (CZH15142Q) and the mean of best QPM and non-QPM checks (Mama MQ623 and PAN53) showed an average grain yield advantage 15% and 13%, respectively. In addition to this, the top yielding QPM hybrid, entry 14 (CZH15142Q), decreased 1.2% in days to anthesis, 2.2% in days to silking, 25.9% in ASI, 8.7% in plant height and 2.7% in ear height compared to the best normal maize check. However, this hybrid had a 6.3% higher ASI and 6.2% higher leaf senescence compared

to the mean of the best normal maize check. The mean of the top ten QPM hybrids increased 12.3% in grain yield and had comparable days to anthesis, days to silking, ASI, ear position and number of ears per plant compared to the mean of the checks.

Days to anthesis ranged between 78.5 to 84.9 with an overall average of 81.1 days, while days to silking varied between 80.5 to 89.7 with an average of 84.4. The range of ASI was higher compared to the optimum and random drought environments. Mean, minimum and maximum ASI days were 3.3, 1.9 and 4.9. Plant height ranged from 181.8 to 214.6 cm with a mean of 196.2 cm. The mean of ear height was 94.1 cm and ranged from 83.5 to 103.2 cm. Ear position ranged from 0.44 to 0.52 with an average 0.48. The average number of ears per plant was 0.9 and ranged from 0.8 to 1.1. The leaf senescence ranged from 2.5 to 4.1 with a mean of 3.2.

Table 3.8 Mean grain yield and agronomic performance of the top 10 QPM hybrids and commercial checks evaluated under low N stress

Entry	Genotype name	GY tha^{-1}	DA days	DS days	ASI days	PH cm	EH cm	EPO ratio	EPP #	Sen (0-10 scale)
14	CZH15142Q	3.2	79.6	82.1	2.5	196	94	0.48	1.0	3.0
34	CZH17203Q	3.0	81.8	85.6	3.8	199.1	102.8	0.51	0.9	3.0
11	CZH15098Q	3.0	78.9	80.9	1.9	200.4	98.1	0.49	1.0	3.1
7	CZH132018Q	2.9	81.7	84.8	3.1	187.6	90.2	0.48	0.9	2.5
27	CZH17196Q	2.8	80.2	83.4	3.2	202.5	98.9	0.49	1.0	2.7
43	MH1634	2.7	78.5	80.8	2.3	188.7	86.1	0.46	1.0	3.1
12	CZH15099Q	2.6	79.4	82.3	2.8	187.6	95.6	0.52	1.0	3.2
9	CZH142237Q	2.6	81.6	83.9	2.3	192.6	91.1	0.47	1.0	3.1
29	CZH17198Q	2.6	81.6	84.6	3	200.1	96.4	0.48	0.9	3.0
10	CZH142238Q	2.6	80.3	82.6	2.3	188.9	83.5	0.44	1.1	3.3
1	ZS261	2.2	79.6	82.2	2.6	186.1	86.4	0.46	0.8	3.4
2	Mama MQ623	2.8	78.6	81.3	2.7	188.3	93.2	0.5	1.0	3.0
3	PAN53	2.8	80.6	83.9	3.4	214.6	96.6	0.45	0.9	2.8
4	SC533	2.1	80.1	82.7	2.6	186.8	87.3	0.47	0.9	3.2
LSD		0.3	1.4	1.6	1.0	9.6	7.7	0.03	0.1	0.5
Mean		2.4	81.1	84.4	3.3	196.2	94.1	0.48	0.9	3.2
Minimum		1.9	78.5	80.8	1.9	181.8	83.5	0.44	0.8	2.5
Maximum		3.2	84.9	89.7	4.9	214.6	103.2	0.52	1.1	4.1
Mean of top 10 hybrids		2.8	80.4	83.1	2.7	194.3	93.7	0.48	1.0	3.0
Mean of checks		2.5	79.7	82.5	2.8	194	90.9	0.47	0.9	3.1
Top 10 hybrids over checks (%)		12.3	0.8	0.7	-3.1	0.2	3.1	2	7.5	-3.7
Top 10 hybrids over best QPM check (%)		0.7	2.2	2.2	1.4	3.2	0.5	-3.8	-0.5	-0.3
Top 10 hybrids over best normal maize check (%)		-1.3	-0.3	-1	-19	-9.5	-3.1	7.2	9.3	5.8
Best hybrid over best QPM check (%)		15.4	1.2	0.9	-7	4.1	0.9	-4.6	3.5	0
Best hybrid over best normal maize check (%)		13	-1.2	-2.2	-26	-8.7	-2.7	6.3	13.6	6.2

GY= grain yield; DA = days of anthesis; DS = days to silking; ASI = anthesis silking interval; PH = plant height; EH = ear height; EPO = ear position; EPP = number of ears per plant; sen = leaf senescence; LSD = least significant difference

3.4.2.5 Hybrid performance in combined environments

Grain yield of the trial ranged from 4.6 t ha^{-1} for commercial QPM check (ZS261) to 6.0 t ha^{-1} for entry 14 (CZH15142Q) with an overall mean of 5.4 t ha^{-1} (Table 3.9). The mean grain yield of the top ten QPM hybrids was 5.7 t ha^{-1} and showed 12.3 and 10.3% yield advantage over the mean of all commercial checks and the mean of the best QPM check, respectively (Table 3.9). Among the top ten hybrids, the top yielding hybrid, entry 14, showed a 15.7% yield advantage over the best QPM check and comparable yield performance with the normal maize check.

Mean days to anthesis and silking varied from 69.8 to 74.5 and 71.4 to 77.1 days with overall means of 71.8 and 73.8 days, respectively. The top ten QPM hybrids showed slightly higher days to anthesis and silking compared to the mean of all checks, and mean of the best QPM check, while they showed similar days to anthesis and silking compared to the best normal maize commercial check. ASI ranged from 1.3 to 3.0 with a grand mean of 2.1. The top ten QPM hybrid values decreased by 3.2, 9.5 and 5.6 when compared with the mean of all checks, the best QPM and normal maize checks, respectively. The highest yielding hybrid, entry 14 (CZH15142Q) showed a 24.1 and 20.9% lower ASI, 5.8 and 3.3% lower ear position and 9.1 and 20.4% higher number of ears per plant, compared to the best QPM and normal maize checks, respectively. This entry also recorded comparable anthesis and silking days with the mean of the best QPM and normal maize commercial checks.

Plant height ranged from 207.2 to 232.1 cm with a grand mean 219.7 cm while ear height ranged from 99.1 cm to 119.4 cm with a grand mean of 109.2 cm. The mean plant height of the top ten QPM hybrids were 219.4 cm, and 1.6 and 5.3% higher compared to the mean of all checks and the best QPM check, respectively. Additionally, the top ten QPM hybrids' mean ear height was 3.7 and 4.2% higher than the mean of commercial checks and the best QPM check, respectively. However, they had 4 and 2.6% lower plant and ear height compared to the best commercial QPM check, respectively. Ear to plant height ratio ranged from 0.47 to 0.53 with an overall mean of 0.49. The mean value of ear position of the top ten QPM hybrids were similar to the mean of all checks and the best commercial QPM and normal maize checks. All top yielding hybrids showed mid ear placement, which can prevent lodging. Number of ears per plant varied from 0.9 to 1.1 with a mean of 1.0 ear per plant. The top ten QPM hybrids' number of ears per plant was 12, 6.8 and 17.8% higher compared to the mean of all checks, the best QPM and normal maize checks, respectively.

Table 3.9 Mean of grain yield and agronomic performance of the top 10 QPM hybrids and commercial checks evaluated across all environments

Entry	Genotype name	GY	DA	DS	ASI	PH	EH	EPO	EPP
14	CZH15142Q	6.0	70.6	72.2	1.6	218.6	103.6	0.47	1.1
9	CZH142237Q	5.8	72.0	73.3	1.3	213.6	105.1	0.49	1.1
44	QS7646	5.8	70.4	73.3	2.9	218.8	107.3	0.49	1.1
10	CZH142238Q	5.8	70.9	72.5	1.6	211.7	100.6	0.47	1.1
12	CZH15099Q	5.7	70.4	72.1	1.6	212.5	108.2	0.51	1.1
34	CZH17203Q	5.7	72.5	74.8	2.3	221.8	116.1	0.52	1.1
23	CZH17192Q	5.7	72.5	74.8	2.3	231.4	116.8	0.50	1.0
11	CZH15098Q	5.6	69.8	71.4	1.6	213.1	104.2	0.49	1.1
17	CZH16021Q	5.6	72.4	74.8	2.4	226.1	111.8	0.49	1.0
6	CZH132044Q	5.6	72.9	74.5	1.7	226.8	113.1	0.49	1.1
1	ZS261 (QPM check)	4.6	70.3	72.4	2.1	214.1	102.1	0.47	1.0
2	Mama MQ623 (QPM check)	5.2	70.2	72.4	2.1	208.3	104.3	0.50	1.0
3	PAN53 (Normal maize check)	5.9	71.9	73.9	2.0	228.5	111.6	0.49	0.9
4	SC533 (Normal maize check)	4.7	70.2	72.0	1.7	212.9	101.3	0.47	0.9
LSD		0.3	0.5	0.6	0.4	5.1	3.9	0.02	0.0
Mean		5.4	71.8	73.8	2.1	219.7	109.2	0.49	1.0
Minimum		4.6	69.8	71.4	1.3	207.2	99.1	0.47	0.9
Maximum		6.0	74.5	77.1	3.0	232.1	119.4	0.53	1.1
Mean of top 10 hybrids		5.7	71.4	73.4	1.9	219.4	108.7	0.50	1.1
Mean of checks		5.1	70.7	72.6	2.0	216.0	104.8	0.50	1.0
Top 10 hybrids over checks (%)		12.3	1.1	1.0	-3.2	1.6	3.7	1.8	12.8
Top 10 hybrids over best QPM check (%)		10.3	1.7	1.4	-9.5	5.3	4.2	-1.5	6.8
Top 10 hybrids over best normal maize check (%)		-2.6	-0.6	-0.8	-5.6	-4.0	-2.6	1.1	17.8
Best hybrid over best QPM check (%)		15.7	0.5	-0.2	-24.1	4.9	-0.7	-5.8	9.1
Best hybrid over best normal maize check (%)		2.2	-1.8	-2.3	-20.9	-4.3	-7.2	-3.3	20.4

GY = grain yield, DA = days to anthesis, DS = days to silking, ASI = anthesis silking interval, PH = plant height, EH = ear height, EPO = ear position, EPP = number of ears per plant, LSD = least significant difference

3.4.3 Estimates of variance components

Genotypic variance (σ^2g) ranged from 0 to 51.8 under optimum, 0 to 64.9 under random drought stress, 0 to 30.1 under managed drought, 0 to 32.3 under low N stress conditions and 0 to 44.2 across all testing environments (Tables 3.10a to 3.10e). Phenotypic variance (σ^2p) ranged from 0 to 59.2 under optimum, 0 to 80.4 under random drought, 0 to 63.9 under managed drought, 0 to 48.4 under low N stress and 0 to 48.3 across combined testing environments. Genotype × environment interaction variance (σ^2g$_{\times}$e) ranged from 0 to 18.9 under optimum, 0 to 19.2 under random drought stress, 0 to 89.1 under managed drought and 0 to 36.8 under low N environments and 0 to 40.0 across all environments. The highest genotypic, phenotypic, and G × E interaction variances were observed for plant and ear height while the lowest variances were observed for ear position and number of ears per plant across all trials.

Genotypic coefficient of variation (GCV) ranged from 0% to 18.2% under optimum conditions, 0 to 32.3% under random drought stress, 0 to 23.1% under managed drought, 0 to 15.6 % under

low N stress and 0 to 22.3% in combined environment analysis (Table 3.10a - e). Phenotypic coefficient of variation (PCV) ranged from 1.8% to 39.5% under optimum, 1.5% to 45.8% under random drought stress, 2.2% to 31.2% under managed drought, 2.2 % to 27.8% under low N stress and 1.2 to 26.0% under combined environments. The highest GCV and PCV estimated values were observed for ASI under almost all management conditions while all other traits showed lower GCV and PCV values except for grain yield, which recorded moderate PCV under random drought stress, managed drought and low N testing sites and leaf senescence also recorded moderate PCV under low N stress conditions. In general, PCV values of grain yield was lower under optimum and random drought stress than managed drought and low N conditions. In addition, PCV values were higher than GCV for grain yield and all other agronomic traits under all management conditions.

3.4.4 Estimates of broad sense heritability and genetic advance

Broad sense heritability (H^2) estimates ranged from 0.54 for ASI to 0.92 for days to anthesis and silking under optimum conditions (Table 3.10a). Heritability values under random drought stress varied from 0.49 for ASI to 0.82 for days to anthesis (Table 3.10b). Under optimum and random drought stress conditions, all traits recorded higher H^2 (> 0.6) values, except for ASI, which had moderate H^2 values. Under managed drought, the highest H^2 values were recorded for days to anthesis (0.73) and silking (0.74), and ear position (0.70). Moderate H^2 values were recorded for ASI (0.56) and ear height (0.60) while grain yield, plant height and number of ears per plant showed lower H^2 values (Table 3.10c). Under low N conditions, the highest H^2 values were recorded for days to anthesis and silking and plant and ear height, whereas the rest of the traits had moderate heritability values (Table 3.10d). Across all environments, H^2 estimates varied from 0.74 for ASI to 0.95 for days to anthesis and silking (Table 3.10e). All traits showed high heritability values under combined environments. In general, higher grain yield heritability values were observed for the combined analysis (0.81), followed by random drought stress (0.69), optimum management (0.64), and low N stress conditions (0.61) while the lowest heritability was recorded under managed drought stress (0.08).

Genetic advance as percent over the mean (GAM) at 5% selection intensity is presented in Tables 3.10a to 3.10e. Under optimum conditions, GAM ranged from 3.3% to 44.1.0%. The highest GAM was recorded for ASI followed by number of ears per plant and ear height, while the remaining traits showed below 10% GAM. Similarly, under random drought stress, ASI also recorded 53.7% GAM followed by grain yield at 14.7% and number of ears per plant at

12.1% while the remaining traits showed lower GAM (<10). All studied traits showed lower GAM under managed drought, except ASI (36.2%) (Table 3.10c). Under low N stress conditions, ASI recorded moderate GAM (18.5%) followed by grain yield at 15.7% and leaf senescence at 10.3% while the remaining traits showed below 10% GAM (Table 3.10d). In combined analysis, the highest and the lowest GAM were observed for ASI (39.3%) and ear position (2.2%), respectively. In general, ASI showed the highest GAM, and ear height and number of ears per plant recorded moderate GAM (Table 3.10e)

Table 3.10 Estimates of variance components, broad-sense heritability and genetic advance for grain yield and other agronomic traits of QPM hybrids and checks evaluated under different environmental conditions

(a) Optimum conditions

Variance	GY	DA	DS	ASI	PH	EH	EPO	EPP
Genotype variance	0.09	1.53	1.54	0.09	48.37	51.77	0.00	0.01
Phenotypic variance	0.15	1.65	1.66	0.17	58.13	59.22	0.00	0.01
G x E variance	0.41	0.72	0.69	0.41	41.41	18.91	0.00	0.01
Residual variance	0.77	1.49	1.81	0.99	171.02	155.77	0.00	0.02
GCV%	3.7	1.7	1.7	28.2	2.8	5.6	0.0	9.3
PCV%	4.7	1.8	1.8	39.5	3.0	6.0	1.8	9.8
Heritability	0.64	0.92	0.92	0.54	0.83	0.88	0.78	0.90
GA	0.51	2.43	2.44	0.46	13.06	13.84	0.01	0.21
GAM%	6.1	3.3	3.4	44.1	5.2	10.7	2.9	18.1
Grand Mean	8.3	72.8	72.7	1.0	252.8	129.2	0.5	1.2

(b) Random drought stress

Variance	GY	DA	DS	ASI	PH	EH	EPO	EPP
Genotype variance	0.14	0.83	0.94	0.30	64.89	20.06	0.00	0.01
Phenotypic variance	0.21	1.01	1.16	0.60	80.43	28.23	0.00	0.01
G x E variance	0.22	0.41	0.46	0.25	19.23	16.85	0.00	0.01
Residual variance	0.68	2.43	2.57	1.31	179.08	97.08	0.00	0.02
GCV%	8.5	1.4	1.4	32.3	3.9	4.7	0.0	6.9
PCV%	10.4	1.5	1.6	45.8	4.3	5.6	2.6	8.2
Heritability	0.69	0.83	0.82	0.57	0.81	0.73	0.74	0.72
GA	0.65	1.70	1.80	0.91	15.01	8.00	0.02	0.12
GAM%	14.7	2.6	2.7	53.7	7.3	8.5	4.0	12.1
Grand Mean	4.4	66.5	67.5	1.7	206.9	94.6	0.5	1.0

(C) Managed drought

Variance	GY	DA	DS	ASI	PH	EH	EPO	EPP
Genotype variance	0.01	1.42	3.19	0.66	14.88	30.14	0.00	0.00
Phenotypic variance	0.17	1.93	4.30	1.20	63.94	50.16	0.00	0.00
G x E variance	0.31	0.96	2.29	0.89	89.08	39.79	0.00	0.01
Residual variance	0.30	1.20	2.11	1.47	116.25	40.57	0.00	0.02
GCV%	4.3	1.9	2.7	23.1	2.2	6.0	6.3	0.0
PCV%	15.2	2.2	3.1	31.2	4.5	7.7	6.8	7.3
Heritability	0.08	0.73	0.74	0.56	0.25	0.60	0.70	0.10
GA	0.07	2.09	3.15	1.27	4.08	8.68	0.05	0.01
GAM%	2.6	3.3	4.7	36.2	2.3	9.5	9.8	1.5
Grand Mean	2.7	63.5	67.0	3.5	177.4	91.4	0.5	0.9

(D) Low N stress

Variance	GY	DA	DS	ASI	PH	EH	EPO	EPP	Sen
Genotype variance	0.06	1.71	2.92	0.28	32.34	17.05	0.00	0.00	0.06
Phenotypic variance	0.09	2.14	3.55	0.89	48.41	24.41	0.00	0.00	0.15
G x E variance	0.17	1.39	2.56	2.56	36.84	6.72	0.00	0.01	0.23
Residual variance	0.23	4.08	4.86	2.22	183.35	89.67	0.00	0.02	0.28
GCV%	9.8	1.6	2.0	15.6	2.9	4.4	0.0	4.9	7.7
PCV%	12.6	1.8	2.2	27.8	3.5	5.2	2.7	7.2	12.2
Heritability	0.61	0.80	0.82	0.32	0.69	0.73	0.50	0.57	0.41
GA	0.38	2.40	3.17	0.63	9.91	7.46	0.01	0.08	0.33
GAM%	15.7	3.0	3.8	18.5	5.1	7.9	2.8	8.4	10.3
Grand Mean	2.4	81.1	84.4	3.4	196.1	94.4	0.48	0.9	3.2

(e) Combined all environments

Variance	GY	DA	DS	ASI	PH	EH	EPO	EPP
Genotype variance	0.08	1.33	1.76	0.21	44.21	30.96	0.00	0.01
Phenotype variance	0.10	1.39	1.85	0.29	48.26	33.44	0.00	0.01
G x E variance	0.31	0.85	1.34	1.05	39.98	19.46	0.00	0.01
Residual variance	0.58	2.38	2.80	1.40	171.31	114.98	0.00	0.02
GCV %	5.1	1.6	1.8	22.3	3.0	5.1	0.0	6.7
PCV %	5.7	1.6	1.8	26.0	3.2	5.3	1.2	7.1
Heritability	0.81	0.95	0.95	0.74	0.91	0.92	0.87	0.90
GA	0.51	2.31	2.66	0.81	13.03	10.97	0.01	0.14
GAM%	9.5	3.2	3.6	39.3	5.9	10.1	2.2	13.2
Grand Mean	5.4	72.3	73.8	2.1	220.5	108.7	0.5	1.0

G × E = genotype × environment interaction; GCV = genotypic coefficients of variation; PCV = phenotypic coefficients of variation; H^2 = broad sense heritability; GA = genetic advance; GAM = Genetic advance as percent over the mean; GY= grain yield; DA = days of anthesis; DS = days to silking; ASI = anthesis silking interval; PH = plant height; EH = ear height; EPO = ear position; EPP = number of ears per plant; Sen = leaf senescence

3.5 Discussion

In this study, significant variability was shown for yield and other important agronomic traits among new QPM hybrids across all environments. This indicates the existence of different genetic responses among genotypes for measured traits under non-stressed and stressed environments. Significant environment effects were observed for grain yield and other agronomic traits in all management conditions. G × E interaction effects were highly significant for grain yield and most agronomic traits under all management conditions. This suggested the occurrence of ranking change among genotypes. The presence of significant genotype and G × E interaction effects in QPM hybrids under different management levels have been reported previously (Wegary et al., 2014; Abakemal et al., 2016; Setimela et al., 2017a; Mebratu et al., 2019a).

Mean grain yield under optimum conditions was the highest, followed by random drought stress conditions and managed drought, while low N had the lowest grain yield. Grain yield is reduced when N level decreases, and drought conditions become more severe (Bänziger et al., 2006). Compared to optimum management conditions, grain yield was reduced by 48% under random drought stress, 68% under managed drought and 71% under low N environments. This is similar to results reported by other researchers, under stressed and non-stressed environments. More than 50% yield reduction was reported by Derera et al. (2008), Makumbi et al. (2011), Tapera (2017) and Ertiro et al. (2017) in non-QPM hybrids and Setimela et al. (2017b) in QPM hybrids under drought and low N stress conditions. Bänziger and Lafitte (1997) also reported yield reduction up to 89% under low N stress in non-QPM genotypes. On the other hand, Mebratu et al. (2019b) reported relatively lower yield reduction (32%) under low N stress when compared to this study in QPM hybrids. In this study, number of ears per plant was reduced by 11% under random drought stress, 24% under managed drought and 20% under low N conditions. The effect of stress on number of ears per plant observed in this study agreed with the results reported by Tapera (2017). Drought and low N stress conditions that occur before or at anthesis, delaying silk emerging time, results in an increase in ASI, causing incomplete or zero fertilization and resulting in absence kernel development. (Magorokosho et al., 2003; Beyene et al., 2013; Ertiro et al., 2017). In this study, ASI was increased by 80% under random drought stress, 218% under managed drought and 203% under low N environments.

Even though all stress types had large negative effects on grain yield and other agronomic traits, the effects of low N and managed drought stress were more severe than random drought stress. Ertiro et al. (2017) also reported that the effect of low N on grain yield, plant height and days to anthesis were higher than for drought stress. Tapera (2017), however, reported that random drought stress effect was more severe than low N stress on grain yield in late maturity hybrids. The highest yield reduction occurs at anthesis compared to other developmental stages by delayed silk elongation, but it often slows silk elongation and results in large ASI for stress susceptible genotypes (Magorokosho et al., 2003). As a result, it is useful using secondary traits, like ASI, EPP as an efficient approach for indirect selection to improve maize grain yield under stress conditions (Magorokosho et al., 2003; Bänziger et al., 2006; Wegary et al., 2012; Beyene et al., 2013; Ertiro et al., 2017).

Among the top ten QPM hybrids, some showed higher than, or comparable grain yield potential to the best non-QPM commercial checks. And almost all of them also showed higher grain yield than the best QPM checks under different management conditions. Compared to the mean of all checks and best QPM check, the top ten QPM hybrids showed high grain yield advantage combined for all environments, and for all separate environments. However, these top ten QPM hybrids also showed comparable or slightly higher grain yield compared to the best normal maize check under stressed and non-stressed environments. For more than three decades, CIMMYT, in collaboration with other institutes, have been developing QPM hybrids that are superior to commercial QPM and normal maize checks under stressed and non-stressed environments (Wegary et al., 2014; Mebratu et al., 2019a; b; Setimela et al., 2017a), and these findings were confirmed in the yield progress reported in this study. Setimela et al. (2017a) reported that QPM genotypes showed varying performance in different management conditions. However, in the current research, QPM hybrids such as 9 (CZH142237Q), 10 (CZH142238Q) and 14 (CZH15142Q) were performed the best under both drought and low N stress conditions, which confirmed results reported by Beyene et al. (2013), who also found that maize varieties developed for drought tolerance also showed good levels of tolerance to low N stress, suggesting the presence of common adaptive mechanisms for both water and low N stresses.

In this study, the genotypic variances (σ^2g) were lower than phenotypic (σ^2p), G × E interaction ($\sigma^2g\times e$) and error (σ^2e) variances for grain yield and most of the studied traits under all production conditions. This indicated that environment and G × E interaction effects were higher than genotype effect on the expression of the traits under stressed and non-stressed conditions. This contrasts with findings of Ababulgu et al. (2018) who reported that the proportion of σ^2g variances were higher than both σ^2e and $\sigma^2g\times e$ for most of the studied traits for QPM inbred lines adapted to transitional highland agro-ecologies of Ethiopia. Tapera (2017) also reported higher σ^2g than $\sigma^2g\times e$ variance for grain yield and other agronomic traits across optimum and stressed testing sites. The σ^2g variances for grain yield and most agronomic traits were lower under random drought, managed drought, and low N environments compared to optimum conditions. This agrees with the results of Ertiro et al. (2017) who reported the presence of low genetic variance for grain yield and other secondary traits and increased error and G × E interaction variances under stressed environments. The low GCV and PCV recorded for almost all studied traits under all management conditions, suggesting

the presence of enough tolerance to drought and low N stress conditions among evaluated QPM hybrids. This is consistent with the findings reported by Tapera (2017).

Compared to optimum environments, all agronomic traits had relatively lower heritability estimates under managed drought and low N environments. Similarly, Ertiro et al. (2017) and Tapera (2017) reported low heritability under drought and low N environments compared to optimum environments. ASI had moderate heritability, high GCV value and genetic advance as percent of the mean across trials under almost all environmental conditions. This indicates the presence of enough genetic variability among genotypes to improve synchronization problems under different management conditions.

3.6 Conclusions

In this study, some QPM hybrids performed better than commercial QPM and normal maize check varieties under stressed and non-stressed environments. Among evaluated QPM hybrids, entries 44 (QS7646), 12 (CZH15099Q), 9 (CZH142237Q), 14 (CZH15142Q) and 10 (CZH142238Q) were selected based on high yield potential under all management conditions and had good agronomic performance. The heritability of grain yield and some agronomic traits were also good under all management conditions except under managed drought condition, indicating that the effects were largely associated with genotypic effect. Commercialization of QPM hybrids that perform better than commercial QPM and non-QPM checks in all environments, can help in improving yield productivity and nutritional problems for smallholder farmers in SSA.

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CHAPTER 4

Protein quality, iron and zinc concentration and bioavailability of quality protein maize hybrids evaluated under stress and non-stress environments

Abstract

Protein quality and mineral concentration with bioavailability improvement of staple crops like maize through biofortification is a powerful approach to mitigate protein quality and mineral deficiency related diseases in developing countries where maize is a major staple food crop. The objectives of this study were to: i) determine total protein, tryptophan, Fe, Zn and phytic acid concentrations and ii) to calculate phytic acid to Fe and Zn molar ratios in QPM hybrids under stress and non-stress conditions. Forty newly developed QPM hybrids and five checks (two QPM, two normal and one local check hybrid) were evaluated at Bako and Ambo in Ethiopia under optimum and low N stress for two years and at Harare under optimum and Gwebi under random drought stress conditions in Zimbabwe, for a year. Significant variation was observed among genotypes for all traits at all locations except protein quality index and molar ratio of Zn to phytic acid at Bako low N stress. In combined analysis, the effects of genotype, environment, and G × E interaction were significant for almost all traits. Broad sense heritability for all nutritional traits was lower under low N conditions compared to optimum and random drought stress conditions. The concentration of total protein and tryptophan in the grain was reduced under low N stress; however, protein quality index was increased under low N stress compared with optimum conditions. Under random drought stress, almost similar concentrations of tryptophan were recorded while the total protein content increased. The concentration of Fe and Zn decreased under low N and random drought stress conditions. Some new QPM hybrids showed high tryptophan and Zn concentration and lower phytic acid and molar ratio of Zn compared with the best QPM and normal hybrid checks. Using such types of QPM hybrids could be a possible solution to improve malnutrition related disease in developing countries.

Keywords: Bioavailability, low N, minerals, protein quality, QPM, random drought stress

4.1 Introduction

Maize is an important cereal crop world-wide and a major source of food and nutritional security in developing countries, while it serves as a source of feed and industrial products in the high-income countries (Prasanna et al., 2021). Especially in SSA, maize is dominantly consumed as a staple food crop, delivering daily energy, proteins, vitamins and minerals to consumers (Prasanna et al., 2020). The demand and consumption rate of maize in SSA is very high compared to other cereal crops. The highest consumption rate is found in southern Africa, at 85 kg per capita per year, followed by 27 kg in east Africa and 25 kg in WCA (Shiferaw et al., 2011). In countries like Lesotho, Malawi, South Africa, Zambia and Zimbabwe the average consumption is over 100 kg per capita per year (Shiferaw et al., 2011). High consumption rate and limited diet diversification in several developing countries cause a lack of some of the essential amino acids such as lysine and tryptophan, as well as minerals like Fe and Zn, which are crucial for human health and wellbeing (Ekpa et al., 2018; 2019; Prasanna et al., 2020). More than two billion people in Asia, Africa, and Latin America suffer one or more nutrient deficiencies that commonly lead to stunted growth, weakened immune system and impaired intellectual development, complications during pregnancy, diminished work and income earning capacity, and increased risk of morbidity and mortality (Rautiainen et al., 2016; Prasanna et al., 2020).

Enhancing the nutritional value of maize through biofortification is a sustainable and cost-effective strategy to reduce the above-mentioned problems (Bouis and Saltzman, 2017; Akhtar et al., 2018). Through extensive manipulation of the *o2* gene with its genetic modifier systems, using modified backcrossing and recurrent selection, maize scientists have developed improved genotypes with enhanced nutritional value such as QPM, that has twice the amount of lysine and tryptophan compared with normal maize (Prasanna et al., 2001; Krivanek et al., 2007; Sofi et al., 2009; Teklewold et al., 2015; Tripathy et al., 2017). Nowadays, some QPM varieties also show comparable or higher productivity potential compared with normal maize (Wegary et al., 2014; Abakemal et al., 2016; Setimela et al., 2017; Mebratu et al., 2019a) and it is also difficult to distinguish them from normal maize using morphological characteristics such as ear height, silk and flower characteristics and kernel texture (Tandzi et al., 2017). The biological value of QPM was reported to be about 90% of milk protein, whereas the value of common maize is only approximately 40% (Vivek et al., 2008; Nuss and Tanumihardjo, 2011). Several QPM feeding studies conducted on children, poultry and pigs also confirmed the superiority of QPM

over conventional maize in improving the weight and height of children and animals (Qi et al., 2004; Vivek et al., 2008; Akalu et al., 2010; Teklewold et al., 2015).

Biofortification of maize for more than one nutrient may be a desirable strategy, because nutrients interact in synergistic ways to enhance nutritional effectiveness (Pixley et al., 2011). For example QPM genotypes were shown to promote Fe and Zn absorption and increase bioavailability compared to non-QPM genotypes (Bänziger and Long, 2000; Prasanna et al., 2020). Chakraborti et al. (2011) reported a higher concentration of Zn in QPM than non-QPM hybrids. However, Hindu et al. (2018) and Prasanna et al. (2020) found that not all QPM germplasms have high kernel Zn concentration, but there is a possibility to identify non-QPM inbred lines, hybrids and OPVs that have high Zn content. In general, the biofortification programme of CIMMYT and IITA identified a few QPM genotypes that can be used as a source of kernel Zn improvement in tropical maize germplasm (Prasanna et al., 2020), indicating the possibility to improve protein quality and mineral concentration simultaneously.

Genetic variation in maize germplasm is necessary for the development of improved cultivars with higher concentrations of certain micronutrients, through biofortification (Menkir, 2008; Bouis and Saltzman, 2017). Kernel micronutrient studies have shown the existence of high and significant genetic variation among maize genotypes (Bänziger and Long, 2000; Oikeh et al., 2004; Menkir, 2008; Prasanna et al., 2011; Queiroz et al., 2011). However, this crop is a major source of phytic acid, a substance that forms insoluble complexes with minerals and prevents the uptake of minerals by monogastric animals, including humans, due to the lack the enzyme phytase in their digestive tract (Gupta et al., 2015). Therefore, improving the concentration of Fe and Zn in maize genotypes is not enough to increase the concentration of micronutrients in the human body, but there is also a need to decrease the amount of antinutrients, such as phytic acid (Welch and Graham, 2004; Beavers et al., 2015). The bioavailability of Fe and Zn decreases when the phytic acid to Fe and Zn molar ratios are more than one and six, respectively (White and Broadley, 2009; Akhtar et al., 2021). Therefore, it is important to integrate low phytate with Fe and Zn biofortification breeding to increase bioavailability of Fe and Zn.

In SSA, maize is mainly grown in small holder farming systems under rain fed conditions with limited inputs. This is associated with various production constraints, including drought, poor soil fertility, insect pests, and diseases (Cairns et al., 2013; Rezende et al., 2020). In the tropics, drought and low soil fertility stresses have been identified as the common constraints and

usually occur together (Nyombayire et al., 2011). The impact of these stresses on grain yield and agronomic traits have been extensively studied for normal maize genotypes (Meseka et al., 2013; Ertiro et al., 2017; Sserumaga et al., 2016; 2018; Das et al., 2019) and for QPM genotypes (Wegary et al., 2014; Badu-Apraku et al., 2016; Setimela et al., 2017; Mebratu et al., 2019a;b). However, research on protein quality and quantity combined with mineral concentration and molar ratios of minerals to phytic acid under drought and low N stress conditions in QPM genotypes has been limited, compared with yield and yield related traits. Therefore, the objectives of this study were: i) to determine the protein quality and quantity in QPM hybrids under stress and non-stress environments, ii) to determine the concentration of Fe, Zn and phytic acid, and iii) to calculate molar ratios of Fe and Zn to phytic acid in QPM hybrids grown under stress and non-stress environments.

4.2 Materials and methods

4.2.1 Testing sites, planting materials and trial management

A detailed description of experimental testing sites, planting materials, experimental design and field management conditions used in this study were given in Chapter 3 in the materials and methods, section 3.2.

4.2.2 Seed sample preparation for nutritional analysis

Seed samples for nutritional analysis were taken from Bako, Ambo, Harare and Gwebi testing sites, representing optimum, low N and random drought stress conditions. The samples were produced by controlled self-pollination using sib-mating from the two border plants of each plot. At maturity, the ears were harvested and shelled manually by bared hand. Shelled seeds were air dried under ambient conditions. For micronutrient analyses, maximum care has given to prevent grain surface contamination with Fe from soil and other contaminants during the harvesting and drying processes. A 500 g sample of each entry was used for determination of protein contents using near-infrared transmission spectroscopy (NIR) with a Perten Grain Analyzer (Model DA 7250, Perten, Instruments AB, Sweden), with three subsamples for each sample. The NIR calibration was confirmed with results from wet chemistry of 50 samples before use.

One hundred kernels of uniform size were randomly selected from the bulked seed samples of each entry and replication and milled into flour using an IKA, A10 Yellowline grinder (Merck Chemicals Pty Ltd) and sieved with a 1 mm stainless steel screen mesh.

4.2.3 Tryptophan analysis

Determination of lysine and tryptophan content is a necessary step to develop QPM cultivars (Nurit et al., 2009). However, these two traits are positively correlated in maize kernels of QPM genotypes (Krivanek et al., 2007; Worku et al., 2007; Nurit et al., 2009). To save laboratory costs and time, analysing only tryptophan content is usually sufficient to select QPM cultivars that have high content of both lysine and tryptophan.

4.2.3.1 Defatting of sample flour

Total lipid was washed from the milled seed samples according to the protocol suggested by Folch et al. (1957). One gram of each sample was weighed and placed into 50 ml Falcon tubes and then a 5 ml mixture of chloroform and methanol in 2:1 v/v ratio was added. After being shaken very well, the tubes were left overnight in a refrigerator (4°C) and then removed and brought to normal room temperature. The samples were washed three times through a filter paper in a funnel with a fresh 5 ml mixture of chloroform and methanol and then left on the filter paper until completely dry. The dried samples were stored in the cold room (4°C) until use for tryptophan analysis.

4.2.3.2 Digestion and calorimetric reaction

The defatted flour samples were used for tryptophan content determination using a colorimetric method based on a glyoxilic acid reaction with the tryptophan present in the flour in the presence of ferric chloride and sulphuric acid (H_2SO_4) based on the protocol suggested by Nurit et al. (2009). First, acetate solution (0.165 M NaH3CCOOH) was prepared from sodium acetate in the ratio of 13.6 g sodium acetate for one liter of distilled water (DH_2O). The solution was adjusted to pH 7.0 with sodium hydroxide (NaOH). After that, the solution was kept as a stock at 4^0C. Papain solution (1 mg ml^{-1}) was prepared by dissolving 40 mg of papain in 40 ml of 0.165 M sodium acetate solution. The papain solution was prepared freshly every time based on the number of samples to be analysed. A 30 N H_2SO_4 solution was prepared by adding 833.30 ml of H_2SO_4 (96% concentration) slowly to 166.7 ml of double distilled water (DDH_2O) on ice to prepare 1000 ml volume solution. The solution was left on ice to cool to room temperature before use. A 7 N H_2SO_4 solution was prepared by mixing 35 ml 30 N of H_2SO_4 with 115 ml of DDH_2O on ice to prepare 150 ml of 7 N H_2SO_4. The solution was left on ice until it cooled to room temperature.

After that, four reagents were prepared. Reagent A: 0.1 M glyoxilic acid was prepared by adding 0.9205g glyoxilic acid placed in a 100 ml flask, then 50 ml of 7 N H_2SO_4 was added and shaken slowly until all the glyoxilic acid completely dissolved and the volume was then adjusted to 100 ml with 7N H_2SO_4. Reagent B: 1.8 mM ferric chloride was prepared by dissolving 0.050g of ferric chloride in 100 ml of reagent A. This reagent was prepared daily, and the amount was determined based on the number of the samples analysed at once. Reagent C was 30 N of H_2SO_4, and reagent D (colorimetric reagent) which was prepared one hour before use by mixing 20 ml of reagent B and 20 ml of reagent C. It was prepared daily and protected from light and oxygen. Tryptophan stock solution was prepared by dissolving 10 mg DL-tryptophan in 100 ml of 0.1 ml sodium acetate solution buffer pH 7. This solution was vortexed thoroughly before dilution for preparing the standard curve. It was prepared weekly and stored at 4^oC.

For tryptophan analysis, 80 mg of defatted flour was placed in a 15 ml Falcon tube and 3 ml papain solution was added for digestion. A blank with only papain solution was included as a control to zero the spectrophotometer reading. The mixture was vortexed 3 to 5 sec and incubated at 64°C for 16 h overnight. The samples were vortexed again one hour after being placed in the water bath, and one hour before the end of the 16h incubation period. After the incubation, the tubes were taken out of the water bath and cooled to room temperature, and then vortexed immediately before being centrifuged at 3600 g for 5 min, to ensure that the supernatant did not have sample particles floating in it. One ml of the hydrolysate (supernatant) was carefully transferred to a clean glass tube, and then 3 ml of reagent D (colorimetric reagent) was added. The samples were vortexed for 3 to 5 sec before incubated for 30 min at 64°C for colour development and were then taken from the water bath and allowed to cool to room temperature. After that the optical density (OD) of each sample was read at 560 nm by using a spectrophotometer (ATAGO® AP-300). Protein quality index was calculated as the proportion of tryptophan to protein content.

4.2.3.3 Standard curve

The tryptophan concentration standard curve was drawn using concentrations of 0, 10, 15, 20, 25, and 30 μg ml^{-1} from dilutions with 0.1 M sodium acetate solution (pH 7) of the tryptophan stock solution. Then 3 ml of colorimetric reagent was added to each test tube and vortexed for 3 - 5 sec before incubation in the water bath at 64°C for 30 min for colour development. Thereafter, the samples were taken from water bath and allowed to cool to room temperature

before reading their OD with the spectrophotometer. The reading was used for drawing a calibration curve with the slope having a unit of OD_{560nm} x µg ml^{-1}. The amount of tryptophan for each sample was estimated using the formula suggested by Nurit et al. (2009)

$$\%Tryptophan = \frac{OD_{560nm}}{slope}\ x\ \frac{Hydrolysis\ volume}{Sample\ weight}\ x\ 100$$

4.2.4 Iron and zinc analysis

Fe and Zn analysis was done according to the dry-ashing method (AOAC, 2000). Maize flour samples (2 g) were placed into crucibles and then ashed at 550^{o}C overnight, after which the samples were removed and allowed to cool to room temperature. Five ml of concentrated hydrochloric acid (HCl) was added to the cooled samples, which were then placed in a hot sand bath to evaporate until completely dry. Samples were removed and allowed to cool to room temperature. Nitric acid (HNO_3) (5 ml) was added to the dried samples for digestion and placed in the sand bath again. Samples were removed and allowed to cool. Thereafter, the samples were filtered through Whatman #4 filter paper into a 100 ml volumetric flask and filled up to 100 ml with distilled H_2O. Fe and Zn concentration was read using an atomic absorption spectrophotometer according to the method described by Garcia et al. (1974).

4.2.5 Phytic acid analysis

Phytic acid content analysis in maize flour was determined according to the protocol described by Dragićević et al. (2011). For each sample, 0.25 g of milled flour was added into test tubes. Phytic acid was extracted by adding 10 ml 5% trichloroacetic acid (TCA) solution into the test tubes containing the samples and shaken for one hour with a mechanical shaker, and vortexed at 10 min intervals; then centrifuged at 12,000 g for 20 min. After that, 0.5 ml supernatant was added to 1.5 ml aliquots of Wade reagent which was prepared by adding 0.3 g $FeCl_3$ and $6H_2O$ with 3 g 5‟-sulphosalicylic acid in 1 l DD H_2O. The solution was centrifuged at 12,000g for 10 min. Phytic acid content was read using a spectrophotometer at a wavelength of 500 nm after it was zeroed using distilled water.

The phytic acid concentration standard curve was drawn using known concentrations of phytic acid (dodecasodium salt, from rice, Sigma P-8810, MW: 660.04 g/mol). The phytic acid stock solution was prepared by dissolving 25 mg of phytic acid in a 200 ml volumetric flask using distilled H_2O with a concentration of 0.5 mg ml^{-1} phytic acid solution. A series of standard phytic acid solutions in 10 ml was prepared from the stock solution by appropriate dilution

with addition of 5% TCA extraction solution. The concentration of phytic acid series were 0, 10, 50, 100; 150; 200; 250; 300; 350; 400 µmol 10 ml^{-1}. After that, 0.5 ml supernatant was added to 1.5 ml aliquots of Wade reagent. Then, the solution was centrifuged at 12,000 g for 10 min; and the absorbance was read at 500 nm with the spectrophotometer.

4.2.6 Molar ratios of Fe and Zn

Fe and Zn bioavailability were estimated using the phytic acid/Fe and phytic acid/Zn molar ratios according to the equations of Norhaizan and Norfaizadatul (2009) and Queiroz et al. (2011).

$$\text{MR} = \frac{(PA/MWPA)}{(Mineral/AWmineral)}$$

MR = molar ratio, PA = phytic acid in the sample, MWPA = phytic acid molecular weight (660 Da), Mineral = Fe or Zn concentration in the sample, AW = Fe (56 Da) or Zn (65 Da) atomic weight.

4.2.7 Data analysis

Analysis of variance for individual and combined locations were done using a general linear mixed effect model procedure of SAS software (SAS, 2002). The genotypes were considered fixed while environments, replications within environments and the incomplete blocks within replications were considered as random factors. Across environments combined analyses were carried out by using adjusted hybrid means from each environment analysis in accordance with the lattice design (Cochran and Cox, 1960). Comparison of hybrid means was done by the LSD test at 5% levels of significance (Gomez and Gomez, 1984). In Ethiopia, Bako and Ambo optimum trials were repeated for two seasons (year 1 and 2). Bako and Ambo low N trials were only planted in year 2, while in Zimbabwe Harare optimum and Gwebi (random drought stress) were planted only in year 1. Harare and Gwebi are only 27 km apart and the climate is considered to be similar at the two locations.

Estimates of genotypic (σ^2g), phenotypic(σ^2p), genotype × environment, ($\sigma^2g\times e$) and error (σ^2e) variances, phenotypic and genotypic coefficients of variation, broad sense heritability and genetic advance expressed as percent of the mean were determined as described in Chapter 3 (Section 3.3.2**)**.

4.3 Results

4.3.1 Analysis of variance and mean performance of total protein and tryptophan contents, and protein quality index at each location

There were significant differences among genotypes for all traits, except protein quality index at Bako low N stress (Table 4.1). The effects of year on total protein and tryptophan concentrations, and protein quality index were significant for Bako and Ambo combined across optimum management conditions. The effect of genotype × year (G × Y) interaction on total protein, tryptophan and protein quality index was highly significant at Bako but not at Ambo (optimum) (Table 4.1).

At Bako, mean total protein concentration in the grain was 8.1% with a range 6.7 to 9.5% under optimum conditions and mean 5.4% with a range 3.8 to 7.1% under low N conditions. Some QPM hybrids had higher total protein content than the best QPM and normal checks and the trial mean. Under optimum conditions, 27, 13 and 53% and under low N stress 55%, 72% and 55% of hybrids recorded higher total protein content than the best QPM and normal checks, and the trial mean, respectively (Figure 4.1a). Mean tryptophan content ranged from 0.06 to 0.107% with a mean 0.081% under optimum conditions and 0.044 to 0.097% with a mean 0.064% under low N stress (Table 4.2). The mean of this trait was reduced under low N conditions at Bako. Almost all QPM hybrids showed higher tryptophan concentration compared with the best normal check and some of them also showed higher tryptophan concentration than the trial mean and best QPM check under both management levels. Under optimum conditions, 97, 35 and 48% and under low N stress, 92, 32 and 47% of hybrids showed higher tryptophan concentration than the best normal maize and QPM checks, and the trial mean, respectively (Figure 4.1b). Protein quality index ranged from 0.67 to 1.47% with an average1.02% under optimum conditions and 0.75 to 1.83% with a mean 1.22% under low N stress (Table 4.2). Protein quality index was increased under low N compared with optimum conditions. Under optimum conditions, 25, 98 and 50% and under low N stress 10%, 78% and 55% of hybrids showed higher protein quality index compared with the best QPM and normal checks, and the trial mean, respectively (Figure 4.1c).

At Ambo, the range of total protein content was 6.7 to 9.7% with a mean 8.0% under optimum conditions and 5.6 to 9.1% with a mean 7.0% under low N stress (Table 4.2). Under optimum conditions, 35%, 8% and 45% and under low N 20, 30 and 55% of hybrids had higher protein content than the best QPM and normal checks, and the trial mean, respectively (Figure 4.1a).

Mean of tryptophan concentration ranged from 0.055 to 0.115% with a mean 0.083% under optimum and 0.046 to 0.092% with a mean 0.071% under low N stress. More than 80% and 50% of QPM hybrids showed higher tryptophan concentration than the best normal checks and the trial mean, respectively, under both management conditions. Compared with the best QPM check, more than 20% of the hybrids showed higher tryptophan concentration under both conditions (Figure 4.1b). Protein quality index ranged from 0.62 to 1.49 with a mean of 1.05 under optimum and 0.50 to 1.54 with a mean 1.04 under low N stress (Table 4.2). At Ambo, the tryptophan concentration decreased under low N stress while comparable values of protein quality index was observed both under optimum and low N stress conditions. Under optimum conditions, around 35%, 100% and 52% and under low N stress 30, 68 and 52% of hybrids showed higher protein quality index compared with the best QPM and normal maize checks and the trial mean, respectively (Figure 4.1c).

At Harare, minimum and maximum values of total protein, tryptophan and protein quality index were 8.1 to 12.0%, 0.06 to 0.11% and 0.54 to 1.02 with means of 10.1%, 0.08% and 0.80, respectively (Table 4.2). At Gwebi, the ranges of total protein, tryptophan content and protein quality index were 7.2 to 11.6%, 0.05 to 0.11% and 0.56 to 1.05 with means of 9.9%, 0.078% and 0.80, respectively. Higher values of protein and tryptophan content and lower values of quality protein index were observed at Harare under optimum conditions than in Gwebi under random drought stress (Table 4.2). The percentage of QPM hybrids at Harare with values higher than the best QPM, normal checks and the trials mean, respectively, were 63, 25 and 55% for protein content, 15, 93 and 45% for tryptophan content and 28, 95 and 48% for protein quality index (Figure 4.1a-c). At Gwebi, of the total QPM hybrids evaluated, 30, 8 and 43% for total protein, 8, 58 and 30% for tryptophan and 5, 90 and 45% for protein quality index showed higher values than the best QPM and normal maize checks and the mean, respectively under random drought stress (Figure 4.1a-c).

Table 4.1 Mean squares for grain protein content, tryptophan concentration and protein quality index of maize hybrids evaluated under optimum, low N and random drought stress conditions at Bako, Ambo, Harare and Gwebi

Source variation	Bako optimum				Bako low N			
	Df	Protein	Tryptophan	QI	Df	Protein	Tryptophan	QI
Year (Y)	1	127.30**	0.002**	0.88**	-	-	-	-
Rep (Y)	2	7.06**	0.003**	0.68**	1	0.01	0.001**	0.42*
Block (Rep × Y)	32	1.06**	0.001**	0.13**	16	1.48*	0.000	0.12
Genotype	43	1.52**	0.000**	0.10**	43	0.93*	0.000*	0.12
G × Y	43	0.58*	0.000**	0.04*	-	-	-	-
Error	54	0.37	0.00	0.03	27	0.43	0.000	0.08
CV (%)		7.49	14.10	16.01		12.31	18.14	23.56
Source of variation	Ambo optimum				Ambo low N			
	Df	Protein	Tryptophan	QI	Df	Protein	Tryptophan	QI
Y	1	31.56**	0.000	0.89**	-	-	-	-
Rep (Y)	2	1.23	0.001	0.05	1	4.95**	0.000	0.06
Block (Rep × Y)	32	0.86	0.001**	0.09**	16	1.47**	0.000	0.08*
Genotype	43	1.72**	0.000**	0.14**	43	1.63**	0.000*	0.09**
G x Y	43	0.83	0.000	0.03		-	-	-
Error	54	0.61	0.000	0.03	27	0.50	0.000	0.03
CV (%)		9.72	16.65	16.90		10.04	15.97	17.60
Source of variation	Harare optimum				Gwebi random drought stress			
	Df	Protein	Tryptophan	QI	Df	Protein	Tryptophan	QI
Rep	1	0.00	0.002**	0.26**	1	0.42	0.000	0.00
Block	16	1.63**	0.000	0.02*	16	0.82*	0.000**	0.03*
Genotype	43	1.75**	0.000**	0.02**	43	1.23**	0.000*	0.03*
Error	27	0.23	0.000	0.01	27	0.40	0.000	0.01
CV (%)		4.75	12.79	11.79		6.42	14.86	14.44

* P ≤0.05, ** P ≤ 0.01; Y = year; Rep = replication; G × Y = genotype by year interaction; CV = coefficient of variation; Low N = low nitrogen; Trp = tryptophan content; QI = protein quality index; Df = degree of freedom

Table 4.2 Means of protein content, tryptophan concentration in grain, and protein quality index of maize hybrids evaluated under optimum, low N and random drought stress conditions at Bako, Ambo, Harare and Gwebi

	Bako Optimum			Bako low N		
Means	Protein	Trp	QI	Protein	Trp	QI
Mean	8.1	0.081	1.02	5.36	0.064	1.22
Minimum	6.7	0.060	0.68	3.78	0.044	0.75
Maximum	9.5	0.107	1.47	7.14	0.097	1.83
LSD	0.9	0.016	0.23	1.35	0.023	0.56
Mean of QPM hybrids	8.1	0.082	1.04	5.39	0.065	1.23
Best QPM hybrid	9.5	0.107	1.47	7.14	0.097	1.83
Best QPM check	9.0	0.085	1.13	5.26	0.068	1.58
Best normal check	8.6	0.090	1.11	5.11	0.051	1.03
Best hybrid over best QPM check (%)	5.7	24.9	30.0	35.8	43.0	15.5
Best hybrid over best normal maize check (%)	10.1	19.0	32.7	39.6	91.1	78.0
	Ambo optimum			Ambo low N		
Means	Pro	Trp	QI	Pro	Trp	QI
Mean	8.0	0.083	1.05	7.0	0.071	1.04
Minimum	6.7	0.055	0.62	5.6	0.046	0.50
Maximum	9.7	0.115	1.49	9.1	0.092	1.54
LSD	1.1	0.020	0.25	1.5	0.023	0.38
Mean of QPM hybrids	8.0	0.083	1.06	7.1	0.072	1.04
Best QPM hybrid	9.7	0.115	1.49	9.1	0.092	1.54
Best QPM check	8.3	0.094	1.14	7.8	0.088	1.15
Best normal check	9.1	0.062	0.70	7.4	0.059	0.92
Best hybrids over best QPM checks (%)	16.9	22.3	31.3	17.6	5.1	33.5
Best hybrids over best normal maize checks (%)	6.7	85.9	114.4	23.5	55.9	67.8
	Harare optimum			Gwebi		
Means	Pro	Trp	QI	Pro	Trp	QI
Mean	10.1	0.080	0.80	9.9	0.078	0.80
Minimum	8.1	0.06	0.51	7.2	0.053	0.51
Maximum	11.9	0.11	1.21	11.6	0.110	1.10
LSD	0.99	0.02	0.23	1.3	0.025	0.27
Mean of QPM hybrids	10.16	0.08	0.80	9.9	0.078	0.80
Best QPM hybrid	11.95	0.11	1.21	11.6	0.110	1.10
Best QPM check	9.85	0.09	0.91	10.1	0.099	0.97
Best normal check	10.90	0.06	0.60	11.0	0.070	0.63
Best hybrids over best QPM checks (%)	21.3	22.3	33.0	14.9	11.2	14.0
Best hybrids over best normal maize checks (%)	9.6	73.8	103.4	5.9	57.6	74.6

Low N = low nitrogen; Pro = total protein content; Trp = tryptophan content; QI = protein quality index; LSD = least significant difference

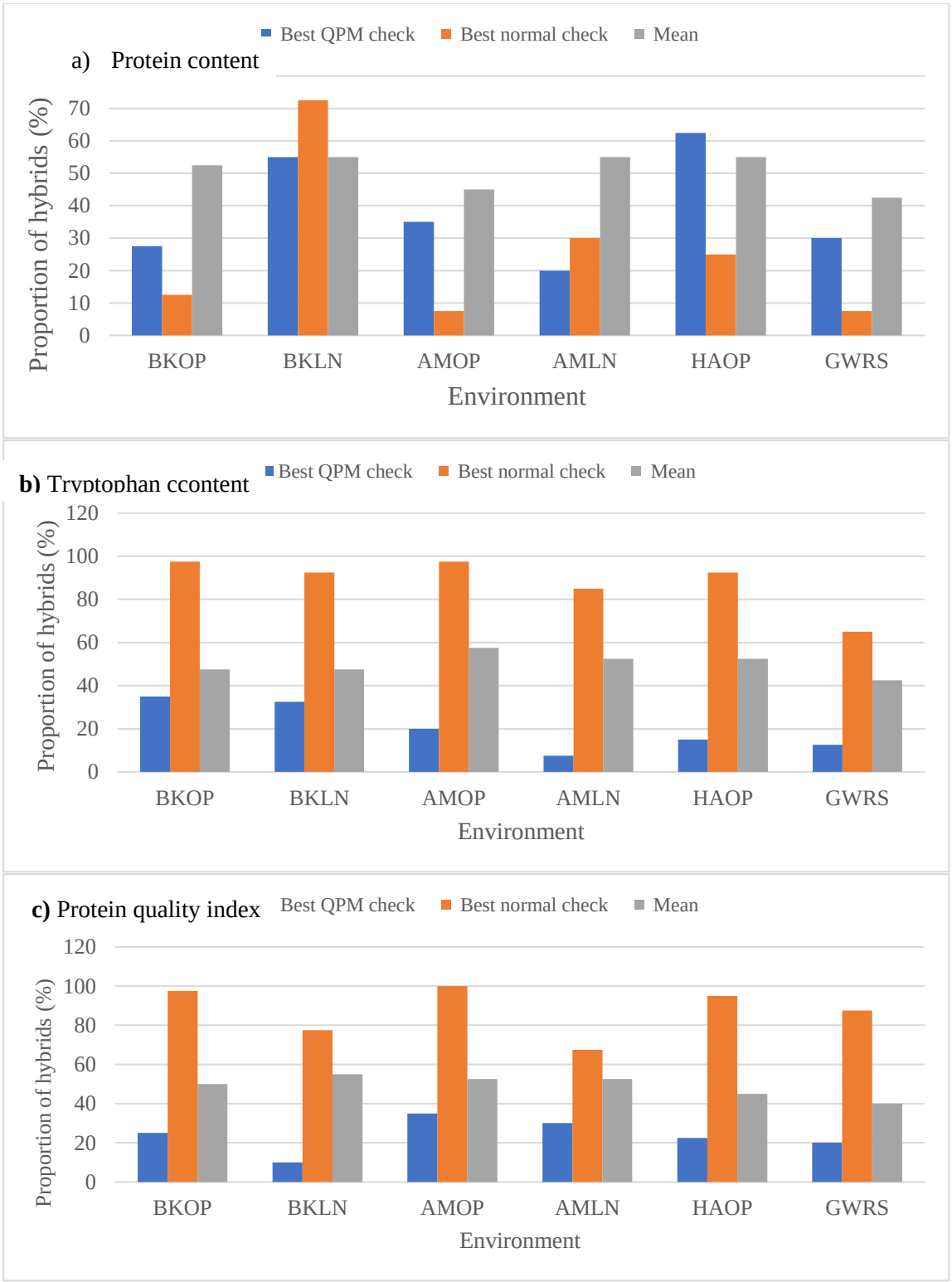


Figure 4.1 Number of new QPM hybrids that showed higher protein content (a), tryptophan content, (b) and protein quality index (c) traits as compare with the best QPM and normal maize checks and the mean under stress and non-stressed conditions

BKOP and BKLN= Bako optimum management and low N stress; AMOP and AMLN = Ambo optimum management and low N stress; HAOP = Harare optimum management and GWRS = Gwebi random drought stress

4.3.2 Combined analysis of variance and means of total protein and tryptophan contents in the grain, and protein quality index

Across all environments, highly significant genotype and environment effects were observed for all studied traits and G × E interaction effect was also significant for total protein and tryptophan contents (Table 4.3). Protein content of the trials in the grain ranged from 7.1 to 9.4%, with a mean of 8.1% (Table 4.4). The mean of total protein content for QPM hybrids (8.1%) was lower than the mean of normal maize (8.6%) while it was slightly higher than the mean of the QPM checks (7.9%). Tryptophan content varied from 0.061 to 0.098% with overall means of 0.079%. Mean of tryptophan concentration for QPM hybrids (0.098%) was higher than the mean of the QPM checks (0.087%) and normal checks (0.059%). Mean of protein quality index also ranged from 0.72 to 1.31 with overall means of 1.01. In general, across all environments, the best QPM hybrid showed higher protein and tryptophan content in the grain, and protein quality index than the best QPM and normal checks (Figure 4.2).

Under optimum conditions, highly significant differences were observed among hybrids and environments for all traits. There was significant G × E interaction effect for only total protein content in the grain (Table 4.3). The total protein content ranged from 7.4 to 10.0% with a mean value 8.5%. Higher total mean protein (8.1%) for QPM hybrids was observed compared with mean of QPM checks (7.9%) (8.6%), while the QPM hybrid mean was lower than the normal maize checks mean. The best QPM hybrids showed 20% and 7% higher protein content than the best QPM and normal maize checks (Figure 4.2). Tryptophan content ranged from 0.064 to 0.104% with a mean of 0.083%. The mean of tryptophan content for QPM hybrids was higher than for normal checks. The best QPM hybrid showed 9% and 71% higher tryptophan concentration than the best QPM and normal checks, respectively (Figure 4.2). The range of protein quality index was 0.71 to 1.32 with a mean of 1.0. Higher mean protein quality index of QPM hybrids was observed compared to normal checks.

Under low N stress, highly significant differences among hybrids and environments were observed for all studied traits. G × E interaction was significant for only total protein content in the grain (Table 4.3). The total protein and tryptophan concentrations in the grain, and quality protein index ranged from 5.2 to 7.9%, 0.052 to 0.087% and 0.80 to 1.48 with means of 6.2%, 0.068% and 1.114, respectively. The mean proportion of protein and tryptophan concentration in the grain decreased, whereas protein quality increased compared with optimum conditions. Protein and tryptophan concentrations in the grain decreased by 36.0% and 20.6% respectively,

while protein quality increased by 12.3% (Table 4.4). Under random drought stress, QPM hybrids showed higher tryptophan and protein quality index than the mean of QPM and normal checks, while they showed comparable means for total protein content (Table 4.4). Although no single hybrid showed high protein and tryptophan content, and protein quality index, the best QPM hybrid showed 19% and 6% higher protein content, 14% and 58% higher tryptophan content and 3% and 75% higher protein quality index than the best QPM and normal checks, respectively (Figure 4.2). Under random drought stress, protein content in the grain decreased by 14.0% while tryptophan and protein quality index increased by 5.3% and 24.7%, respectively, compared with optimum management conditions (Table 4.4). Under random drought stress, the mean of total protein and tryptophan contents were also higher than the mean under low N stress conditions, while protein quality index values were lower than under optimum and low N stress conditions (Table 4.4).

Table 4.3 Mean squares of total protein and tryptophan concentrations and protein quality index of QPM hybrids and commercial checks evaluated under optimum, random drought stress and low N stress conditions

Source variation	Combined				Optimum			
	Df	Protein	Trp	QI	Df	Protein	Trp	QI
Environment (E)	7	224.00**	0.004**	1.97**	4	113.23**	0.001**	1.41**
Rep (E)	8	2.74**	0.001**	0.26**	5	3.31**	0.002**	0.34**
Block (Rep × E)	128	1.16**	0.000	0.09**	80	1.09**	0.000	0.10**
Genotype	43	4.05**	0.001**	0.32**	43	3.27**	0.001**	0.22**
G × E	301	0.88**	0.000	0.04	172	0.78**	0.000	0.03
Residuals	216	0.44	0.000	0.03	135	0.44	0.000	0.03
CV (%)		8.21	15.199	17.82		7.8	14.71	16.32
Source variation	Low N stress				Random stress			
	Df	Protein	Trp	QI	Df	Protein	Trp	QI
Environment	1	124.99**	0.002**	1.44**	-	-	-	-
Rep (E)	2	2.48**	0.001*	0.20*	1	0.42	0.000	0.00
Block (Rep × E)	32	1.48**	0.000	0.10*	16	0.82*	0.000	0.03*
Genotype	43	1.49**	0.000*	0.13**	43	1.23**	0.000*	0.03*
G × E	43	1.06**	0.000	0.08	-	-	-	-
Residuals	54	0.47	0.000	0.05	27	0.40	0.000	0.01
CV (%)		11.03	16.57	20.39		6.42	14.86	14.44

* P ≤0.05, ** P ≤ 0.01; Rep = replication; Blk = block within replication; G × E = genotype by environment interaction; CV = coefficient of variation; Low N = low nitrogen; Trp = tryptophan content; QI = protein quality index; Df = degree of freedom

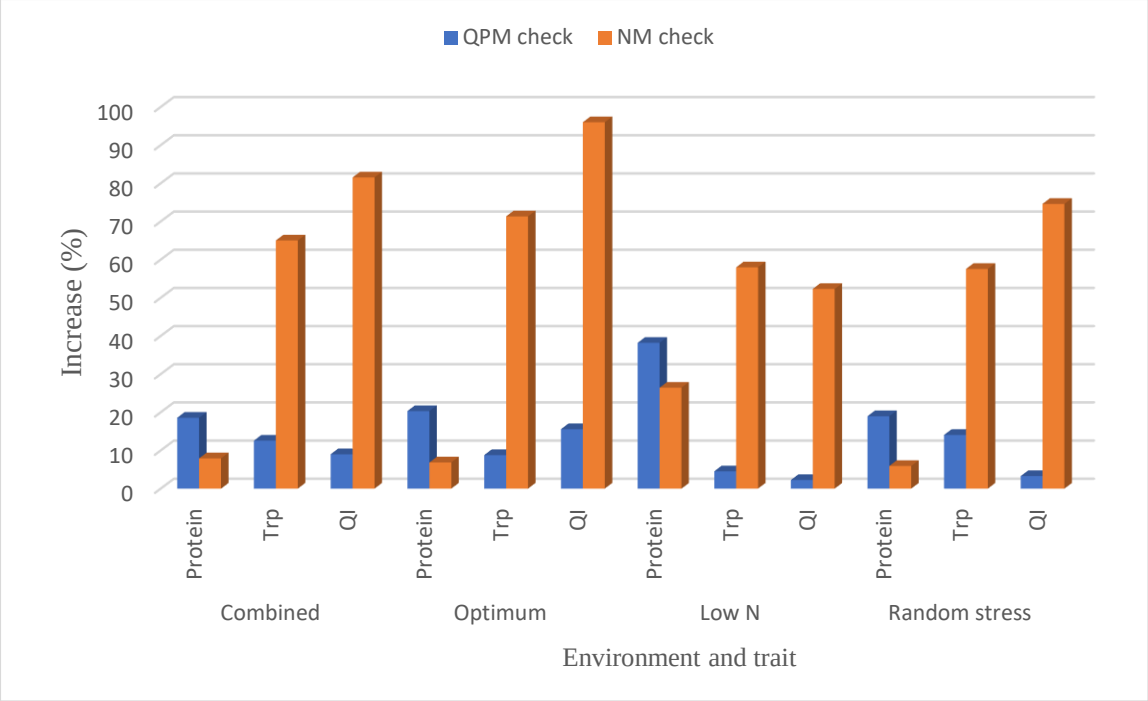


Figure 4.2 Performance of 40 new QPM hybrids compared with the best QPM and normal maize (NM) checks for total protein content (%), tryptophan content (Trp, %), protein quality index (QI,%) under optimum, low nitrogen (low N) and random drought stress conditions

Table 4.4 Means of total protein and tryptophan concentration, and protein quality index of quality protein QPM hybrids and commercial checks evaluated optimum, low N and random drought stress conditions

Character	Combined mean			Optimum			Low N			Random drought stress		
	Pro	Trp	QI	Pro	Trp	QI	Pro	Trp	QI	Pro	Trp	QI
Grand Mean	8.1	0.079	1.01	8.5	0.083	1.001	6.2	0.068	1.14	9.9	0.078	0.80
Minimum	7.1	0.061	0.72	7.4	0.064	0.71	5.2	0.052	0.80	7.15	0.059	0.57
Maximum	9.4	0.098	1.31	10.0	0.104	1.32	7.9	0.087	1.48	11.60	0.110	1.10
Mean of QPM hybrids	8.1	0.077	0.98	8.5	0.081	0.97	6.2	0.067	1.11	9.9	0.076	0.78
Mean of QPM checks	7.9	0.082	1.06	8.3	0.087	1.05	5.8	0.066	1.17	10.0	0.089	0.89
Mean of normal checks	8.6	0.058	0.70	9.2	0.060	0.66	6.0	0.053	0.89	10.7	0.061	0.57
Best QPM hybrid	9.4	0.098	1.309	10.0	0.104	1.32	7.9	0.087	1.478	11.6	0.110	1.1
Best QPM check	7.9	0.087	1.143	8.3	0.088	1.09	6.0	0.078	1.37	10.1	0.099	0.965
Best normal check	8.7	0.06	0.72	9.4	0.06	0.67	6.3	0.055	0.97	11.0	0.070	0.63
LSD	0.46	0.008	0.12	0.59	0.011	0.14	0.97	0.016	0.33	1.30	0.024	0.23
Change from optimum mean (%)							36.0	20.6	-12.3	-14.0	5.3	24.7

Pro = total protein content; Trp = tryptophan content; QI = protein quality index; max = maximum, min = minimum, LSD = least significant difference, negative values indicate an increase under low N and random drought stress for that trait

4.3.3 Analysis of variance and mean performance of iron, zinc phytic acid and molar ratios of iron and zinc to phytic acid at each location

Bako showed highly significant differences among genotypes for all studied traits under both optimum and low N stress conditions, except phytic acid to Zn molar ratio under low N stress (Table 4.5). The effects of year and G × Y interaction were also highly significant for all traits, except year for phytic acid content under optimum conditions (Table 4.5). The concentrations of Fe and Zn ranged from 19.58 to 41.16 mg kg^{-1} and 10.86 to 31.44 mg kg^{-1} with means of 28.82 mg kg^{-1} and 19.85 mg kg^{-1} under optimum and 8.53 to 28.94 mg kg^{-1} and 7.86 to 23.47 mg kg^{-1} with means 17.95 mg kg^{-1} and 12.15 mg kg^{-1} under low N stress, respectively (Table 4.6). None of QPM hybrids showed higher Fe content than the best normal checks under optimum conditions; however, 45% and 10%, and 52% and 57% of QPM hybrids showed higher Fe and Zn concentrations than the best QPM check, and the trial mean, respectively, under optimum conditions (Figures 4.3a-b). Ten, 23 and 45% and 23, 45 and 48% of hybrids also showed higher Fe and Zn values than the best QPM and normal maize checks, and the trial mean, respectively (Figures 4.3a-b). Based on the grand mean, Fe and Zn concentrations decreased by 6.0% and 6.3% under low N stress. At Bako, phytic acid and phytic acid to Fe and Zn molar ratios ranged from 4.66 to 6.49 mg g^{-1}, 12.26 to 26.31 and 18.16 to 51.0 with means of 5.64 mg g^{-1}, 18.58 and 31.65 under optimum and 3.84 to 5.6 mg g^{-1} for phytic acid, 15.02 to 48.19 with means 4.7mg g^{-1}, 24.18 and 41.42 under low N stress, respectively (Table 4.6). Some QPM hybrids showed lower content of phytic acid and molar ratios of Fe and Zn to phytic acid than the best QPM and QPM normal maize checks, and the trial mean both under optimum and low N stress conditions (Figures 4.3c-e).

Highly significant differences ($P \leq 0.01$) were observed among genotypes for all studied traits under optimum and low N stress conditions at Ambo, except phytic acid to Zn molar ratio (Table 4.5). The effects of year and G × Y interaction were also significant ($P \leq 0.01$) for all traits under optimum conditions, except year, which did not affect Zn (Table 4.5). The ranges of Fe and Zn were 13.92 to 42.37 mg kg^{-1}, 12.31 to 33.88 mg kg^{-1}, with means of 27.38 mg kg^{-1} and 20.26 mg kg^{-1} under optimum and 7.34 to 31.7mg kg^{-1} for Fe, 8.71 to 23.51 mg kg^{-1} with means 17.12 mg kg^{-1} and 16.61 mg kg^{-1} under low N stress, respectively. In this location, some hybrids showed higher Fe and Zn concentrations than the best QPM and normal checks, and the trial mean (Figures 4.3a-b). Phytic acid and molar ratios of Fe and Zn to phytic acid ranged from 5.19 to 6.95 mg g^{-1}, 15.0 to 45.47, and 18.73 to 70.12 with means of 6.09 mg g^{-1}, 23.72 and 32.62, respectively, under optimum and 4.01 to 6.07 mg g^{-1}, 12.6 to 62.98 and 20.75 to

52.45 with means 5.05 mg g^{-1}, 30.80 and 32.75, respectively under low N stress conditions (Table 4.6). More than 50% and 30% of hybrids showed lower phytic acid to Fe and Zn molar ratios than the best QPM and normal maize checks, respectively (Figures 4.3d-e). Fe, Zn and phytic acid concentrations were decreased by 59.9%, 22.0% and 20.6% and molar ratio of Fe to phytic acid increased by 23% under low N stress conditions. Phytic acid to Zn molar ratio was similar for both conditions (Table 4.6).

The effect of genotype was significant for all studied traits at Harare under optimum and Gwebi under random drought stress conditions, except molar ratio of Zn to phytic acid at Harare (Table 4.5). The ranges of Fe, Zn, phytic acid, phytic acid to Fe and Zn molar ratios at Harare were 13.56 to 24.02 mg kg^{-1}, 15.44 to 22.12 mg kg^{-1}, 3.72 to 5.72 mg g^{-1}, 16.76 to 29.64 and 20.24 to 29.22, respectively (Table 4.6). At Harare, 35, 20 and 47% of the hybrids showed higher Fe content than the best QPM and normal maize checks, and the trial mean, respectively, while 67, 22 and 50% of hybrids also showed higher Zn content than the best QPM and normal maize checks, and the trial mean, respectively. On average, half of the hybrids showed lower phytic acid content and molar ratios of Fe and Zn to phytic acid than the best QPM and normal checks, and the trial mean, respectively (Figures 4.3c-e).

At Gwebi ranges of Fe, Zn, phytic acid, phytic acid to Fe and Zn molar ratio were 12.76 to 19.24 mg kg^{-1}, 14.86 to 22.76 mg kg^{-1}, 4.22 to 6.53 mg g^{-1}, 21.36 to 38.67 and 21.41 to 43.0, respectively. At Gwebi, 50% and 37% of hybrids showed higher Fe and Zn than the mean of the trial, respectively while around 10% of hybrids showed higher Fe and Zn content than the best QPM and normal maize checks (Figures 4.3a-b). On the other hand, 55, 15 and 45% of hybrids showed lower phytic acid content than the best QPM and normal maize checks, and the trial mean, respectively (Figure 4.3c). Around 90% of hybrids also had lower phytic acid to Zn molar ratio compared with the best QPM and normal maize checks, and the trial mean, respectively (Figure 4.3e).

Table 4.5 Mean squares of iron, zinc, phytic acid and phytic acid to iron and zinc molar ratios of QPM hybrids and commercial checks evaluated under optimum, low N and random drought stress conditions

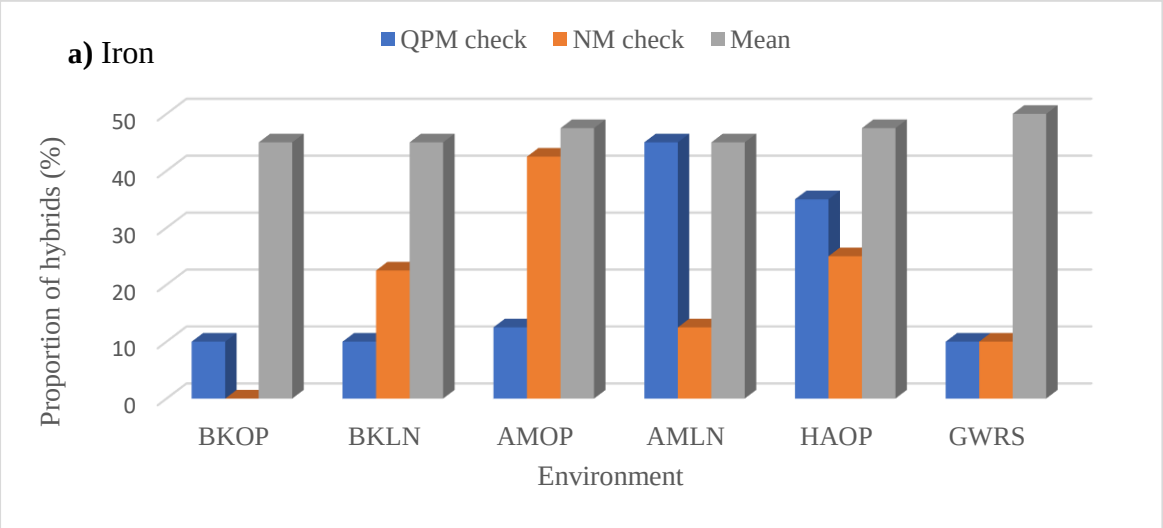
Source variation	Bako optimum						Bako low N					
	Df	Fe	Zn	PA	MRFe	MRZn	Df	Fe	Zn	PA	MRFe	MRZn
Year (Y)	1	2902.82**	1488.35**	0.05	1369.89**	3913.36**						
Rep (Y)	2	415.95**	234.41**	1.15**	344.26**	294.59**	1	194.33**	36.19*	0.15	536.09**	748.40**
Block (Rep × Y)	32	98.83**	58.67**	0.44**	65.91**	194.01**	16	11.94	19.44**	0.35**	34.88	284.27
Genotype	43	103.61**	58.08**	0.49**	37.93**	156.27**	43	36.41**	15.59**	0.26**	82.51**	150.07
G ×Y	43	86.99**	45.80**	0.21**	44.31**	105.79**						
Residual	54	17.03	7.27	0.10	12.18	34.53	27	13.10	6.07	0.11	36.27	113.85
CV (%)		14.32	13.58	5.54	18.78	18.56		20.16	20.28	6.95	24.91	25.76
Source variation	Ambo optimum						Ambo low N					
	Df	Fe	Zn	PA	MRFe	MRZn	Df	Fe	Zn	PA	MRFe	MRZn
Year	1	9220.43**	0.14	3.92**	9432.11**	1304.35**						
Rep (Y)	2	669.57**	111.60**	5.20**	347.44**	455.91**	1	2.06	31.87*	0.20	3.39	386.61*
Block (Rep × Y)	32	179.91**	60.72**	0.84**	148.72**	251.34**	16	94.15**	39.69**	0.39**	315.50**	252.08**
Genotype	43	114.59**	45.62**	0.62**	171.42**	272.59**	43	77.23**	21.97**	0.23*	300.66**	127.62*
G ×Y	43	117.78**	26.96**	0.64**	171.31**	172.69**						
Residual	54	16.79	9.38	0.15	23.74	41.02	27	6.53	7.38	0.11	45.59	75.68
CV (%)		14.96	15.12	6.26	20.54	19.63		14.92	16.36	6.42	21.92	26.56
Source variation	Harare optimum						Gwebi random drought stress					
	Df	Fe	Zn	PA	MRFe	MRZn	Df	Fe	Zn	PA	MRFe	MRZn
Rep	1	7.45	12.97	0.08	3.37	8.21	1	0.06	0.63	0.01	0.05	3.82
Block (Rep)	16	2.32	4.39	0.41**	9.26	21.43	16	3.36	5.91**	0.33**	13.45**	20.28**
Genotype	43	7.72**	5.12*	0.20**	12.05*	8.73	43	4.63**	5.64**	0.28**	18.97**	19.74**
Residual	27	2.01	2.50	0.07	5.38	6.24	27	1.68	1.69	0.12	4.40	4.50
CV (%)		8.56	8.58	5.90	9.89	10.24		8.12	7.10	7.13	8.11	8.09

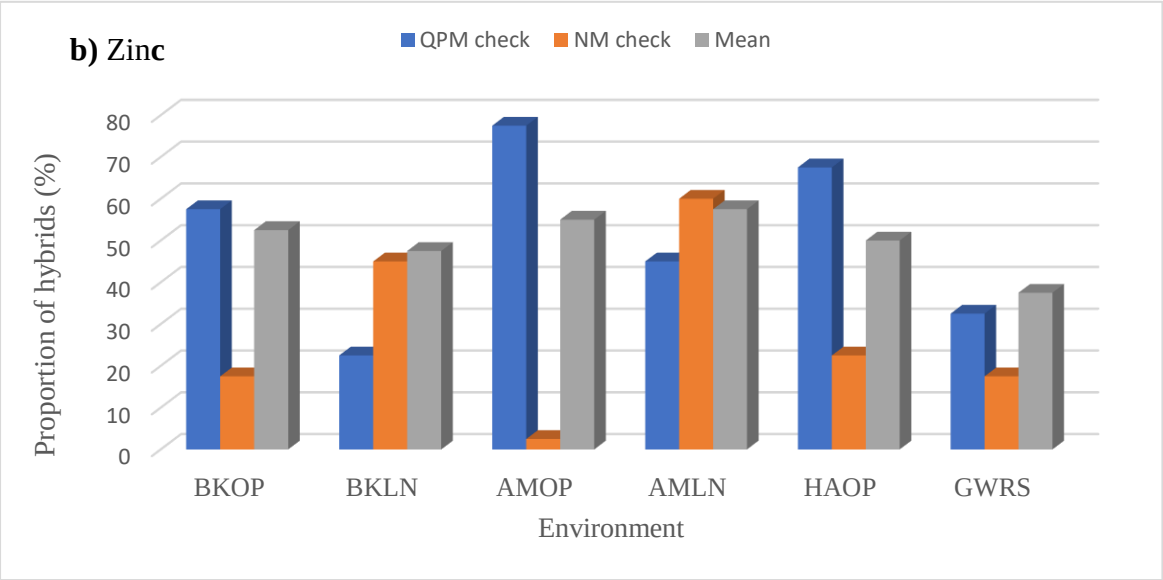
* P ≤0.05, ** P ≤ 0.01; Rep = replication; G × Y = genotype by year interaction; Df = degree of freedom; CV = coefficient of variation; Fe = iron; Zn = zinc; PA = phytic acid; MRFe = phytic acid to Fe molar ratio; MRZn = phytic acid to zinc molar ratio

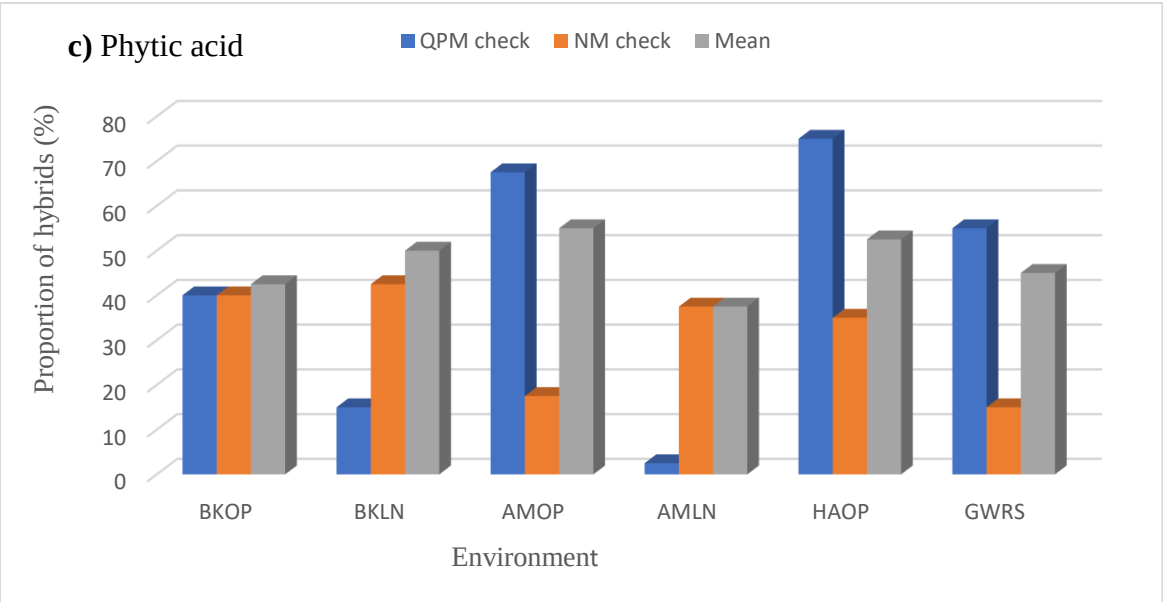
Table 4.6 Mean iron, zinc and phytic acid concentrations and phytic acid to iron and zinc molar ratios of QPM hybrids under optimum, low N and random drought stress conditions

	Bako optimum					Bako low N				
	Fe (mg kg^{-1})	Zn (mg kg^{-1})	PA (mg g^{-1})	MRFe	MRZn	Fe (mg kg^{-1})	Zn (mg kg^{-1})	PA (mg g^{-1})	MRFe	MRZn
Grand mean	28.82	19.85	5.64	18.58	31.65	17.95	12.15	4.70	24.18	41.42
Minimum	19.58	10.86	4.66	12.26	18.16	8.53	7.86	3.84	15.02	19.10
Maximum	41.16	31.44	6.49	26.31	51.00	28.81	23.47	5.60	48.19	68.56
Mean of QPM hybrids	28.55	20.08	5.63	18.73	31.23	17.80	12.16	4.70	24.46	41.64
Mean of QPM checks	28.47	16.23	5.62	18.03	37.62	20.05	12.50	4.38	19.33	35.15
Mean of normal checks	34.43	18.86	5.85	16.15	34.10	18.94	11.44	4.96	23.53	43.28
Best QPM hybrid	37.19	31.4	6.49	26.3	51	28.8	23.5	5.6	48.2	68.56
Best QPM check	34.96	19.22	5.66	22.18	46.09	23.39	13.52	4.41	22.71	37.74
Best normal check	41.16	23.37	6.16	20.04	39.25	20.36	12.25	5.31	26.56	43.30
LSD	5.85	3.82	0.44	4.95	8.33	7.43	5.05	0.67	12.36	21.89
Reduction (%)						6.0	6.3	2.0	-2.3	-2.4
	Ambo optimum					Ambo low N				
	Fe (mg kg^{-1})	Zn (mg kg^{-1})	PA (mg g^{-1})	MRFe	MRZn	Fe (mg kg^{-1})	Zn (mg kg^{-1})	PA (mg g^{-1})	MRFe	MRZn
Grand mean	27.38	20.26	6.09	23.72	32.62	17.12	16.61	5.05	30.80	32.75
Minimum	13.92	12.31	5.19	15.00	18.73	7.34	8.71	4.01	12.60	20.75
Maximum	42.37	33.88	6.95	45.47	70.12	31.70	23.51	6.07	62.98	52.45
Mean of QPM hybrids	27.43	20.17	6.06	23.66	32.63	16.89	16.86	5.05	31.08	32.23
Mean of QPM checks	27.35	17.45	6.62	25.61	39.41	13.02	13.13	4.86	38.61	40.07
Mean of normal checks	26.45	24.86	6.25	23.09	25.75	25.93	14.96	5.18	17.37	35.85
Best QPM hybrid	42.37	33.88	6.93	45.47	70.12	31.70	23.51	6.07	62.98	52.45
Best QPM check	28.50	26.41	6.84	28.51	38.30	17.38	17.56	5.49	56.55	48.73
Best normal check	28.50	26.41	6.84	24.29	26.87	27.07	15.94	5.31	18.51	38.07
LSD	6.11	4.42	0.53	7.35	9.36	6.11	4.42	0.53	7.35	9.36
Proportion of reduction (%)						59.9	22.0	20.6	-23.0	-0.4
	Harare optimum					Gwebi random drought stress				
	Fe (mg kg^{-1})	Zn (mg kg^{-1})	PA (mg g^{-1})	MRFe	MRZn	Fe (mg kg^{-1})	Zn (mg kg^{-1})	PA (mg g^{-1})	MRFe	MRZn
Grand mean	16.56	18.44	4.52	23.45	24.39	15.97	18.29	4.82	25.86	26.23
Minimum	13.56	15.44	3.72	16.76	20.24	12.76	14.86	4.22	21.36	21.41
Maximum	24.02	22.12	5.72	29.64	29.64	19.24	22.76	6.53	38.67	43.00
Mean of QPM hybrids	16.58	18.51	4.51	23.38	24.22	15.81	18.20	4.82	26.13	26.36
Mean of QPM checks	16.37	16.64	4.78	24.87	28.37	17.38	18.38	5.00	24.43	26.85
Mean of normal checks	16.46	18.87	4.53	23.47	23.67	17.91	19.89	4.62	21.89	23.02
Best QPM hybrid	24.02	22.12	5.72	29.64	29.22	19.24	22.76	6.53	38.67	43.00
Best QPM check	17.43	17.51	4.82	26.27	29.64	18.22	18.89	5.13	25.01	26.88
Best normal check	17.75	19.91	4.64	24.70	24.34	18.28	19.94	4.83	22.43	24.17
LSD	2.91	3.25	0.55	4.76	5.13	2.66	2.66	0.70	4.30	4.35
Reduction (%)						3.68	0.85	-6.07	-9.32	-7.03

Fe = iron; Zn = zinc; PA = phytic acid; MRFe = phytic acid to iron molar ratio; MRZn = phytic acid to zinc molar ratio; LSD = Least significant difference; Low N = Low nitrogen, negative values indicate checks had higher value than the best hybrids for that trait


a) Iron
QPM check
NM check
Mean
Proportion of hybrids (%)
50
40
30
20
10
0
BKOP
BKLN
AMOP
AMLN
HAOP
GWRS
Environment


b) Zinc
QPM check
NM check
Mean
Proportion of hybrids (%)
80
70
60
50
40
30
20
10
0
BKOP
BKLN
AMOP
AMLN
HAOP
GWRS
Environment


c) Phytic acid
QPM check
NM check
Mean
Proportion of hybrids (%)
80
70
60
50
40
30
20
10
0
BKOP
BKLN
AMOP
AMLN
HAOP
GWRS
Environment

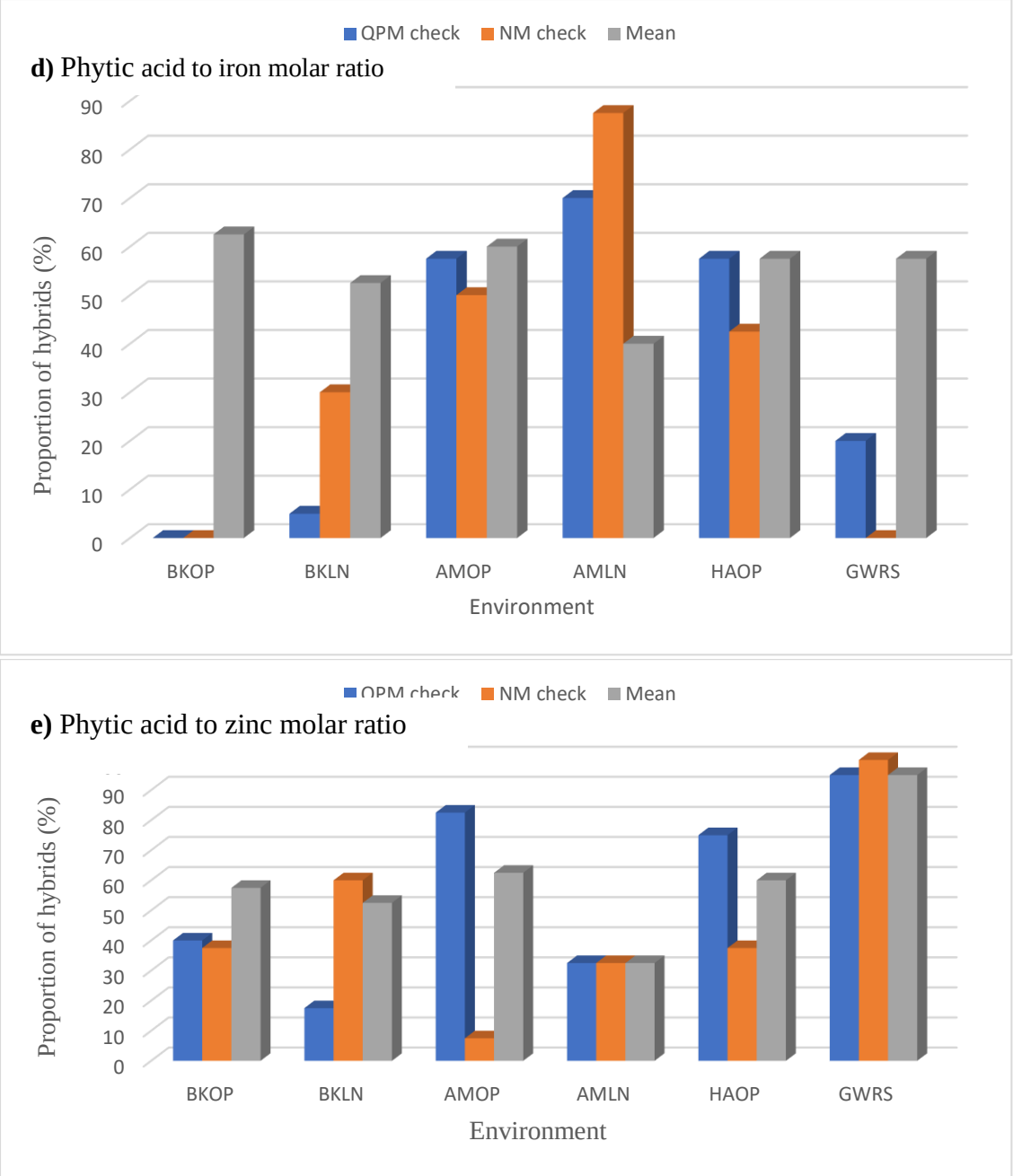


Figure 4.3 Number of new QPM hybrids that showed higher iron (a) and zinc (b) concentration and lower phytic acid (d) and phytic acid to iron (e) and zinc (d) molar ratios as compare with best QPM and normal maize checks and the mean under stress and non-stressed conditions

BKOP = Bako optimum management; BKLN = Bako low N stress; AMOP = Ambo optimum management; AMLN = Ambo low N stress; HAOP = Harare optimum management and GWRS = Gwebi random drought stress

4.3.4. Combined analysis of variance and mean performance of iron, zinc and phytic acid content, and molar ratios of phytic acid to iron and zinc

There were significant differences among genotypes for all traits under optimum, low N and random drought stress conditions and across all environments, except Fe under optimum conditions (Table 4.7). The effects of environment and G × E interaction were highly significant for all studied traits under both optimum and low N stress conditions, except under optimum conditions where environment effect was non-significant for Fe and under low N stress where G × E interaction effects were non-significant for phytic acid to Zn molar ratio (Table 4.7). Across all environments, Fe concentration ranged from 17.38 to 26.81 mg kg^{-1} with a mean 22.38 mg kg^{-1}, Zn concentration ranged from 13.24 to 23.90 mg kg^{-1} with a mean of 18.28 mg kg^{-1}. Phytic acid concentration ranged from 4.64 to 65.74 mg g^{-1} with a mean of 5.31 mg kg^{-1}. Maximum and minimum molar ratios of Fe and Zn to phytic acid were 29.28 and 18.61 and 43.97 and 23.51 with means 23.73 and 31.52, respectively (Table 4.8). Some QPM hybrids showed high Fe and Zn concentration and lower phytic acid, and molar ratios of Fe and Zn across all environments (Figure 4.4).

Under optimum conditions, the ranges of Fe and Zn were 20.35 to 34.52 mg kg^{-1}, 13.23 to 27.08 mg kg^{-1}, with a mean of 25.71 and 19.80 mg kg^{-1}, respectively. The ranges of phytic acid and phytic acid to Fe and Zn molar ratios were 4.74 to 6.12 mg g^{-1}, 16.53 to 32.25 and 22.22 to 44.73, with the means 5.58 mg g^{-1}, 21.63 and 30.39, respectively (Table 4.8). Fe and Zn under optimum conditions for QPM hybrids was higher than the mean of QPM checks and lower than means of normal checks (Table 4.8). Lower phytic acid content for QPM hybrids was observed under optimum conditions compared with the mean of QPM and normal checks. The best QPM hybrid showed 11% and 16% higher Fe concentration, 52% and 15% higher Zn concentration and 17% and 12% lower phytic acid content, and 17% and 13% lower phytic acid to Fe molar ratio and 34% and 18% lower phytic acid to Zn molar ration than the best QPM and normal maize checks, respectively (Figure 4.4).

Under low N stress, mean of Fe and Zn concentrations varied from 10.77 to 26.55 mg kg^{-1} and 9.33 to 21.30 mg kg^{-1} with means, 17.34 and 14.51 mg kg^{-1}, respectively. Phytic acid and phytic acid to Fe and Zn molar ratios also varied from 4.2 to 5.43 mg g^{-1}, 15.6 to 42.92 and 24.0 to 53.35 with the means 4.88 mg g^{-1}, 27.77 and 36.93, respectively. Some of the QPM hybrids showed high Fe and Zn concentration and low phytic acid and molar ratios of Fe and Zn to phytic acid ratio compared with the best QPM and normal checks (Figure 4.4). The mean Fe,

Zn and phytic acid concentration in the grain decreased by 48.2%, 36.4% and 14.3% respectively, while molar ratios of Fe and Zn to phytic acid increased by 22.1% and 17.7%, respectively, compared with mean of optimum conditions (Table 4.8). Under random drought stress Fe, Zn and phytic acid content in the grain, and molar ratio of Zn to phytic acid decreased by 62.6%, 8.77%, 15.8% and 15.29%, respectively, while molar ratio of Fe to phytic acid increased by 17% (Table 4.8).

Table 4.7 Combined analysis mean squares of iron, zinc, phytic acid and phytic acid to iron and zinc molar ratios of QPM hybrids evaluated under optimum, random and low N stress conditions

Source variation	Optimum condition					
	Df	Fe	Zn	PA	MRFe	MRZn
Environment (E)	4	5418.35**	421.31**	37.16**	3374.76**	2382.80**
Rep (E)	5	435.70**	141.00**	2.56**	277.35**	301.84**
Block (E × Rep)	80	111.96**	48.63**	0.60**	87.70**	182.43**
Genotype (G)	43	89.15	67.04**	0.69**	98.26**	226.45**
G × E	172	85.39	28.64**	0.37**	84.69**	122.40**
Residuals	135	13.93	7.16	0.11	15.44	31.47
CV (%)		14.47	13.56	5.97	18.18	18.34
Source of variation	Low N stress					
	Df	Fe	Zn	PA	MRFe	MRZn
E	1	30.38	875.54**	5.44**	1927.21**	3302.06**
Rep (E)	2	98.19**	34.03**	0.17	269.74**	567.50**
Block (E × Rep)	32	53.05**	29.56**	0.37**	175.19**	268.17**
G	43	52.90**	24.49**	0.27**	162.12**	174.19*
G × E	43	60.74**	13.07*	0.22**	221.05**	103.50
Residuals	54	9.81	6.72	0.11	40.93	94.76
CV (%)		17.86	18.04	6.68	23.27	26.25
Source of variation	Random drought stress					
	Df	Fe	Zn	PA	MRFe	MRZn
Rep	1	0.07	0.63	0.01	0.047	3.822
Block	16	3.36	5.91**	0.33**	13.45**	20.28**
G	43	4.63**	5.64**	0.28**	18.97**	19.74**
Residuals	27	1.683	1.68	0.12	4.400	4.501
CV (%)		8.120	7.10	7.13	8.111	8.089
Source of variation	Combined					
	Df	Fe	Zn	PA	MRFe	MRZn
Environment	7	4935.42**	880.70**	35.01**	2897.18**	3016.29**
Rep (E)	8	296.87**	96.71**	1.64**	240.79**	331.01**
Block (E × Rep)	128	83.66**	38.53**	0.51**	100.29**	183.60**
G	43	83.46**	64.28**	0.56**	116.05**	228.36**
G × E	301	66.50**	22.93**	0.34**	103.30**	112.16**
Residuals	216	11.37	6.37	0.11	20.44	43.92
CV (%)		14.99	13.86	6.26	19.15	20.93

* P ≤0.05, ** P ≤ 0.01; Rep = replication; G × E = genotype by environment interaction; Df = degree of freedom; CV = coefficient of variation; Fe = iron; Zn = zinc; PA = phytic acid; MRFe = phytic acid to Fe molar ratio; MRZn = phytic acid to zinc molar ratio

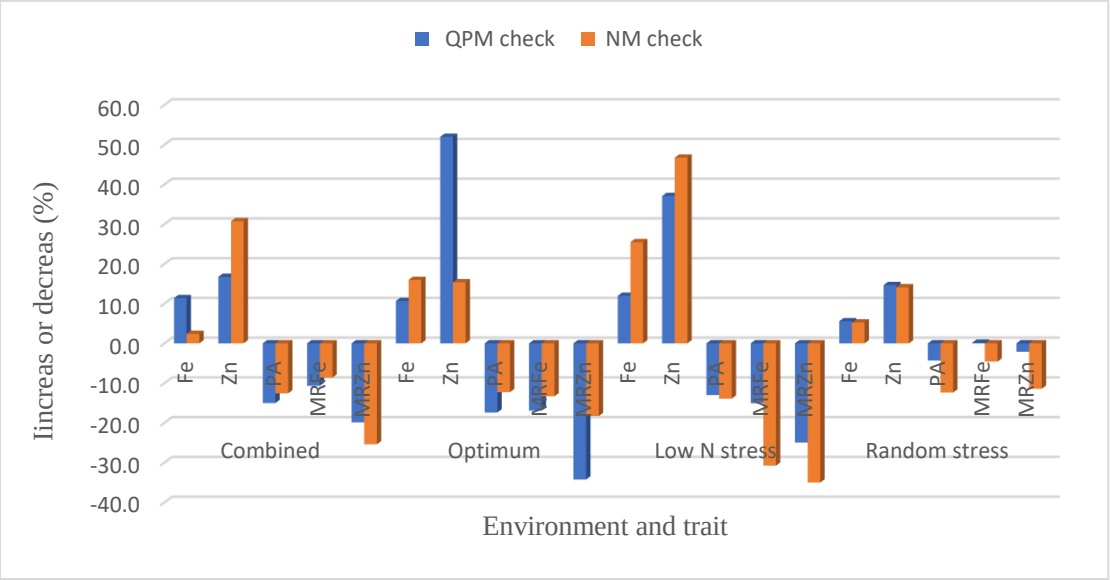


Figure 4.4 Performance of the QPM hybrids compared with the best QPM and normal maize (NM) checks for iron, zinc and phytic acid concentration in maize grain, and molar ratios of phytic acid to iron (MRFe) and zinc (MRZn) under optimum, low N and random drought stress, and combined for all environments

Table 4.8 Mean values for iron, zinc and phytic acid concentration and molar ratios of iron and zinc with phytic acid of QPM hybrids and commercial checks evaluated across combined environments

	Combined					Optimum				
Mean	Fe (mg kg^{-1})	Zn (mg kg^{-1})	PA (mg g^{-1})	MRFe	MRZn	Fe (mg kg^{-1})	Zn (mg kg^{-1})	PA (mg g^{-1})	MRFe	MRZn
Grand mean	22.38	18.28	5.31	23.73	31.52	25.71	19.80	5.58	21.63	30.39
Minimum	17.38	13.24	4.64	18.61	23.51	20.35	13.23	4.74	16.53	22.22
Maximum	26.81	23.90	5.74	29.28	43.97	34.52	27.08	6.12	32.25	44.73
Mean of QPM hybrids	22.46	18.23	5.31	23.67	31.61	25.71	19.80	5.58	21.63	30.39
Mean of QPM checks	21.01	18.29	5.51	24.66	32.59	25.60	16.80	5.85	22.43	36.49
Mean of normal maize checks	24.28	17.98	5.31	22.04	31.77	27.64	21.26	5.75	20.39	28.67
Best QPM hybrid	26.81	23.90	4.64	18.61	23.51	34.52	27.08	4.74	16.53	22.22
Best QPM check	24.07	20.47	5.46	20.83	29.35	31.19	17.82	5.74	19.89	33.80
Best normal check	26.18	18.28	5.31	20.35	31.52	29.77	23.47	5.41	19.07	27.19
LSD	2.35	1.76	0.23	3.15	4.62	3.30	2.37	0.30	3.48	4.96
	Low N stress					Random drought stress				
Mean	Fe (mg kg^{-1})	Zn (mg kg^{-1})	PA (mg g^{-1})	MRFe	MRZn	Fe (mg kg^{-1})	Zn (mg kg^{-1})	PA (mg g^{-1})	MRFe	MRZn
Grand mean	17.34	14.51	4.88	27.77	36.93	15.81	18.20	4.82	26.13	26.36
Minimum	10.77	9.33	4.20	15.60	24.00	12.76	14.86	4.22	21.40	21.41
Maximum	26.55	21.30	5.43	42.92	53.35	19.24	22.76	6.53	38.67	43.00
Mean of QPM hybrids	17.37	14.43	4.86	27.62	37.07	15.81	18.20	4.82	26.13	26.37
Mean of QPM checks	18.20	14.41	4.87	29.00	35.23	17.88	19.37	4.77	22.61	24.37
Mean of normal maize checks	19.25	13.81	5.09	25.15	38.80	17.04	19.07	4.82	24.28	25.26
Best QPM hybrid	26.55	21.30	4.20	15.60	24.00	19.24	22.76	4.22	21.40	21.41
Best QPM check	23.71	15.54	4.83	18.37	31.99	18.22	19.85	4.41	21.36	21.87
Best normal check	21.16	14.51	4.88	22.53	36.93	18.28	19.94	4.82	22.43	24.17
LSD	4.44	3.68	0.46	9.07	13.80	2.66	2.66	0.70	4.30	4.35
Proportion of reduction (%)	48.2	36.4	14.3	-22.1	-17.7	62.6	8.8	15.8	-17	15.29

Fe = iron; Zn = zinc; PA = phytic acid; MRFe = phytic acid to iron molar ratio; MRZn = phytic acid to zinc molar ratio; max = maximum, min = minimum, LSD = Least significant difference; negative values indicate checks had higher value than the best hybrids for that trait

4.4.5 Estimates of variance components for nutritional traits

The estimates of genotypic and phenotypic variance, GCV and PCV, broad sense heritability and genetic advance as percent of mean for total protein content, tryptophan concentration, protein quality index, phytic acid level, Fe and Zn concentrations and molar ratios of Fe and Zn to phytic acid are presented in Table 4.9a-d. GCV ranged from 0 to 10.9% under optimum conditions, 6.2 to 14.9% under random drought stress, 0 to 12.3% under low N stress and 0.6 to 12.3% across all environments. PCV ranged from 0 to 17.3% under optimum, 8.2% to 18.9% under random drought stress, 5.9% to 28.4% under low N stress and 4.0% to 16.2% across all environments. None of the traits showed consistently high GCV and PCV values under all management conditions except Fe (23.9) and molar ratio of Fe to phytic acid (28.4) which recorded the highest PCV values under low N stress. Medium GCV were observed for protein quality index, Zn and molar ratio of Zn to phytic acid under optimum conditions, tryptophan content, protein quality index, molar ratios of Fe and Zn to phytic acid under random drought stress, QI and Zn under low N stress and tryptophan, QI and Zn for combined environment analysis. Low GCV and PCV were observed for total protein and phytic acid contents in all management conditions.

3.4.6 Estimates of broad sense heritability and genetic advance for nutritional traits

Broad sense heritability (H^2) estimates ranged from 0 for Zn to 0.89 for protein quality index (QI) under optimum conditions; 0.57 for Zn to 0.77 for molar ratio of Zn to phytic acid (MRZn) under random drought stress; 0 for Fe and molar rato of Fe to phytic acid (MRFe) to 0.5 for QI under low N stress and 0 for MRFe to 0.9 for QI across all environment analysis. Under optimum conditions the highest heritability was recorded for total protein (0.78) and tryptophan (0.82) contents, and QI (0.89) while moderate heritability was also recorded for phytic acid, Zn and MRZn. The lowest H^2 was recorded for Fe and MRFe. Across random drought stress environments, all traits recorded high H^2 values, except for Zn, with a moderate H^2 value. Under low N environments, moderate H^2 values (0.3 to 0.6) were recorded for tryptophan content, QI and Zn concentration, whereas the rest of the traits had low heritability values. In combined environments, the highest H^2 estimates were recoded for total protein content, tryptophan concentration, QI and Zn while moderate H^2 values were observed for phytic acid and MRZn, whereas Fe and MRFe showed the low heritability values.

Under optimum conditions, genetic advance as percent of the mean (GAM) varied from 0 to 31.5%. The highest GAM was recorded for QI followed by Zn, MRZn and total protein content

while the remaining traits showed below 10% GAM. Under random drought stress, GAM ranged from 8.3 to 18.8%. All traits showed moderate GAM except Zn and phytic acid. Under low N stress conditions, tryptophan, QI and MRZn recorded moderate GAM (>10) while the remaining traits had below 10% GAM. In combined analysis, the highest GAM was observed for QI (30%) followed by tryptophan (21.9%). Moderate GAM was also recoded for protein content, Zn and MRZn while phytic acid, Fe and MRFe showed low GAM.

Table 4.9 Estimates of genetic variability components for nutritional traits of new QPM hybrids and checks evaluated under different environmental conditions

(a) Optimum								
Variance	Protein	Tryptophan	QI	PA	Fe	Zn	MRFe	MRZn
Genotypic variance	0.30	0.00	0.03	0.05	0.00	4.41	0.00	10.97
Phenotypic variance	0.39	0.00	0.03	0.09	10.96	8.16	11.36	22.38
G x E variance	0.21	0.00	0.00	0.16	47.25	15.29	49.51	42.58
Residual variance	0.45	0.00	0.02	0.12	15.13	6.87	14.54	28.94
GCV%	6.4	0.0	16.3	3.8	0.0	10.6	0.0	10.9
PCV%	7.3	0.0	17.3	5.3	12.8	14.5	15.6	15.6
Heritability	0.78	0.82	0.89	0.51	0.00	0.54	0.00	0.49
GA	1.00	0.00	0.31	0.31	0.00	3.16	0.00	4.77
GAM%	11.8	0.0	31.5	5.6	0.0	16.0	0.0	15.7
Grand mean	8.5	0.082	0.99	5.6	25.8	19.8	21.6	30.4
(b) Random drought stress								
Variance	Protein	Tryptophan	QI	PA	Fe	Zn	MRFe	MRZn
Genotypic variance	0.53	0.00	0.01	0.10	1.93	1.69	8.32	9.07
Phenotypic variance	0.71	0.00	0.02	0.16	2.70	2.96	10.55	11.83
Residual Variance	0.37	0.00	0.02	0.11	1.54	2.54	4.46	5.53
GCV%	7.3	12.6	14.9	6.6	8.7	7.1	11.2	11.5
PCV%	8.5	16.3	18.9	8.2	10.3	9.4	12.6	13.1
Heritability	0.74	0.60	0.62	0.66	0.71	0.57	0.79	0.77
GA	1.11	0.01	0.15	0.43	2.04	1.53	4.68	4.74
GAM%	11.2	15.6	18.8	9.0	12.8	8.3	18.1	18.1
Grand mean	9.89	0.08	0.80	4.82	15.97	18.28	25.87	26.23
(c) Low N stress								
Variance	Protein	Tryptophan	QI	PA	Fe	Zn	MRFe	MRZn
Genotypic variance	0.11	0.00	0.02	0.02	0.00	2.27	0.00	13.31
Phenotypic variance	0.40	0.00	0.04	0.08	17.51	6.53	61.06	45.86
G x E Variance	0.32	0.00	0.01	0.08	30.22	5.25	103.61	21.09
Residual Variance	0.51	0.00	0.05	0.10	9.60	6.51	37.03	88.03
GCV%	5.4	9.9	12.3	2.8	0.0	10.5	0.0	9.8
PCV%	10.2	14.1	17.5	5.9	23.9	17.8	28.4	18.2
Heritability	0.28	0.49	0.50	0.22	0.00	0.35	0.00	0.29
GA	0.36	0.01	0.20	0.13	0.00	1.83	0.00	4.04
GAM%	5.8	14.2	17.9	2.7	0.0	12.7	0.0	10.9
Grand mean	6.2	0.068	1.13	4.9	17.5	14.4	27.5	37.2

(d) **Across locations**

Variance	Protein	Tryptophan	QI	PA	Fe	Zn	MRFe	MRZn
Genotype Variance	0.24	0.00	0.02	0.02	0.25	3.31	0.02	9.18
Phenotypic variance	0.30	0.00	0.03	0.05	5.63	5.11	8.43	17.51
G x E Variance	0.25	0.00	0.01	0.14	37.01	11.24	57.78	45.10
Residual Variance	0.46	0.00	0.03	0.11	12.13	6.26	18.92	42.94
GCV%	6.0	11.4	15.4	2.8	2.2	10.0	0.6	9.6
PCV%	6.7	12.3	16.2	4.0	10.5	12.4	12.3	13.2
Heritability	0.80	0.87	0.90	0.47	0.04	0.65	0.00	0.52
GA	0.89	0.02	0.30	0.21	0.21	3.01	0.01	4.51
GAM%	11.0	21.9	30.0	3.9	1.0	16.5	0.1	14.2
Grand mean	8.10	0.08	1.00	5.32	22.50	18.21	23.61	31.69

QI = Protein quality index; PA = Phytic acid content; Fe = Iron; Zn = Zinc; MRFe = Molar ratio of Fe to phytic acid; MRZn = Molar ratio of Zinc; GCV = Genotypic coefficient of variation; PCV = Phenotypic coefficient of variation; GA = Genetic advance; GAM = genetic advance as percent of the mean

4.5 Discussion

Determination of protein quality and quantity, and essential minerals such as Fe and Zn concentration and their bioavailability for newly developed QPM hybrids is an important component of the maize breeding programme to identify nutritionally rich maize hybrids for addressing the present malnutrition challenges in SSA. Results of this study showed significant differences among QPM hybrids at each location under optimum, low N, and random drought stress conditions, and across all environments. This indicated the possibility of selection of superior QPM hybrids under different production conditions. A broad range of variation in total protein and tryptophan concentrations were observed among QPM hybrids under stress and non-stress conditions. Variation among QPM genotypes for protein quality and quantity has been reported (Bantte and Prasanna, 2004; Alamerew, 2008; Wegary et al., 2011; Ngaboyisonga et al., 2012). The ranges of protein content observed were comparable with results reported by Shawa et al. (2021) for ten QPM hybrids and two non-QPM local check hybrids evaluated under optimum and low N stress condition and Ngaboyisonga et al. (2012) for 41 new and one check maize genotypes evaluated under random drought stress. However, there were slightly lower than results reported by Wegary et al. (2011) and Ngaboyisonga et al. (2012) under optimum and low N stress conditions. High ranges of tryptophan and protein quality index were observed between the lowest and the highest hybrids. A comparable result of tryptophan concentration was reported from tropical QPM genotypes (Ngaboyisonga et al., 2012; Shawa, 2019) and temperate QPM genotypes (Ignjatovic-Micic et al., 2020). The mean of protein quality index under optimum, low N, random drought stress and across all environments of this reseach were higher than results reported by Ngaboyisonga et al. (2012).

For a maize genotype to be classified as QPM, it must have a quality index equal or above 0.80 (Wegary et al. 2014; Tandzi et al. 2017). The average values were all above one, except for drought stress, which was 0.8, but the minimum values under combined and optimum conditions fell slightly short of this, and under drought conditions the lowest value was well below this at 0.57. This indicates that drought had a highly negative influence on protein quality index.

The effect of year was significant for total protein and protein quality index at both Bako and Ambo. This indicated that the hybrid performances varied from year to year. Similar results were reported by Ignjatovic-micic et al. (2020). The effect of environment was highly significant for all traits under both optimum and low N stress conditions, while G × E interaction was significant only for total protein under optimum and low N stress conditions, suggesting that G × E interaction was very small in the case of tryptophan and protein quality index compared to total protein content, which was more sensitive to environmental conditions (Wegary et al., 2011; Ngaboyisonga et al., 2012; Ngaboyisonga and Kiarie, 2014).

Protein content was generally more sensitive to environmental conditions than tryptophan concentration. The total protein content in the grain was reduced under low N stress but increased under random drought stress, whereas tryptophan concentration was reduced under low N stress and comparable under random drought stress conditions. This indicated that low N and drought stress significantly affected protein quantity and quality in these genotypes. According to Ngaboyisonga et al. (2012) most QPM kernels become opaque in water deficit environments with increased protein and tryptophan concentration, while low N environments cause significantly reduced concentrations of protein and tryptophan. Consistent results were reported in tropical and sub-tropical maize genotypes evaluated under stressed and non-stressed conditions (Wegary et al., 2011; Ngaboyisonga et al., 2012). Some QPM hybrids showed up to 10% higher total protein content compared to normal checks. In this study the concentration of tryptophan was more stable than the concentration of total protein across environments, which is consistent with results reported by Wegary et al. (2011) and Ngaboyisonga et al. (2012).

Significant variation among QPM hybrids for Fe and Zn concentrations under optimum, low N, random drought stress and across all environments, indicated wide genetic variability among QPM genotypes that can be used for improving kernel micronutrient traits. Previously, the presence of significant genetic variation for Fe and Zn concentrations were reported in QPM

and non QPM genotypes (Bänziger and Long, 2000; Oikeh et al., 2003; Simic et al., 2009; Queiroz et al., 2011; Akhtar et al., 2021). The effects of year/environment and G × E interaction were also significant for Fe and Zn concentration, indicating that concentrations of these minerals were influenced by environment. Pixley et al. (2011) found that large variation due to location effects tends to limit the usefulness of the trait in breeding programme. Prasanna et al. (2011) confirmed that micronutrient concentration can be affected by different factors including soil type and fertility, moisture, environmental conditions, and interactions among nutrients. Oikeh et al. (2004) and Prasanna et al. (2011) reported significant G × E interaction effects for grain Fe and Zn concentrations for diverse tropical maize genotypes. Contrary to this, Menkir (2008) reported no significant genotype × location interaction for concentration of Fe, Zn and other minerals for low land and mid-altitude inbred lines.

In this study, there were some QPM hybrids that showed consistently high concentrations of Zn in all trials. Similarly, Chakraborti et al. (2011) reported that the concentration of Zn in QPM hybrids was higher than in non-QPM genotypes. Hindu et al. (2018) and Prasanna et al. (2020) reported the possibility of identifying QPM genotypes that have high Zn concentration. They also noted that it is possible to find normal maize genotypes that have high Zn content. The concentrations of Fe and Zn in this study were 20.35 to 34.52 mg kg^{-1} and 13.23 to 27.08 mg kg^{-1} under optimum, 10.77 to 26.55 mg kg^{-1}, 9.33 to 21.30 mg kg^{-1} under low N stress and 12.76 to 19.24 mg kg^{-1} and 14.86 to 22.76 mg kg^{-1}, respectively. This is comparable with results reported by Queiroz et al. (2011) for 22 tropical maize inbred lines and Shawa (2019) for 10 QPM genotypes and two normal checks evaluated under optimum and low N stress conditions. On the other hand, Oikeh et al. (2003) reported slightly lower values. Some studies showed higher concentration of Fe and Zn than in this study (Bänziger and Long, 2000; Prasanna et al., 2011). The effects of low N and drought stress were significant on the concentration of Fe and Zn. Akhtar et al. (2021) also reported that low N stress significantly affected the concentration and bioavailability of Zn. Pfeiffer and McClafferty (2007) and Prasanna et al. (2011) also reported that planting season and management practices can have a significant impact on the concentration of Fe and Zn.

The mean value of phytic acid for QPM hybrids was lower than the mean of non-QPM checks under optimum conditions while they showed higher values under low N stress and had similar values under random drought stress. This is in agreement with the results of Shawa (2019) for trials evaluated under low N stress. The concentration of phytic acid in the current study ranged

from 4.74 to 6.12 mg g^{-1} under optimum, 4.20 to 5.43 mg g^{-1} under low N and 4 22 mg g^{-1} to 6.53 mg g^{-1} under random drought stress conditions. These results are comparable with results reported by Shawa (2019) and Akhtar et al. (2021). In general, the effect of low N on the concentration of phytic acid was not as much as the effect on Fe and Zn concentrations. For this reason, the molar ratios of Fe and Zn to phytic acid were increased under low N conditions. This indicated that the concentration of Fe and Zn was more sensitive than phytic acid concentration to N deficiency.

Highly significant variation was observed among genotypes for phytic acid to Fe and Zn molar ratios, which indicated the possibility of breeding for enhanced Fe and Zn bioavailability along with increased Fe and Zn concentrations as a strategy to improve the nutritional value of maize. Different researchers also reported the presence of significant variation among maize genotypes under stress and non-stressed environment conditions for Fe and Zn bioavailability (Oikeh et al., 2003; Simic et al., 2009; Pixley et al., 2011; Queiroz et al., 2011; Akhtar et al., 2021). In the present study, highly significant variation was observed for genotype, environment, and G × E interaction for phytic acid to Fe and Zn molar ratios. The molar ratios of Fe and Zn ranges of the current study were comparable with results reported by Queiroz et al. (2011) among 22 tropical maize inbred lines and Shawa (2019) among ten QPM and two normal checks evaluated under optimum and low N stress conditions. Akhtar et al. (2021) reported similar results of phytic acid to Fe and Zn molar ratios for 18 line by tester crosses evaluated under optimum and low N stress conditions. The proportion of phytic acid to Fe and Zn molar ratios increased under low N, while under random drought stress phytic acid to Zn molar ratio increased while phytic acid to Fe molar ratio decreased. In the low N stress trials, small and shrivelled seeds were observed, which might have affected the concentration of Fe, Zn and phytic acid (Akhtar et al., 2021).

The amount of variation present in a population is measured by genotypic and phenotypic coefficient of variation (Sarankumar et al., 2019). According to Deshmukh et al. (1986) genotypic and phenotypic coefficient of variation were categorized as low (0 to 10%), moderate (10 to 20%) and high (> 20%). Based on this, none of the traits in this study showed high GCV values, however moderate GCV was recorded for QI under all management conditions. On the other hand, the highest PCV was recorded for Fe and Zn only under low N stress conditions whereas most traits showed moderate PCV under all management conditions. Phytic acid showed the lowest GCV and PCV under all management conditions and in combined

environments analysis. Sharma et al. (2017) reported consistent results with the results of this study in terms of GCV and PCV for total protein and contrary results for tryptophan content under optimum environments. In general, the values of PCV were higher than GCV values for all nutritional quality traits across all management conditions. This clearly showed that there was a large environmental effect on the expression of the traits.

The value of heritability and genetic advance as percent of the mean is important to show the scope for improvement in various quality traits (Singh et al., 2017). Broad sense heritability of total protein and tryptophan contents, and protein quality index was high under optimum and random drought stress conditions, indicating that the genotypic effect was high for the expression of these traits. High broad sense heritability of total protein and tryptophan has also been reported in other studies under optimum management conditions (Sharma et al., 2017; Amegbor et al., 2022). On the other hand, heritability of total protein and tryptophan contents was lower under low N stress conditions compared to optimum and random drought stress conditions, indicating that the N level strongly affected the total protein and tryptophan contents of these experimental genotypes. Consistent with this result, Ertiro et al. (2022) reported the broad sense heritability of protein content under low N conditions was reduced up to 14% compared with optimum conditions. Wegary et al. (2011), Ngaboyisonga et al. (2012) and Ngaboyisonga and Kiarie (2014) reported that low N environments significantly reduced the total protein and tryptophan contents in maize grain. In combined environment analysis, tryptophan concentration and protein quality index had high heritability and genetic advance. This indicated that these traits are controlled by additive genes and can therefore be exploited in maize breeding to improve tryptophan content in the grain. Similar results for tryptophan concentration under optimum management conditions have been reported by Sharma et al. (2017). Low or zero heritability and GAM shown for Fe and molar ratio of Fe to phytic acid in optimum, low N and combined environment analysis indicated that environment had a high impact on these traits. Previous research also reported large variation among maize genotypes for Fe due to environmental effects and concluded that breeding is not effective for the improvement of this trait (Pixley et al., 2011; Prasanna et al., 2011).

4.6 Conclusions

Low N stress had a significant impact on total protein and tryptophan concentration in the grain, and protein quality index, causing decreases. Total protein content was more sensitive than tryptophan concentration to low N stress because protein quality index was increased under low N. Under random drought stress a comparable concentration of tryptophan was recorded to that under optimum conditions while the total protein content increased. QPM hybrids, such as 24 and 25 for protein content and 10, 14 and 16 for tryptophan were showed higher total protein and tryptophan concentration than the total protein and tryptophan concentrations of the best QPM and normal maize checks under all management conditions. Poor soil fertility and drought had a significant effect on the concentration and bioavailability of Fe and Zn. As a result, the concentrations of Fe and Zn decreased under low N and random drought stress conditions. Although no single hybrid showed consistently higher Fe and Zn concentration and lower phytic acid and molar ratios of Fe and Zn to phytic acid among evaluated genotypes, there were QPM hybrids, 10 and 12 under optimum, 15 and 28 under low N stress and 30, 33 and 28 under random stress had the highest Fe concentration and lower phytic acid to Fe molar ratio. In addition, QPM hybrids such as 15 and 14 under optimum, 28 and 9 under low N and 14 and 7 under random stress conditions had high Zn concentration and lower phytic acid to Zn molar ratio compared with the best QPM and normal checks. In general, using these kinds of QPM hybrids is a possible solution to improve macro and micronutrient deficiencies in SSA countries for people who depend on maize, with little or no diet diversification.

4.7 References

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CHAPTER 5

Grain yield stability of quality protein maize hybrids under non-stressed and stressed environments

Abstract

Shortages of moisture and low soil fertility are the most important abiotic stress factors causing significant yield reduction in maize growing areas in SSA. Evaluation of QPM hybrids under multiple environments, including drought and low N stressed testing sites is an important breeding approach, to identify well adapted and stable QPM hybrids, and to identify discriminating and representative testing environments. This study was undertaken to determine G × E interaction and stability for grain yield of newly developed QPM hybrids under non-stressed and stressed environments to select desirable QPM varieties in SSA for commercialization. Totally 45 hybrids, including 40 new QPM hybrids, and two QPM, two non-QPM and one local check were used for this study. These experimental hybrids were evaluated at 14 optimum, nine random drought stress, three managed drought stress and eight low N testing sites in Ethiopia, Zimbabwe, Zambia, Mozambique, and Malawi in 2018 to 2020 using a 5 × 9 alpha lattice design with two replications per genotype at each site. AMMI, AMMI stability value (ASV), and GGE biplot models were used for data analysis. Highly significant ($P \leq 0.01$) environment (E), genotype (G), and G × E interaction effects were observed for grain yield under optimum, random drought, managed drought, and low N stress environments. The proportion of total variation explained by E, G and G × E were 36, 10 and 54% under optimum, 54, 11 and 35% under random drought stress, 20, 28 and 52% under managed stress and 70, 8 and 22% under low N conditions, respectively. Based on AMMI and GGE biplot analysis, entries 10 (CZH142238Q) and 14 (CZH15142Q) under optimum; 23 (CZH17192Q) under random drought stress; 19 (CZH17188Q) and 40 (CZH17209Q) under managed drought and 14 (CZH15142Q) under low N were identified as the best yielding and most stable hybrids. Among these entries 10 (CZH142238Q) and 14 (CZH15142Q) were also identified as ideal hybrids under both stressed and non-stressed environments. GGE biplot analysis was effective in defining mega-environments and identifying top yielding hybrids in each testing environment. Testing sites like Kwekwe (KWE), Bindura (BIN), Chokwe (CHO) and Bako second year (BK2) were identified as discriminating and representative sites for optimum conditions, random drought, managed drought and low N stress environments, respectively.

The selected QPM hybrids in this study should be advanced to variety verification for commercial release to improve the livelihood of smallholder farmers in SSA.

Keywords: AMMI, GGE biplot, G × E interaction, Grain yield, QPM, yield stability

5.1 Introduction

Maize is one of the most important strategic cereal crops for improving food security and economic wellbeing of millions of people in the developing world. It is a major staple food and the most important calorie source for more than 300 million people in SSA (Bankole et al., 2017; Wegary et al., 2018). In addition, maize is a source of proteins, vitamins, minerals, insoluble and soluble dietary fiber, and polar and non-polar lipids, that provide health benefits and prevent diseases (Prasanna et al., 2020). However, the concentrations of nutrients such as essential amino acids lysine and tryptophan in commonly used maize (conventional maize germplasm) are insufficient (Vasal, 2000; Prasanna et al., 2001). The discovery of the *o2* gene brought an exciting opportunity for the development of QPM varieties that have double the amount of lysine and tryptophan of non-QPM (Wegary et al., 2011; Setimela et al., 2017a). QPM varieties are also good sources of essential micronutrients like Fe and Zn (Chakraborti et al., 2011; Phalafala, 2013; Prasanna et al., 2020). Hence, the development and release of nutritionally enhanced maize varieties through biofortification is a sustainable and cost-effective approach to improve food and nutritional security in low income communities that have limited access to essential nutrient sources like animal products (Vivek et al., 2008; Wegary et al., 2014; Abakemal et al., 2016; Bouis and Saltzman, 2017; Akhtar et al., 2018; Mebratu et al., 2019).

The average maize yield productivity in SSA is less than 2.0 t ha^{-1} which is significantly lower than other maize-growing regions in the developing world and average world productivity (Cairns et al., 2013; Beyene et al., 2016; Rezende et al., 2020). This is associated with various production constraints, including drought, poor soil fertility, insect pests, and diseases (Cairns et al., 2013; Rezende et al., 2020). In the tropics, drought and low soil fertility stress has been identified as common constraints and frequently occur together (Nyombayire et al., 2011). Drought causes several changes in morpho-physiological traits and metabolism of plants (Rezende et al., 2020) and significant yield losses result when these stress factors occur either combined or separately with soil nutrient deficiency (Meseka et al., 2013). According to Fisher et al. (2015) around 40% of maize fields in SSA face occasional drought stress, which can

cause 10 to 25% yield losses every year. Around 25% of maize growing areas suffer from frequent drought which causes up to 50% yield losses (Fisher et al., 2015). This may lead to food insecurity and can place the livelihood of farmers and their families at risk (Ertiro et al., 2017). Since SSA maize production areas are mainly dependent on rainfed conditions (Rezende et al., 2020), accurate prediction of rainfall patterns and supplementary irrigation has the potential to improve maize production in drought prone areas (Nyombayire et al., 2011). However, due to poor infrastructure, the high cost of irrigation facilities and inadequate capacity to accurately predict rainfall patterns, this leaves the majority of smallholder farmers with limited ability to cope with drought (Ertiro et al., 2017).

Low N availability in SSA is also a widespread problem on small scale farms (Bänziger et al, 2004; Ertiro et al., 2020; Rezende et al., 2020). In SSA, application of fertilizer is less than 10 kg ha^{-1}, which is very low compared to the 100 and 96 kg ha^{-1} used in Asia and Latin America, respectively (Das et al., 2019), and far below the recommended rate in the SSA region of 90 to 120 kg ha^{-1} yr^{-1} (Badu-Apraku et al., 2017; Setimela et al., 2017b; Tapera, 2017; Ertiro et al., 2020). Unaffordability and unavailability of fertilizer at planting are the main reasons for low use by most of the smallholder farmers (Tapera, 2017). This has negatively impacted the application of the required amount and best type of fertilizers for the normal growth and development of maize (Bänziger et al., 2004; Das et al., 2019; Ertiro et al., 2020). Therefore, the most effective strategy is the development of maize genotypes that are tolerant to drought with high nitrogen use efficiency (NUE). This is a cost effective and environmentally safe approach that could increase yields and have a major impact on livelihoods, food security and sustainability in SSA (Meseka et al., 2013; Ertiro, 2018).

In maize breeding programmes, evaluation of newly developed hybrids in multi-location trials for several years can help to determine their performance, adaptability and stability before commercial release as the performance of hybrids may vary when grown in different environments or agro-ecological conditions (Lule et al., 2014; Makumbi et al., 2015; Abakemal et al., 2016; Rezende et al., 2020). Hybrids that are superior in one environment may not be superior in other environments, due to G×E interaction (Mendes et al., 2012; Makumbi et al., 2015; Sserumaga et al., 2016; 2018). Consequently, it is difficult to identify desirable genotypes and ideal testing environments to be used for the selection of superior genotypes (Wolde et al., 2018; Mebratu et al., 2019). Hybrids adapted to several environments can be considered as generally adaptable, whereas those that perform well only in specific

environments, are known as specific adaptable varieties (Lule et al., 2014; Shaibu et al., 2016). Several statistical methods have been developed for analysing G × E interaction and stability. AMMI (Zobel et al., 1988) and GGE biplot (Yan, 2001; Yan and Kang, 2003; Yan and Tinker, 2006) analyses are the most widely used analytical and statistical tools to determine the pattern of genotypic responses across environments (Lule et al., 2014; Abakemal et al., 2016; Mebratu et al., 2019). AMMI increases the precision of yield estimation and allows selection for higher yielding genotypes (Crossa et al., 1990). It is also used to determine stability of genotypes across locations using PCA and can detect the G × E patterns graphically. GGE biplot analysis is also efficient to select well adapted and stable hybrids and to identify discriminating and representative environments (Yan and Holland, 2010).

Several studies have reported the significant effects of G × E interaction on grain yield in different maize genotypes evaluated under different environmental conditions. Mebratu et al. (2019) and Setimela et al. (2017a) reported the presence of high G × E interaction variation in QPM hybrids under optimum, low N, and drought environments. Abakemal et al. (2016) reported that G × E interaction contributed up to 40.1% of the total variation in 68 QPM maize hybrids evaluated across seven transitional highland agro-ecologies in Ethiopia. Mengesha et al. (2019) reported G × E interaction variation for both grain yield and provitamin A content in 21 maize synthetics evaluated at nine locations for 4 years. They identified stable and high provitamin A content genotypes recommended for production in the low-land tropics of western and central Africa with similar environments.

CIMMYT, in collaboration with the NARS, have been screening a number of QPM and non-QPM genotypes under drought and low N environmental conditions for a number of years and significant progress has been made in the identification of stress tolerant genotypes (Wegary et al., 2013; 2014; Ertiro et al., 2017; Sserumaga et al., 2016; 2018; Mebratu et al., 2019). As a result, several stress tolerant maize varieties have been released and disseminated for commercial production in partnership with various public and private organizations in SSA (Prasanna, 2013; Fisher et al., 2015; Teklewold et al., 2015; Abate et al., 2017). Compared to the conventional maize varieties, however, a very limited number of stress-tolerant (mainly drought and low N) QPM varieties have been released and commercialized. Therefore, the objectives of this study were (1) to determine G × E interaction and stability of newly developed QPM hybrids and (2) to identify the most representative and discriminating testing environments (both stress and optimal) in the eastern and southern Africa (ESA) region.

5.2 Materials and methods

5.2.1. Testing sites and trial management

Detailed description of experimental testing sites and management conditions used in this study were given in the materials and methods section in Chapter 3.

5.2.2 Planting materials

A total of 45 hybrids, including 40 new QPM hybrids, and two QPM, two non-QPM and one local check were used for this study. However, different local checks were used at different locations, and hence, were excluded from the analysis. A detailed description of the hybrids was given in the materials and methods section of Chapter 3.

5.2.3 Experimental design and collected data

A detailed description was presented in the materials and methods section of Chapter 3.

5.2.4 Data analysis

Yield data of individual and combined locations ANOVA for each management type was done using a mixed effect model procedure of the SAS statistical package (SAS, 2002). The presence of significant G × E interaction was confirmed before AMMI and GGE biplot analysis. The hybrids were considered as a fixed factor, while environments, replications within environments and the incomplete blocks within replications were considered random factors.

The AMMI and GGE models were used to determine the G × E interaction and stability of hybrids based on grain yield. The AMMI analysis was performed by using the raw data of a lattice design with GEA-R (Genotype by Environment Analysis with R) version 4.1 software (Pacheco et al., 2015). The model separates the additive effects for genotype and environment from the multiplicative effects for G × E interaction by applying PCA (Pacheco et al., 2015).

The results of AMMI analysis were also presented graphically in the form of biplots drawn by placing both the genotype and environment means on the axis (abscissa) and the respective eigenvectors or scores called the PCA on the y-axis (ordinate), according to Gauch and Zobel (1996).

The basic model is: $yij = \mu + \ g_i \ + \ e_{in} + \sum_{n=1}^{N}(\tau_n \ \gamma_{in} \ \delta_{in}) \ + \ e_{ij}$

Where Y_{ij} is the yield of the i^{th} genotype (i=1,..,I) in the j^{th} environment (j=1,..,J); μ is the grand mean; g_i and e_j are the genotype and environment deviations from the grand mean, respectively; τ_n is the eigenvalue of the PC analysis axis n; γ_{in} and δ_{jn} are the genotype and environment principal components scores for axis n; N is the number of principal components retained in the model and e_{ij} is the error term.

ASV for grain yield stability analyses were calculate based on the formula proposed by Purchase et al. (2000). Hybrids with ASV values closer to zero are considered more stable than hybrids with higher positive or negative ASV values.

$$\text{ASV} = \sqrt{\left[\frac{IPCA1\ SS}{IPCA2\ SS}\ (IPCA1\ score)\right]^2 + (IPCA2\ score)^2}$$

ASV= AMMI stability value; SS= sum of squares; IPCA1 and IPCA2= the first and the second interaction principal component axis, respectively

GGE biplot analysis was also done to determine stability of QPM hybrids based on the linear-bilinear sites (environments) regression model (SREG) (Yan and Kang, 2003) using GEA-R Version 4.1 software (Pacheco et al., 2015). The GGE biplot graph was generated using singular value decomposition (SVD) of the first two principal component axis derived from environment-centered data (axis1 and axis2). Graphical visualization of the "which won where" pattern shows the relationship among testing environments and hybrids (Yan, 2001; 2002). The average environment coordinate (AEC) observes the performance and stability of hybrids, identifies ideal hybrids and environments, and representativeness and discriminating ability of the environments (Yan, 2001).

5.3 Results

5.3.1 AMMI analysis

Combined analysis for each management condition showed significant difference among G, E, and G × E interaction for grain yield as was shown in Chapter 3, Table 3.3. Mean squares from the AMMI analysis showed highly significant G, E, and G × E interaction effects for grain yield under optimum, random drought, managed drought, and low N stress conditions (Table

5.1a-d). The proportion of total variation explained by E, G and G × E were 36, 10 and 54% under optimum, 54, 11 and 35% under random drought stress, 20, 28 and 52% under managed stress and 70, 8 and 22% under low N conditions, respectively.

The G × E interaction source of variation was further partitioned into interaction principal components (IPCA) (Table 5.2). Under optimum conditions, G × E interaction was partitioned into eight significant IPCAs, which explained 86.1% of the total G × E interaction variation. Under random drought stress, G × E interaction was partitioned into four significant IPCAs. These IPCAs explained a total of 76.9% of the G × E interaction variation. Under managed drought, G × E was partitioned into two highly significant IPCAs, which explained 100% of the variation. Under low N conditions, G × E interaction was partitioned into six significant IPCAs, which explained 97.1% of the variation. The first two IPCAs in each management type explained 38.5%, 51.9%, 100% and 60.3% of variation under optimum, random drought, managed drought, and low N environments, respectively.

5.3.2 Mean grain yield and IPCA scores of hybrids and environments

Mean grain yield, IPCA scores and AMMI biplots of 44 hybrids, including commercial checks, for optimum, random drought, managed drought and low N environments are presented in Table 5.2 and Figures 5.1a to 5.1d. Entries, 6, 10, 14, 20 and 24 produced good mean grain yield and had IPCA1 scores close to zero, suggesting that these hybrids were stable under optimum conditions (Table 5.2, Figure 5.1a). On the other hand, entries 3, 12,23 and 44 had high IPCA1 scores and yielded above the grand mean, so these hybrids were considered as unstable and adapted to specific environments at Ambo (AM1) and Lionsden (LIO). Entries 11, 15, 17, 37 and 41 had relatively high negative IPCA1 scores with above mean grain yield and were hence unstable and adapted specifically to Bako (BK2). Entries such as 1 (check) and 31 had low mean grain yield and close to zero IPCA1 values, indicating consistent, but poor yield performance across optimum environments. The highest positive IPCA1 scores were recorded at Harare (HAR), Chinhoyi (CHI), AM1 and LIO and the highest negative IPCA1 score was also recorded at BK2 under optimum conditions. The smallest positive and negative IPCA1 scores were recorded at Lusaka (LUS), Rattray-Arnold (RAT), Msekera (MSE) and Chitala (CHT) (Table 5.3 and Figure 5.1a). All these environments yielded low compared to the mean of all sites except CHI.

Table 5.1 AMMI analysis of variance for grain yield of 40 QPM hybrids and four commercial checks evaluated under optimum and stressed environments in ESA from 2018 to 2020

(a) Optimum management

Source of variation	Df	SS	MS	Total variation	% G×E interaction	% total G×E interaction
Environment	13	635.39	48.88**	35.8		
Genotype	43	183.02	4.26**	10.3		
E × G	559	956.35	1.71**	53.9		
PC1	55	214.26	3.90**		22.5	22.5
PC2	53	152.77	2.88**		16.0	38.5
PC3	51	123.81	2.43**		13.0	51.5
PC4	49	92.57	1.89**		9.7	61.2
PC5	47	87.80	1.87**		9.2	70.4
PC6	45	53.64	1.19**		5.6	76.1
PC7	43	50.80	1.18*		5.3	81.4
PC8	41	44.88	1.09*		4.7	86.1
Residuals	616	611.85	0.99			

(b) Random drought stress

Source of variation	Df	SS	MS	Total variation	% G×E interaction	% of total G×E interaction
Environment	8	647.54	80.94**	54.0		
Genotype	43	132.73	3.09**	11.1		
E × G	344	418.89	1.22**	34.9		
PC1	50	109.47	2.19**		27.5	27.5
PC2	48	96.87	2.02**		24.4	51.9
PC3	46	53.67	1.17**		13.5	65.4
PC4	44	45.74	1.04*		11.5	76.9
Residuals	396	384.24	0.97			

(c) Managed drought stress

Source of variation	Df	SS	MS	Total variation	% G×E interaction	% of total G×E interaction
Environment	2	35.56	17.78**	20.4		
Genotype	43	48.10	1.12**	27.6		
E × G	86	90.73	1.06**	52.0		
PC1	44	51.30	1.17**		59.2	59.2
PC2	42	35.36	0.84**		40.8	100.0
Residuals	132	71.37	0.54			

(d) Low nitrogen stress

Source of variation	Df	SS	MS	Total variation	% G×E interaction	% of total G×E interaction
Environment	7	607.31	86.76**	69.8		
Genotype	43	66.64	1.55**	7.7		
E × G	301	196.36	0.65**	22.5		
PC1	49	70.07	1.43**		34.6	34.6
PC2	47	51.92	1.10**		25.7	60.3
PC3	45	27.69	0.62**		13.7	74.0
PC4	43	20.29	0.47**		10.0	84.0
PC5	41	13.69	0.33*		6.8	90.8
PC6	39	12.78	0.33*		6.3	97.1
Residuals	352	141.93	0.40			

*P $\leq$ 0.05; **P $\leq$ 0.01; Df = degree of freedom; MS = mean squares; SS = sum of squares; G × E = genotype by environment interaction; PC = principal component

Entries such as 3, 8, 9, 23 and 44 showed high mean grain yield and IPCA1 scores close to zero, indicating that these hybrids were relatively stable and high yielding under random drought stress conditions. Entries 10, 14, 18, 34 and 38 produced high mean grain yield and high positive IPCA1 values, suggesting that these hybrids were unstable and more adapted to the high potential at Bindura (BIN) site (Tables 5.2, 5.3 and Figure 5.1b). Entries like 7, 20, 25, 32 and 43 had low mean grain yield and highly negative IPCA1 values, while checks 2 and 4 and entry 5 also had low mean yield and high positive IPCA1 values. The highest positive IPCA1 score was seen at BIN location, which contributed to the instability of the evaluated hybrids, but the mean grain yield of this location was higher than the average of trials under random drought stress conditions. The lowest IPCA1 values close to zero were observed at Magobo (MAG) and ZamSeed Farm (ZAM) locations and contributed to the stability of hybrids, but the mean grain yield of MAG was very low, while ZAM showed high yield compared to other locations (Table 5.3 and Figure 5.1b).

Among the 44 entries, 7, 19, 35 and 40 showed high mean grain yield and had IPCA1 scores close to zero, indicating relatively stable and wide adaptability under managed drought environments. Entries 4, 6, 16, 26, 33 and 37 had lower mean grain yield than the average of the trial but had IPCA1 scores close to zero. This indicates that these hybrids were stable, but they yielded poorly across environments (Table 5.2 and Figure 5.1c). Entries 8, 10, 15 and 23 showed higher yield than the mean of the trial and had high positive IPCA1 values, while entries 3 (check), 9, 14 and 22 also showed above mean yield, but highly negative IPCA1 scores. These hybrids were considered as unstable and adapted to specific environments. On the other hand, entries 21, 31 and 36 yielded below the grand mean of the trial and had high negative IPCA1 values, indicating that these hybrids were also unstable (Figure 5.1c). Chisumbanje (CHI) showed high negative IPCA1 while Chokwe (CHO) and Makoholi (MAK) locations showed positive IPCA1 scores under managed drought environments (Table 5.3).

Across low N environments, entry 3 (PAN53), 11 (CZH15098Q) and 14 (CZH15142Q) showed above trial average yield and had low IPCA1 values, indicating that these hybrids were relatively more stable and adaptable to all low N environments (Table 5.2 and Figure 5.1d). Entries 23, 30, 32, 33 and 36 showed low mean grain yield and IPCA1values close to zero, indicating that these varieties were also stable (Figure 5.1d). Entries 7, 10, 27 and 34 had high positive and negative IPCA1 values and were therefore unstable and adapted to specific environments under low N conditions. Across low N environments, AM1 and AREX sites

showed relatively high IPCA1 values while HAR, BK2 and BK1 and AM2 had low IPCA1 scores (Table 5.3).

5.3.3 AMMI stability values

AMMI stability values showed variation in grain yield stability among the 44 hybrids under different management conditions (Table 5.2). Stable genotypes have ASV values close to zero (Purchase, 1997; 2000). Across optimum conditions, entries 1 (ZS261) check, 6 (CZH132044Q), 20 (CZH17189Q), 24 (CZH17193Q), 35 (CZH17204Q), 14 (CZH15142Q) and 28 (CZH17197Q) had the lowest ASV values and are considered as more stable hybrids. Among these entries, 6, 14, 20 and 24 showed above overall trial mean grain yield while entries 1 and 35 showed the lowest mean yield. Across random drought stress trials entries 37 (CZH17206Q), 12 (CZH15099Q), 8 (CZH142236Q) and 23 (CZH17192Q) had relatively lower ASV values and high mean grain yield and these hybrids were considered more stable and widely adaptable across random drought stress environments. On the other hand, entries 3 (PAN53), 9 (CZH142237Q) and 44 (QS764638) were top yielding, but with the high ASV values. These hybrids were considered unstable but adapted to specific environments under random drought stress conditions.

Entries 16 (CZH16022Q), 26 (CZH17195Q), 40 (CZH17209Q), 33 (CZH17202Q), 7 (CZH132018Q), 6 (CZH132044Q) and 24 (CZH17193Q) showed the lowest ASV values compared to the remaining entries, indicating these were relatively more stable under managed drought environments. Entry 40 was also both stable and a widely adaptable hybrid under managed drought environments. Entries 9 (CZH142237Q) and 10 (CZH142238Q) exhibited the highest ASV values, but were top yielding hybrids, which indicates that these hybrids were unstable, but could adapt to specific environments under managed drought environments. Across low N environments, entries 30 (CZH17199Q), 42 (MH1633), 39 (CZH17208Q), 32 (CZH17201Q) and 37 (CZH17206Q) showed relatively low ASV values and mean yield below the average mean of the trial, except entries 42 and 32 that had comparable grain yield with the average mean of the trial, whereas entries 3 (PAN53), 14 (CZH15142Q), 11 (CZH15098Q) and 34 (CZH17203Q) had relatively high ASV values and showed high mean grain yield. This indicates that these hybrids were adapted to specific testing environments under low N stress conditions.

Table 5.2 Mean grain yield and IPCA scores and ASV values of 40 QPM hybrids and four commercial checks evaluated in optimum and stressed environments in ESA from 2018 to 2020

Entry no.	Hybrids	Optimum				Random drought stress				Managed drought				Low N stress			
		GY	IPCA1	IPCA2	ASV	GY	IPCA1	IPCA2	ASV	GY	IPCA1	IPCA2	ASV	GY	IPCA1	IPCA2	ASV
1	ZS261	7.3	-0.01	-0.02	0.02	3.2	-0.24	-0.32	0.42	2.6	-0.16	0.41	0.47	2.2	-0.28	0.32	0.50
2	Mama MQ623	7.8	-0.31	-0.38	0.57	4.1	0.30	-0.25	0.42	2.8	0.42	0.16	0.63	2.8	-0.42	0.22	0.61
3	PAN53	8.8	0.50	0.36	0.79	4.9	-0.12	-0.28	0.31	3.0	-0.61	0.12	0.90	2.8	0.08	-1.00	1.01
4	SC533	7.6	0.25	-0.31	0.47	3.5	0.37	-0.29	0.50	2.1	-0.14	-0.39	0.44	2.1	0.34	-0.51	0.69
5	CZH04032	7.8	0.18	-1.00	1.03	3.7	0.22	0.03	0.25	2.6	0.36	0.22	0.57	2.4	0.17	0.78	0.81
6	CZH132044Q	8.7	-0.01	0.19	0.19	4.5	0.05	-0.39	0.40	2.3	-0.15	-0.06	0.23	2.6	0.39	-0.06	0.52
7	CZH132018Q	8.2	-0.20	0.12	0.30	4.1	-0.50	-0.26	0.62	3.0	0.09	-0.17	0.21	2.9	-0.84	0.41	1.21
8	CZH142236Q	8.4	0.19	0.19	0.33	4.7	0.15	0.09	0.19	3.1	0.81	0.48	1.27	2.2	0.54	-0.12	0.74
9	CZH142237Q	8.8	-0.22	-0.09	0.32	4.8	0.16	0.79	0.81	3.6	-0.81	-0.12	1.18	2.6	0.30	-0.14	0.42
10	CZH142238Q	8.7	-0.19	0.84	0.88	4.8	0.62	-0.01	0.70	3.5	0.69	0.13	1.01	2.6	0.63	-0.18	0.87
11	CZH15098Q	8.6	-0.74	0.15	1.05	4.4	-0.50	-0.66	0.86	2.6	0.08	0.31	0.33	3.0	-0.29	0.29	0.49
12	CZH15099Q	8.9	0.43	0.41	0.73	4.5	-0.01	0.18	0.18	3.2	-0.27	-0.17	0.43	2.6	0.32	0.02	0.43
13	CZH15117Q	8.5	0.46	-0.08	0.65	4.4	-0.60	-0.61	0.91	3.0	-0.30	0.41	0.60	2.6	-0.26	0.52	0.63
14	CZH15142Q	8.7	0.11	0.13	0.20	5.3	0.77	-0.20	0.89	3.2	-0.49	-0.14	0.72	3.2	-0.29	0.51	0.64
15	CZH16006Q	8.5	-0.68	0.38	1.02	4.2	-0.27	0.15	0.34	2.8	0.77	-1.00	1.49	2.3	-0.30	0.42	0.59
16	CZH16022Q	8.4	0.52	-0.47	0.87	4.6	0.23	-0.32	0.41	2.3	-0.01	-0.07	0.07	2.6	0.32	-0.17	0.47
17	CZH16021Q	8.8	-0.41	-0.56	0.80	4.5	-0.23	0.43	0.50	2.5	0.18	0.27	0.37	2.5	-0.38	0.06	0.52
18	CZH17187Q	8.1	0.13	0.22	0.28	4.6	0.65	0.20	0.76	2.3	-0.21	0.02	0.30	2.1	0.86	0.12	1.17
19	CZH17188Q	8.1	-0.13	-0.50	0.54	4.6	-0.17	0.90	0.92	3.0	-0.01	0.68	0.68	2.4	0.43	0.02	0.59
20	CZH17189Q	8.7	0.01	-0.09	0.09	4.0	-0.66	0.29	0.81	2.7	0.29	0.35	0.54	2.4	-0.22	0.24	0.38
21	CZH17190Q	7.9	0.25	-0.33	0.48	3.8	-0.48	0.60	0.81	2.5	-0.37	0.37	0.65	2.1	0.34	0.57	0.73
22	CZH17191Q	8.4	0.37	0.23	0.56	4.3	-0.10	0.42	0.43	3.1	-0.63	-0.04	0.92	2.3	0.28	0.08	0.39
23	CZH17192Q	8.6	0.40	-0.19	0.59	4.9	-0.06	-0.05	0.09	3.3	0.67	-0.54	1.11	2.2	-0.03	0.47	0.47
24	CZH17193Q	8.6	-0.07	0.09	0.13	4.4	0.68	0.10	0.77	2.2	0.18	-0.10	0.28	2.0	0.46	0.15	0.63
25	CZH17194Q	7.9	-0.20	0.50	0.57	3.9	-0.51	0.34	0.67	1.8	0.28	-0.42	0.58	2.5	-0.35	-0.38	0.60
26	CZH17195Q	7.7	0.29	0.03	0.41	4.1	0.04	-0.03	0.05	1.9	-0.05	-0.11	0.13	2.1	0.16	-0.59	0.63
27	CZH17196Q	8.2	-0.43	0.09	0.61	3.8	-0.38	-0.18	0.46	1.9	-0.31	-0.66	0.80	2.8	-0.58	-0.38	0.88
28	CZH17197Q	8.3	0.11	-0.03	0.16	4.2	0.05	-0.59	0.60	2.8	0.38	-0.35	0.65	1.9	0.31	0.15	0.44
29	CZH17198Q	8.4	-0.26	0.01	0.37	4.3	0.09	-0.48	0.49	2.7	-0.06	-0.36	0.37	2.6	-0.87	-0.72	1.37
30	CZH17199Q	8.1	0.26	-0.15	0.40	4.2	0.10	-0.25	0.28	2.5	0.18	0.33	0.42	2.0	-0.12	0.11	0.19
31	CZH17200Q	8.0	0.01	-0.74	0.74	4.1	-0.02	0.09	0.10	2.3	-0.54	-0.65	1.02	2.1	0.27	-0.01	0.37
32	CZH17201Q	7.8	0.36	0.32	0.60	4.2	-0.83	-0.19	0.95	2.3	0.21	-0.46	0.55	2.4	0.04	-0.25	0.26
33	CZH17202Q	8.3	-0.53	0.03	0.74	3.8	-0.49	0.29	0.62	2.5	0.06	0.12	0.15	2.2	0.02	-0.53	0.53
34	CZH17203Q	8.5	0.75	0.17	1.07	4.7	0.79	-0.17	0.91	2.3	-0.27	0.02	0.39	3.0	-0.36	-0.44	0.66
35	CZH17204Q	8.1	-0.10	-0.02	0.14	4.2	0.07	1.00	1.00	3.1	0.14	0.38	0.44	2.5	-0.24	0.15	0.35
36	CZH17205Q	8.1	-0.37	-0.25	0.57	4.5	0.43	-0.54	0.73	2.4	-0.46	-0.06	0.67	1.9	0.09	0.56	0.57
37	CZH17206Q	8.5	-0.75	-0.18	1.06	4.5	-0.08	0.17	0.19	2.4	-0.02	0.37	0.37	2.2	-0.21	0.13	0.32
38	CZH17207Q	8.7	-0.21	0.30	0.42	4.7	0.57	0.60	0.88	2.8	-0.47	0.02	0.68	2.2	0.59	-0.09	0.81
39	CZH17208Q	7.8	0.45	0.33	0.71	4.6	0.61	0.26	0.74	2.4	0.32	0.16	0.50	2.0	0.13	-0.18	0.25
40	CZH17209Q	7.8	-0.28	0.19	0.43	4.1	0.09	0.05	0.12	3.3	0.08	0.09	0.15	2.4	0.17	-0.40	0.46
41	CZH17210Q	8.4	-0.73	0.11	1.03	4.0	-0.24	0.00	0.28	2.7	-0.21	-0.19	0.37	2.0	0.35	0.04	0.47
42	MH1633	7.9	0.32	0.16	0.48	4.3	0.23	-0.53	0.59	2.7	0.35	0.22	0.56	2.4	-0.17	0.00	0.23
43	MH1634	8.3	0.13	-0.40	0.44	4.0	-0.60	-0.05	0.68	2.6	0.28	0.06	0.41	2.7	-0.52	0.26	0.75
44	QS7646	8.9	0.32	0.24	0.51	4.9	-0.19	-0.32	0.39	2.8	-0.26	0.34	0.50	2.5	-0.82	-0.44	1.19

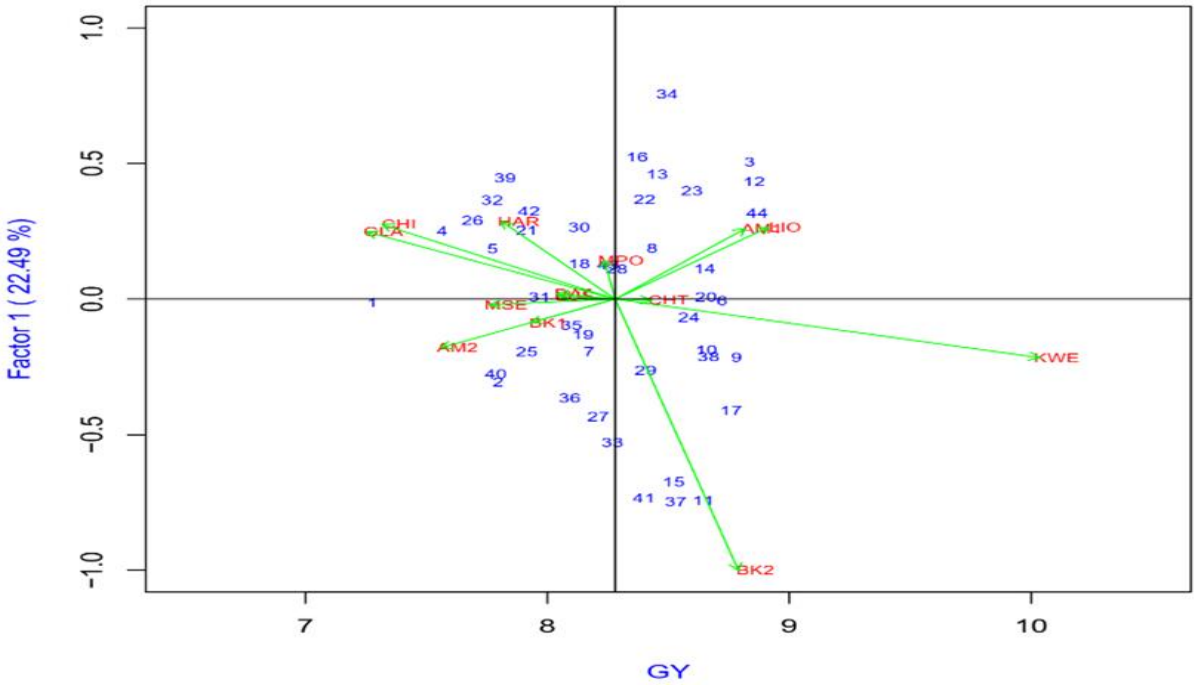
GY (t ha^{-1}) = mean grain yield ton per hectare; ASV= additive main effects and multiplicative interaction stability value; IPCA = interaction of principal component analyse

Table 5.3 Mean grain yield and IPCA scores of environments evaluated in optimum and stressed environments in ESA from 2018 to 2020

(a) Optimum management					
Environment	Code	GY	1PCA1	IPCA2	IPCA3
Ambo 2018/19	AM1	8.9	0.26	-0.31	-0.16
Ambo 2019/20	AM2	7.6	-0.18	0.03	-0.51
Bako 2018/19	BK1	8.0	-0.09	0.42	0.17
Bako 2018/20	BK2	8.9	-1.00	-0.25	-0.05
Chinhoyi	CHI	7.4	0.28	0.41	-0.31
Chitala	CHT	8.5	0.00	-0.25	0.27
Glandale	GLA	7.3	0.25	-0.37	0.54
Harare	HAR	7.9	0.29	0.10	-0.20
Kwekwe	KWE	10.1	-0.22	0.63	0.45
Lionsden	LIO	9.0	0.27	-0.15	0.00
Lusaka	LUS	8.1	0.01	0.10	-0.02
Mpongwe	MPO	8.3	0.14	-0.06	0.16
Msekera	MSE	7.8	-0.02	-0.30	-0.08
Rattray-Arnold	RAT	8.1	0.02	-0.01	-0.25
(b) Random drought stress					
Environment	Code	GY	IPCA1	IPCA2	IPCA3
Bindura	BIN	5.2	1.00	0.36	-0.01
ETG Farm	ETG	3.6	-0.33	0.03	-0.15
Gwebi	GWB	5.2	-0.30	-0.05	0.14
Gweru	GWR	3.5	-0.20	-0.11	0.18
Lichinga	LIC	4.9	-0.26	-0.17	0.26
Magobo	MAG	2.6	-0.06	-0.15	-0.15
Meru	MER	4.2	0.35	-0.85	-0.28
Umbuluzi	UMB	5.4	-0.26	0.62	-0.62
ZamSeed Farm	ZAM	4.4	0.05	0.32	0.64
(c) Managed drought					
Envronment	Code	GY	IPCA1	IPCA2	IPCA3
Chisumbanje	CHS	2.9	0.29	0.90	-0.00
Chokwe	CHO	3.0	-1.00	-0.22	-0.00
Makoholi	MAK	2.2	0.71	-0.68	-0.00
(d) Low N stress					
Environment	Code	GY	IPCA1	IPCA2	IPCA3
Ambo 2018/19	AM1	3.5	-1.00	0.27	0.15
Ambo 2018/20	AM2	2.6	0.08	0.04	0.14
AREX	AREX	1.5	0.39	0.12	0.07
Bako 2018/19	BK1	1.4	0.11	0.14	0.08
Bako 2019/20	BK2	2.7	0.01	0.16	-0.83
Gwebi	GWB	2.1	0.32	0.11	0.19
Harare	HAR	4.0	-0.13	-0.99	-0.02
Rattray	RAT	1.3	0.21	0.16	0.23

GY (t ha^{-1}) = grain yield, ton per hectare; IPCA = interaction of principal component analyses

(a) Optimum management



(b) Random drought stress

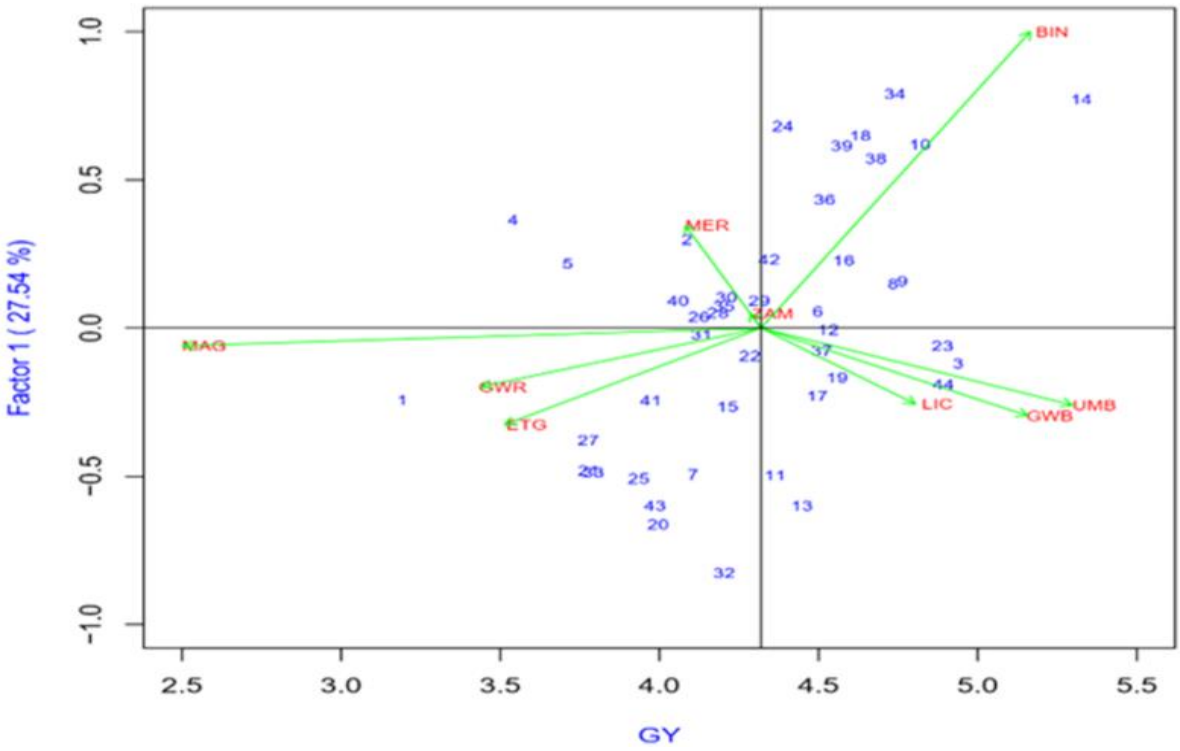
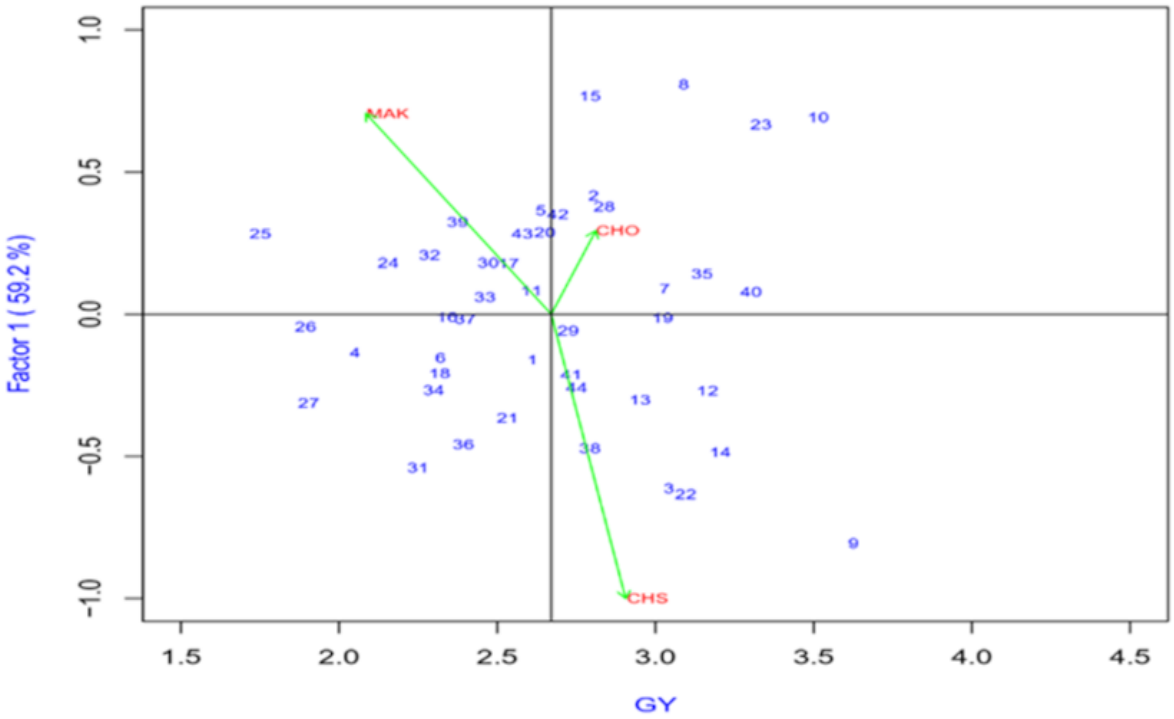


Figure 5.1 Grain yield (main effect) and PC1 biplot of 40 QPM hybrids and four commercial checks evaluated under (a) optimum management, (b) random drought stress, (c) managed drought stress and (d) low nitrogen stress. Environment codes are explained in Table 5.3.

(c) Managed drought



(d)Low nitrogen stress

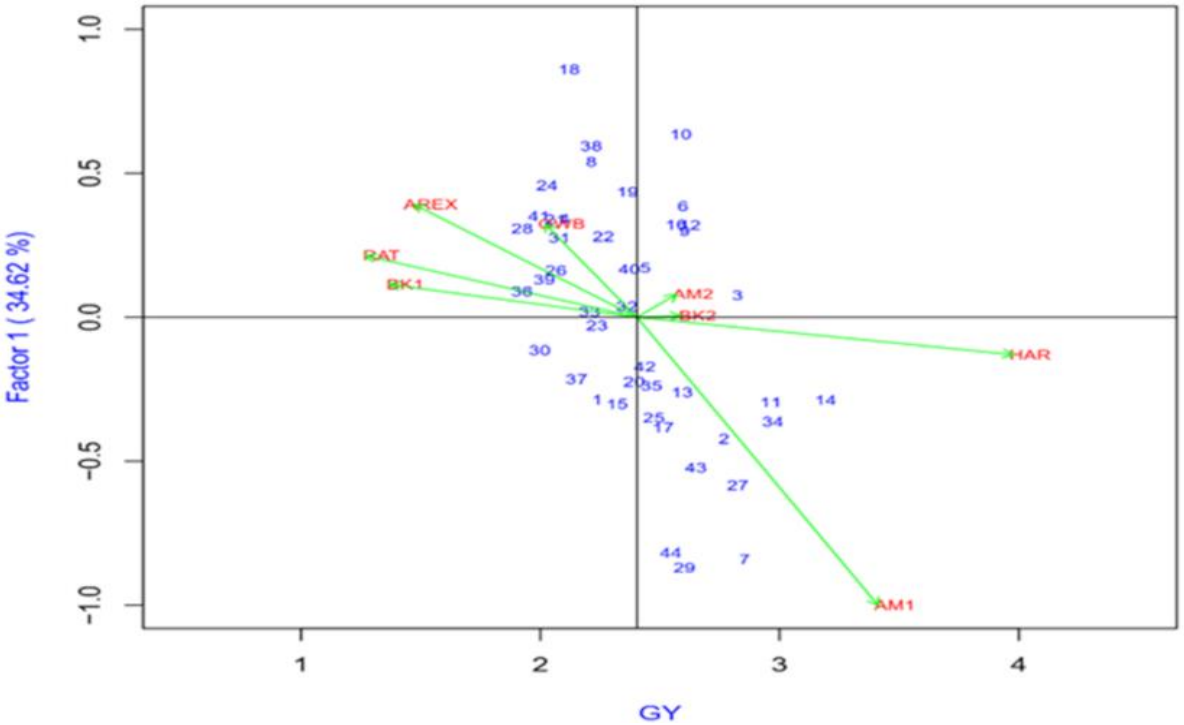


Figure 5.1 Continued

5.3.4 GGE biplot analysis

GGE biplot analysis for grain yield for 40 QPM hybrids and four checks are presented in Figures 5.2a to 5.2d. The first two principal axis explained 39.74% (PC1 = 20.98% and PC2 = 18.76%) under optimum, 49.41% (PC1 = 30.85 and 18.56%) under random drought stress, 74.86% (PC1 = 41.95 and PC2 = 32.91%) under managed drought and 59.15% (PC1 = 39.11 and PC2 = 20.15%) under low nitrogen of the total G × E interaction variations.

5.3.4.1 Which won where pattern

The which-won-where polygon view of GGE biplots in Figures 5.2a to 5.2d showed which genotypes performed well at which site. The line that starts from the origin of the biplot and passes perpendicular to the side of the polygon was divided into different sectors. Under optimum conditions, ten sectors; under random drought stress, seven sectors; under managed drought, six sectors and under low N conditions six sectors were formed (Figures 5.2a to 5.2d). Hybrids that formed the vertex of the polygon were the highest yielding in corresponding environments within each sector. On the other hand, hybrids located far away from all the test environments were considered as poor yielding at each testing sites. The vertex entries 15, 41 and 12 were the highest yielding hybrids at AM2, BK1 and CHI sites, respectively, whereas entries 1, 4, 5 and 34 were low yielding under optimum conditions (Figure 5.2a).

Entries 14 and 19 were on the corners of the polygon and the highest yielding at BIN and MER, and UMB testing sites, respectively, under random drought stress conditions (Figure 5.2b). Similarly, entries 9 and 10 were top yielding hybrids at CHI, and MAK and CHO respectively, while hybrids 31, 27, 25 and 15 were on the lower yielding vertex of the polygon under managed drought conditions (Figure 5.2c). Under low N conditions, entries 3, 7 and 34 were top yielding hybrids at the corresponding sites of HAR, AM1 and BK2, respectively, whereas entries 18 and 36 were on the low yielding vertex of the polygon (Figure 5.2d). Entries like 23, 7,18,43, 35 and 25 under optimum; 12, 31 and 24 under random drought; 33, 37, 29 and 11 under managed drought; 5, 40 and 35 under low N stress were relatively closer to the origin of the biplot and considered as more stable than entries far from the origin, in either positive or negative direction.

A strong correlation exists between hybrids and testing sites that fall in the same sector. Under optimum conditions, entries like 15, 38 and 9 at AM2, entries 41, 11, 17 and 37 at BK2 testing sites showed better performance than other hybrids. Most of the testing sites, such as RAT,

MPO, LUS, LIO, AM1, BK1, GLA HAR, KWE and CHI were found in one sector. Hybrids well adapted to these sites included entries 3, 12, 10, 14, 44 and 23 (Figure 5.2a). Under random drought stress, testing sites fell only in three sectors and formed three mega environments with different winner hybrids (Figure 5.2b). Including the vertex hybrid, more than 15 hybrids showed good performance at BIN, MER, GWB, LIC, MAG and GWE testing sites. UMB and ETG fell in another sector and entries 9, 17 and 19 showed good performance at these testing sites. Under managed drought, most of the hybrids were found around the origin of the biplot and were considered stable (Figure 5.2c). All testing sites fell in two of the six sectors. MAK and CHO were found in one sector and entries 10, 8, 23, 7, 35 and 40 exhibited the best grain yield performance at these sites. CHI was alone in one sector and more than ten hybrids, including the vertex hybrid, were adapted to this site. Under low N conditions, HAR, AREX, and GWB were found in one sector and entries 3, 10, 12, 9 and 32 were more adapted to these testing sites (Figure 5.2d). In the second sector only entry 34 was found and this hybrid was well adapted to BK2 and RAT sites, whereas more than 12 hybrids were well adapted to AM1, AM2 and BK1 sites.

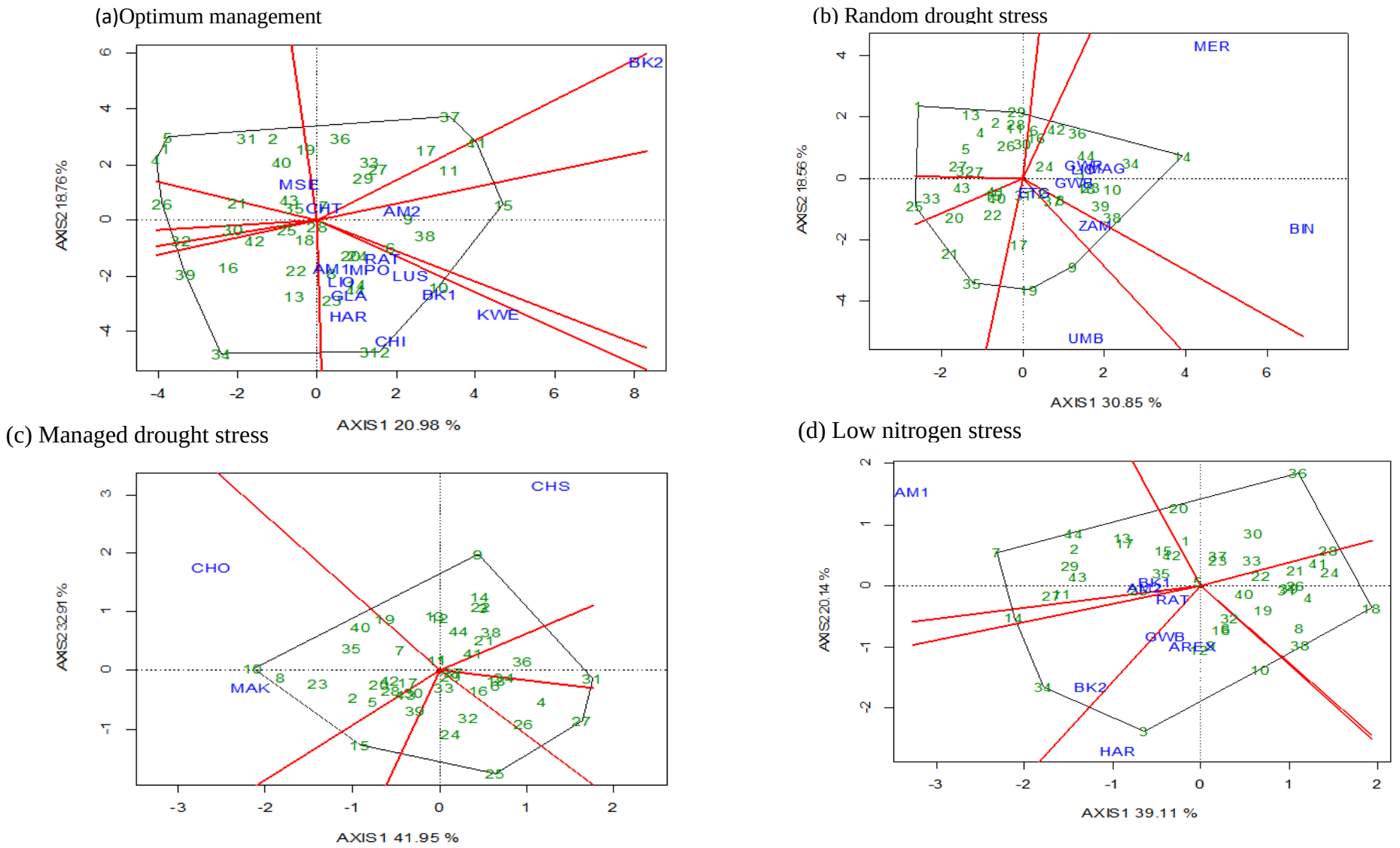


Figure 5.2 "Which won where" polygon view of the GGE biplot for 40 QPM hybrids and four standard checks evaluated across a) optimum management, b) random drought stress, c) managed drought stress and d) low nitrogen stress. Environment codes are explained in Table 5.3

5.3.4.2 Grain yield stability under different growing conditions

Average environment coordination (AEC) view of the GGE biplot was drawn to see the stability and adaptability of hybrids evaluated under optimum, random drought, managed drought, and low N conditions (Figures 5.3a to 5.3d). The axis of the AEC ordinate is a line that passes through the origin of biplot and is perpendicular to the AEC abscissa to separate genotypes that had below average grain yield from those showing higher grain yields. The hybrids were ranked along the average environment axis with the arrow pointing to a higher value based on mean performance across all testing environments. The top yielding hybrids according to their projections onto the AEC, in descending order, were 10, 12, 3, 15, 38 and 6 under optimum (Figure 5.2a), 14, 34, 38, 10, 39, 23, 9 and 3 under random drought stress (Figure 5. 2b), 9, 10, 19,40, 8 and 35 under managed drought (Figure 4.2c) and 34, 14, 3, 7, 27 and 1 under low N (Figure 5.2d) conditions. Among these top yielding hybrids, 3, 12 and 15 under optimum, 14 and 9 under random drought stress, 9, 8 and 10 under managed drought and 3 and 7 under low N conditions had large projections onto the AEC abscissa, hence these hybrids were unstable and might be adapted to specific environments. Entries like 10, 14, 44, 6, 9, 38 and 20 under optimum, 38, 23, 10, 3 (check) and 39 under random drought, 19, 40, 35, 13 and 12 under managed drought and 34, 14, 1 (check), 23 and 35 under low N environments had above average grain yield, located on the AEC abscissa with low projections, hence these hybrids were considered as stable and might have wide adaptability across corresponding environments. Among checks, entry 3 (PAN53) was the highest yielding normal maize hybrid in this study but it was not stable under all management conditions except under random drought stress. In contrast, entries 1 (ZS261) and 4 (SC533) were the lowest yielding and most stable QPM and normal maize checks, respectively under most conditions.

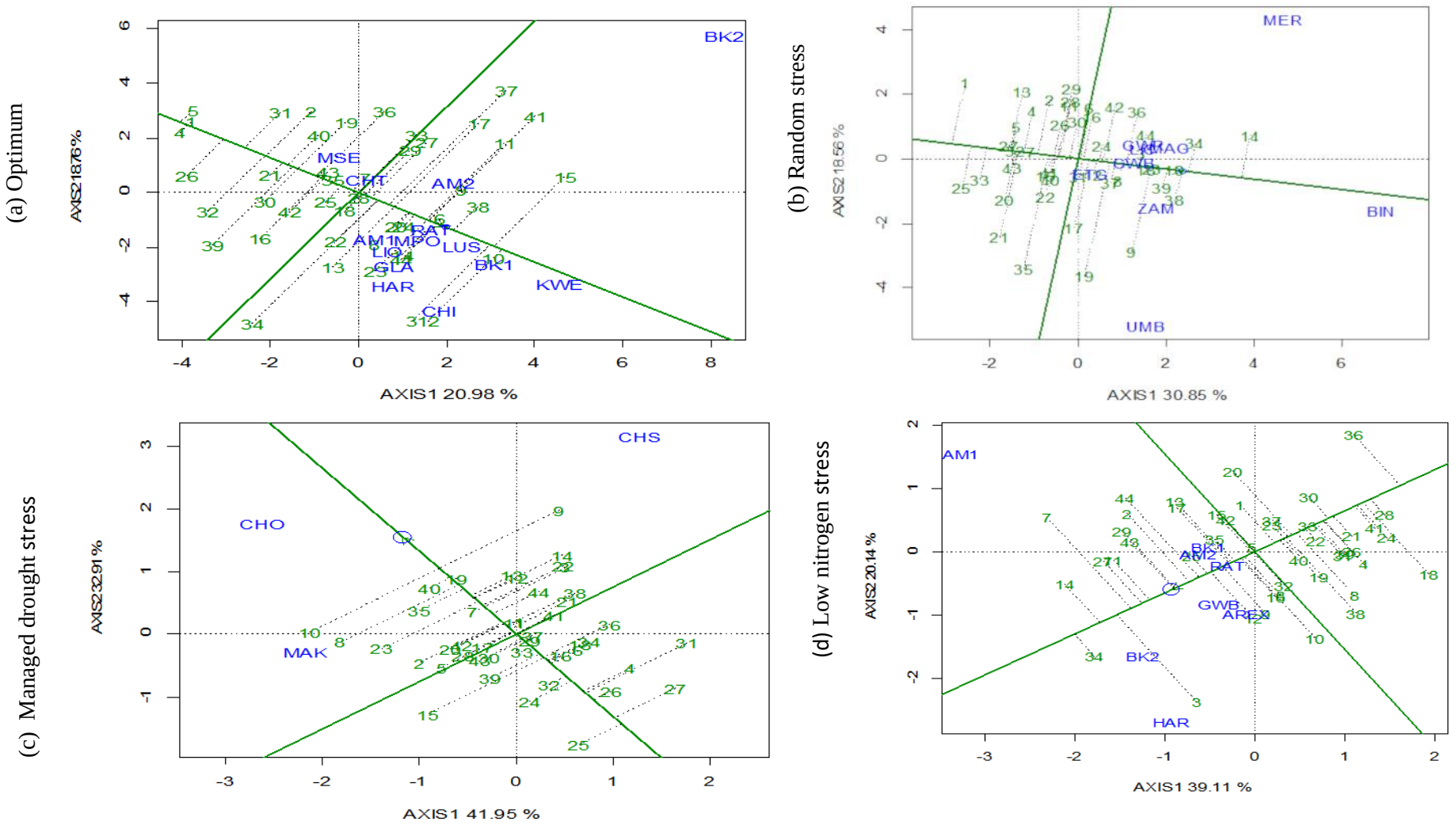


Figure 5.3 Mean vs. stability view of GGE biplot based on grain yield of 40 new QPM hybrids and four standard checkes evaluated across a) optimum management, b) random drought stress, c) managed drought stress and d) low nitrogen stress. Environment codes are explained in Table 5.3.

5.3.4.3 Genotype ranking

The small circle on the AEC axis, with an arrow pointing to it and drawn based on the genotype-focused biplot, represents the ideal hybrid. In this study, entry 10 under optimum (Figure 5.4a), entry 14 and 10 under random drought (Figure 5.4b), entries 19 and 40 under managed drought (Figure 5.4c), and entries 14 and 34 under low N (Figure 5.4d) stress conditions were the most ideal hybrids. Contrary to this, entries 1 (ZS261), QPM check 4 (normal maize check) and 5 were the least ideal genotypes under optimum and random drought conditions and entries 25 and 36 were the least ideal hybrids under managed drought and low N conditions, respectively.

5.3.4.4. Representativeness, discriminating ability and association among testing sites

The AEC axis indicated the most representative and discriminating testing environments among the testing sites (Figures 5.5a to 5.5d). A test environment with a small angle with the AEC axis is more representative than other environments and a longer vector length of the environment is considered as more discriminative (Yan and Tinker, 2006). In this study, BK2 was the most discriminating, but it was the least representative environment under optimum conditions, whereas KWE, BK1 and CHI were the most representative and discriminating environments (Figure 5.5a). In contrast, MSE and CHT were non-representative and non-discriminating environments (Figure 5.5a). Under random drought stress, LIC was the most representative and non-discriminating environment. UMB and MER were the most discriminating and non-representative environments (Figure 5.5b). Under managed drought, CHO was the most representative, while CHI was the most discriminating testing site (Figure 5.5c). Under low N conditions, RAT was the most representative and the least discriminating environment, whereas HAR was the most discriminating, while AM1 was also a representative and non-discriminating environment (Figure 5.5d).

The angle between two environments shows the relationship among testing environments and this was visualized by vectors drawn by a dotted line that connects each testing site with the biplot origin (Figures 5.5a - 5.5d). An angle between two environments of $< 90^{o}$ indicates a strong positive correlation between them, whereas an angle of equal to 90^{o} indicates no correlation between them. A degree angle of $> 90^{0}$ and $< 270^{o}$, indicates negative correlation between the environments (Yang et al., 2009). Under optimum conditions, testing sites such as KWE, RAT, LUS, MPO, BK1, AM1 showed strong positive association because they had a less than 90^{o} angle among them, whereas BK2 and HAR had a negative association ($> 90^{o}$ angle).

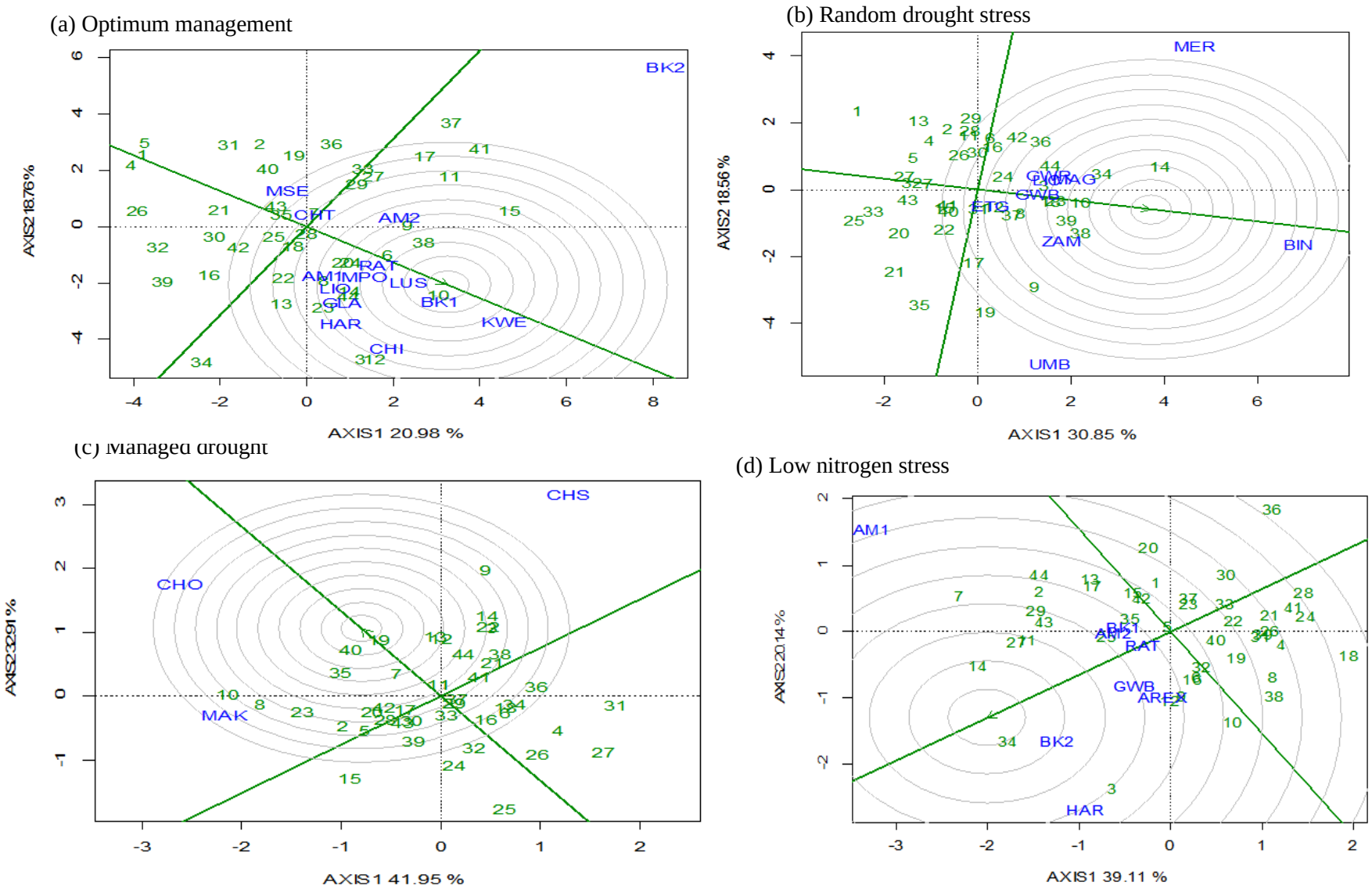


Figure 5.4 GGE biplot view showing ranking of hybrids based on grain yield of 40 new QPM hybrids and four standard checkes evaluated across a) optimum management, b) random drought, c) managed drought and d) low N environments. Environment codes are explained in Table 5.3.

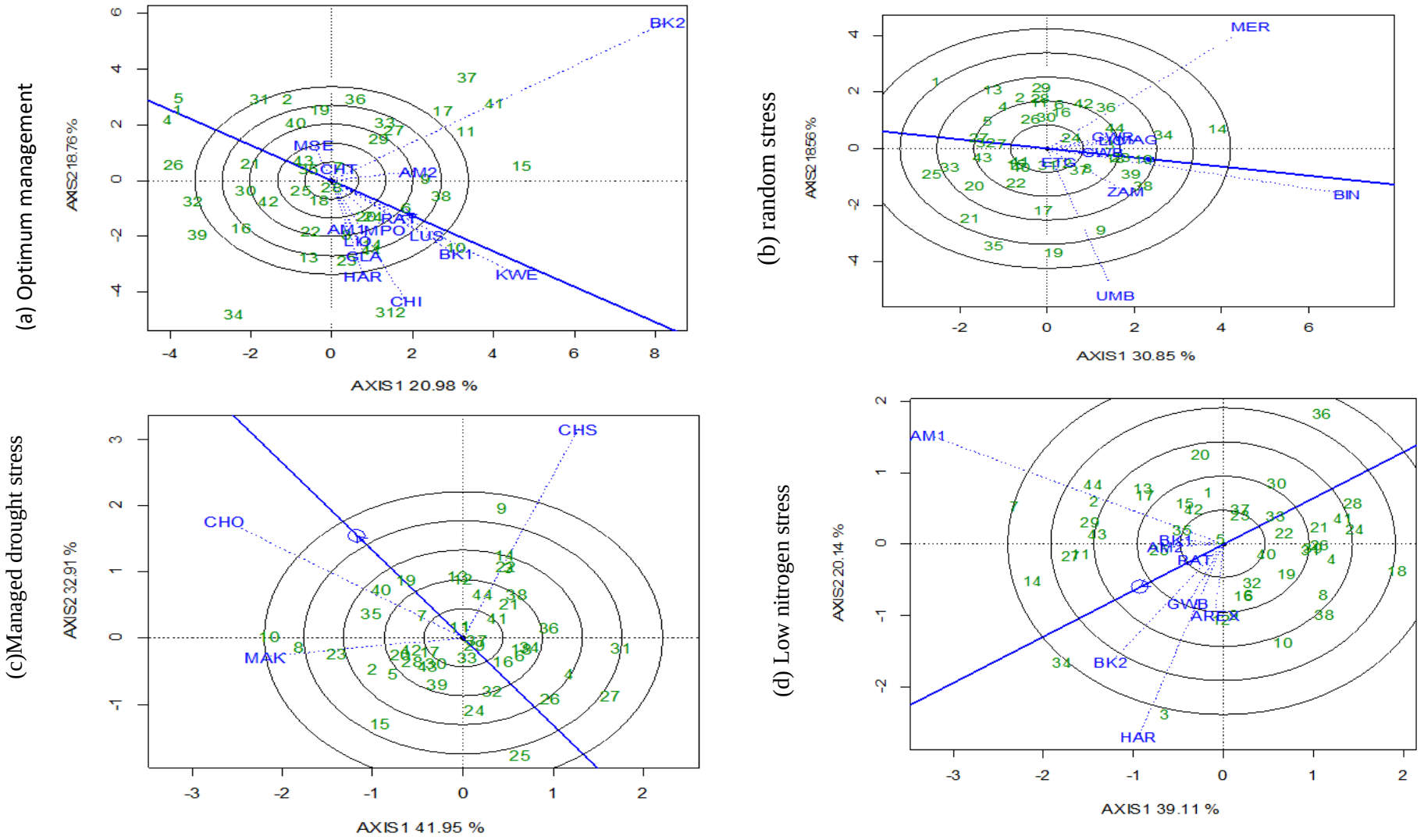


Figure 5.5 Discriminativeness vs. representativeness view of the GGE biplots based on grain yield of 40 new QPM hybrids and four standard checks evaluated across a) optimum management, b) random drought stress, c) managed drought stress and d) low nitrogen stres. Environment codes are explained in Table 5.3.

Under random drought stress, MER and UMB showed negative association, while strong positive association was observed among LIC, BIN, MAG, GWB, GWE testing sites. Across managed drought testing sites, CHO and MAK showed positive association while CHI with MAK showed negative association. No correlation was observed between CHI and CHO sites. Under low N environments, BK2, GWB, AREX and HAR sites had strong positive association, whereas negative association was observed between AM1 with BK2 and AM1 with HAR. Testing sites which had strong negative association indicate the presence of high G × E interaction. In this study Ethiopian sites Bako and Ambo were included for two seasons under both optimum and low N environmental conditions. Both Bako and Ambo sites showed positive association during 2018/19 and 2019/20 under optimum and low N conditions (Figures 5.5a and 5.5d).

5.4 Discussion

The results of the AMMI ANOVA indicated the presence of differing grain yield performance among new QPM hybrids across testing sites under non-stressed and stressed conditions. Significant environmental variation and G × E interactions reflected the diversity of the testing environments and the reaction of the hybrids to this. This is consistent with previous results reported for QPM hybrids evaluated under non-stressed and stressed conditions (Setimela et al., 2017a; Mebratu et al., 2019). Abakemal et al. (2016) also reported the existence of high environmental effects on the performance of QPM hybrids adapted to transitional highland agro ecologies. G × E interaction was partitioned into eight significant IPCAs for optimum conditions, four for random drought, two for managed drought and six for low N environments.

In this study, entries 6 (CZH132044Q), 14 (CZH15142Q), 20 (CZH17189Q) and 24 (CZH17193Q) under optimum, 23 (CZH17192Q), under random drought stress, 40 (CZH17209Q) under managed drought and local check, entry 3 (PAN53) under low N stress were identified based on high mean grain yield, lowest IPCA1 scores and ASV values. This indicated that these hybrids showed better stability than the rest of the hybrids. Several authors also reported the presence of high mean yield and stability of QPM hybrids (Abakemal et al., 2016; Setimela et al., 2017a; Mebratu et al., 2019), of normal maize hybrids (Sserumaga et al., 2016; Ertiro et al., 2017; Wolde et al., 2018) and of PVA genotypes (Mengesha et al., 2019). Badu-Apraku et al. (2017) also identified high-yielding and stable early and extra early maize genotypes that were evaluated across multiple stress environments.

The GGE biplot provided a polygon view to identify best performing genotypes in different testing environments by dividing the biplot into different sectors (Mebratu et al., 2019). The line that starts from the origin of the biplot and passes perpendicular to the side of the polygon divided the polygon into different sectors. A group of testing environments found within one sector are seen as one mega-environment. On the other hand, environments that fall in different sectors are in different mega environments. Each sector has different wining genotypes (Yan and Tinker, 2006) indicating the presence of crossover (G × E interaction). Under optimum conditions, the polygon was divided into ten sectors, but testing sites fell into three of them that were represented by BK2, AM2 and CHI with winner hybrids 41 (CZH17210Q), 15 (CZH16006Q) and 12 (CZH15099Q), respectively. Under random drought stress, the polygon was divided into seven sectors, but the testing sites fell only into two of them that were represented by MER and UMB sites, with winner hybrids 14 (CZH15142Q) and 19 (CZH17188Q), respectively. Similarly, under managed drought, the polygon was divided into six sectors, but the testing sites fell into two of them that were represented by CHO and CHI with winner hybrids 10 (CZH142238Q) and 9 (CZH142237Q), respectively. Under low N conditions, the polygon was also divided into six sectors, but the testing sites fell into three of them that were represented by AM1, BK2, HAR each with winner hybrids 7 (CZH132018Q), 34 (CZH17203Q) and 3 (PAN53), respectively. This kind of information is important to recommend stable and widely adaptable genotypes for multi-environments and can identify specifically adapted genotypes for specific environments (Abakemal et al., 2016; Tapera, 2017; Wolde et al., 2018; Mebratu et al., 2019). On the other hand, genotypes that were placed far away from all the test environments indicated that they were poor yielding at these testing environments (Makumbi et al., 2015; Wolde et al., 2018). In this study, hybrids 5 (CZH04032), 4 (SC533), and 34 (CZH17203Q) under optimum, 1 (ZS261), 25 (CZH17194Q) and 35 (CZH17204Q) under random drought, 25 (CZH17194Q), 27 (CZH17196Q) and 31(CZH17200Q) under managed drought and 36 (CZH17205Q) and 18 (CZH17187Q) under low N stress were poor performing hybrids. These results are consistent with the finding of several researchers who reported on grouping of testing sites into different mega-environments by using AMMI and GGE biplots (Abakemal et al., 2016; Sserumaga et al., 2016; Setimela et al., 2017b; Mebratu et al., 2019)

Based on genotype ranking GGE biplot analysis, genotypes that were closest to the concentric circle were considered as ideal genotypes that have both high mean grain yield and are stable in multi-environments (Yan and Kang, 2003; Yan and Tinker, 2006; Wolde et al., 2018). As

indicated in Figures 5.4a to 5.4d, the most desirable hybrids closest to the ideal were 10 (CZH142238Q) under optimum, 14 (CZH15142Q) under random drought, 19 (CZH17188Q) and 40 (CZH17209Q) under managed drought, 34 (CZH17203Q) and 14 (CZH15142Q) under low N conditions. In addition to this, entries 10 and 14 showed good yield performance across non-stressed and stressed environments, indicating that these hybrids had inherently high yielding potential and wide adaptability across different management conditions. Entry 1 (ZS261), the QPM check, was the least ideal genotype in optimum and random drought environments compared with experimental QPM hybrids, indicating that this hybrid was inherently low yielding and poorly adapted to some or all the testing sites, which is similar with results reported by Mebratu et al. (2019).

The vector length drawn from the origin of biplot to the marker of the environment and the angle between the AEC abscissa and vector that connects the environments with the biplot origin are important to measure its discriminating ability and representativeness of the environments (Yan, 2001; Yang et al., 2009). A test environment that has a small angle with the AEC is more representative, whereas a long vector length from the biplot origin is considered as a more discriminative environment (Yan, 2001; Yan and Tinker, 2006). Environments that had both a long vector and a small angle with the AEC abscissa are more discriminating and representative environments and are considered "ideal testing sites" and these are important to identify desirable hybrids (Mebratu et al., 2019). The most representative environments were KWE and BK1 under optimum conditions, LIC under random drought, CHO under managed drought and RAT under low N stress, whereas BK2, UMB, CHI and AM1 were the most discriminating environment under optimum, random drought, managed drought, and low N environments, respectively. Similarly, KWE under optimum conditions, BIN under random drought, CHO under managed drought and BK2 under low N stress were both discriminating and representative environments, hence, these sites were recognized as ideal environments in the respective management conditions. An environment which has the ability to discriminate the genotypes and is representative of the target environment is considered as the ideal environment (Yan and Tinker, 2006). So these test environments can be used to select the top yielding hybrids that will perform consistently across environments (Tolessa and Gela, 2014; Mebratu et al., 2019). Testing environments which had strong negative association indicates the presence of cross-over among genotypes (Yan and Tinker, 2006). Even though most of the environments had positive association, negative association was shown between BK2 and HAR environments under optimum conditions, MER with UMB

under random drought, CHI with MAK under managed drought and AM1 with BK2 and AM1 with HAR under low N conditions. If two testing sites associated consistently across seasons/years, one could be dropped without significant loss of information about the genotypes (Tukamuhabwa et al., 2012; Lule et al., 2014). In this study Bako and Ambo testing sites showed positive association during 2018/19 and 2019/20 both under optimum and low N conditions, but negative correlation was observed between BK2 and AM1 under low N. This kind of inconsistency might have been due to variation in weather conditions like rainfall, temperature, soil conditions, and other unpredictable factors.

5.5 Conclusions

AMMI analysis indicated significant effects of environment, genotype, and G × E interaction on grain yield of newly developed QPM hybrids. Though the performance of hybrids was highly influenced by environmental effects across all management types, environmental effects were more pronounced under random drought stress and low N stress environments. AMMI and GGE biplots were effective in determining the magnitude and pattern of G × E interaction effect and visualising the yield performance and stability of QPM hybrids, and to determine the discrimination ability and representativeness of the testing environments. Based on AMMI and GGE biplot stability analysis, entries 6, 10, 14 and 20 under optimum, 3 and 23 under random drought, 19, 35 and 40 under managed drought and 14 under low N conditions were identified as the highest yielding and stable hybrids in both stressed and non-stressed environments. The selected QPM hybrids in this study should be advanced into variety verification for commercialization to improve food and nutritional security of smallholder farmers in SSA. GGE biplot analysis was very effective in defining mega-environments and identifying top yielding hybrids in each of the testing environments. The polygon of each management type was divided into different sectors. Based on this, four mega environments under optimum, three mega environments under random drought, two mega environments under managed drought and three mega-environments under low N were identified. KWE, BIN and BK2 were identified as the most discriminating and representative locations for optimum, random drought and low N stress conditions, respectively hence, these sites were recognized as ideal environments in the respective management conditions.

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CHAPTER 6

Association of grain yield, agronomic and nutritional traits in QPM hybrids evaluated under stressed and non-stressed environments

Abstract

Breeding for improved grain yield and concentration of essential minerals and amino acids in staple crops like maize, can help in addressing food insecurity and malnutrition related diseases in developing countries where maize is a major staple food crop. The objective of this study was to determine the relationships among grain yield, agronomic traits, tryptophan, Fe and Zn contents in QPM hybrids evaluated under stress and non-stressed environments. Forty-five QPM hybrids, including four checks (two QPM and two normal maize and one local check) were evaluated under optimum, low N and random drought stress environments in Ethiopia, Zimbabwe, Zambia, Mozambique, and Malawi in 2018 to 2020. Correlations were calculated and PCA done to determine the relationship between grain yield, agronomic and nutritional traits in QPM hybrids under optimum, low N and random drought stress conditions. The correlation of grain yield with some secondary traits like number of ears per plant, days to anthesis and silking, and ASI. It is advised that these traits be taken into consideration as crucial for developing high yielding and early maturing types of genotypes, especially for low N stress environments. Grain yield showed non-significant correlation with tryptophan, phytic acid and Zn, and molar ratios of Fe and Zn to phytic acid under all management conditions, indicating the need for independent selection for enhancing grain yield and these nutritional traits. Significant negative correlation was observed between total protein and tryptophan content under optimum conditions. Fe and Zn were significantly correlated under random drought and low N stress conditions, but not under optimum conditions. Significant and positive correlations were observed among optimum, random drought stress and low N conditions for grain yield, total protein, tryptophan and Zn content, indicating the same genes influence genetic variation under stress and non-stress environments.

Keywords: Correlation, grain yield, agronomic traits, QPM, minerals, molar ratios

6.1 Introduction

Maize is one of the leading cereal crops worldwide in terms of production, and ranks second after rice in terms of economic value (Makumbi et al., 2018). It plays an important role in the human diet in many developing countries by providing daily energy, protein and micronutrients with intake ranging from 52 to 450 g per person per day (Ekpa et al., 2019; Prasanna et al., 2021). Maize production in SSA increased threefold since 1961, however, this increase is mainly associated with a large increase in the area under maize production rather than increased productivity (Prasanna et al., 2021). Low yield is mainly associated with abiotic, biotic and socio-economic constraints, mainly poor soil fertility and drought (Shiferaw et al., 2011; Cairns et al., 2013; Abate et al., 2017; Rezende et al., 2020).

Drought is one of major abiotic stresses that has a significant impact on maize yield and yield related traits in SSA (Shiferaw et al., 2011; Ertiro et al., 2017). It can occur at any growth stage of the crop and has different effects on the crop growth. If the stress coincides with the most drought-sensitive stages, such as flowering and grain-filling, yield losses can be even more severe (Magorokosho et al., 2003; Bänziger et al., 2006; Badu-Apraku et al., 2011; Wegary et al., 2014).

Despite N being one of the most essential micronutrients for plant growth and development (Makumbi, 2005), N deficiency is a persistent problem in SSA due to the inherently poor soil fertility status (Abu et al., 2021a) and because fertilizer application is far below the optimal rate, or not commonly used (Bänziger and Lafitte, 1997; Makumbi, 2005; Meseka et al., 2013). This scenario can cause a significant yield reduction (Wegary et al., 2014; Ertiro et al., 2017; Setimela et al., 2017; Mebratu et al., 2019) and has a large impact on total protein content and protein quality, such as lysine and tryptophan content (Wegary et al., 2011; Ngaboyisonga et al., 2012; Ngaboyisonga and Kiarie, 2014), and minerals like Fe and Zn (Prasanna et al., 2011). Therefore, development of QPM germplasm with tolerant to low N is crucial for increasing maize yield and nutritional quality for a resource poor small scale farmers residing in SSA (Betrán et al., 2003; Badu-Apraku et al., 2011; Wegary et al., 2011; Ngaboyisonga et al., 2012; Shawa et al., 2021).

Indirect selection for grain yield under drought and low N stress conditions based on secondary traits can be done, because the heritability estimates of grain yield under these stress conditions are usually very low (Bänziger and Lafitte, 1997; Badu-Apraku et al., 2011; 2012; Abu et al.,

2021b). Under these conditions, secondary traits which have a strong association with yield may increase selection efficiency (Bolanos and Edmeades, 1996). Several studies have been conducted to assess the association between grain yield and secondary traits under drought and low N stress by CIMMYT, IITA and NARS and they identified the most reliable traits for selection of genotypes tolerant to drought and low N stress (Lafitte and Edmeades, 1994; Monneveux et al., 2005; Badu-Apraku et al., 2011; Oyekunle and Badu-Apraku, 2018). However, association studies on grain yield, agronomic and nutritional quality traits, under low N and drought stress for QPM genotypes in SSA breeding programmes have been limited. In addition, some studies conducted previously also showed inconsistent information on the relationship between grain yield and nutritional quality traits. For example, Bänziger and Long (2000) reported the presence of negative association between grain yield and mineral concentrations (Fe and Zn) and indicated the difficulty of simultaneous improvement of these traits. Chakraborti et al. (2011) reported the existence of positive and significant correlation between Fe and Zn, while none of associations of Fe and Zn was significant and negative with grain yield and suggesting the possibility of simultaneous improvement of Fe and Zn without lowering grain yield. On the other hand, Chakraborti et al. (2009) reported that kernel Fe concentration was negatively correlated with grain yield. Prasanna et al. (2011) reported that Fe and Zn were not significantly correlated. These studies indicated that the correlations of traits are genotype dependent, making it necessary to determine the relationships between traits of newly developed QPM hybrids. Pearson correlation and PCA are effective statistical tools that show the associations among traits and help to identify important traits for indirect selection and identify the most suitable genotypes for different management conditions (Badu-Apraku et al., 2011; Abu et al., 2021b) and designing breeding programmes as well as establishing breeding objectives.

Since poor soil fertility and drought are the main constraints of maize cultivation in SSA, it is important to assess the relationship of grain yield, agronomic and nutritional quality traits in QPM genotypes under stressed and non-stressed environments before release for commercial production, to identify traits which can be improve simultaneously. Therefore, the objective of this study was to determine relationship of grain yield, yield related and nutritional traits in QPM hybrids evaluated under stress and non-stressed environments.

6.2 Materials and methods

6.2.1 Testing sites

Detailed description of experimental testing sites used in this study are given in the materials and methods section in Chapter 3.

6.2.2 Planting materials

A detailed description was given in the materials and methods section of Chapter 3.

6.2.3 Experimental design and field management

A detailed description was given in the materials and methods section of Chapter 3.

6.2.4 Nutritional trait analysis

A detailed description was given in the materials and methods section of Chapter 4.

6.2.5 Statistical analysis

The mean values of the genotypes for all testing sites for each management condition for grain yield, agronomic and nutritional quality traits were used for analyses. Correlation coefficient values and PCA were computed using Genstat 18th Edition statistical software (VSN International, 2015). PCA was performed using the correlation matrix to determine principal components, eigenvalues and the scores of the principal components. Since the traits were measured in different units, the genotype mean values were standardized using the standard deviation method (Yan and Tinker, 2006). Principal components which had an eigenvalue of less than one were removed from the analysis (Chatfield and Collins, 1980).

6.3 Results

6.3.1 Correlation analysis

6.3.1.1 Across optimum conditions

The association of grain yield across optimum testing environments was non-significant with all measured agronomic and nutritional traits, but yield was significantly positively correlated with protein quality index (Table 6.1). Days to anthesis correlated significant and positively with days to silking, plant height and total protein content. Days to silking had significant positive correlation with ASI, plant height and ear height, while significant negative correlation was observed with number of ears per plant and protein quality index. The association of plant and ear height with ear position and protein content were significant and positive, while they

correlated significantly negatively with tryptophan and protein quality index. Number of ears per plant was correlated significantly and positive with protein quality index and molar ratio of Zn to phytic acid, while it showed negative and significant correlation with protein content, phytic acid and Zn. The association of protein content with phytic acid was significant and positive, whereas the associations with tryptophan content and protein quality index were negative and significant.

6.3.1.2 Across low N stress conditions

Grain yield showed significant and positive correlation with number of ears per plant and significant negative correlation with days to anthesis and silking and ASI (Table 6.2). None of the quality traits were significantly correlated with grain yield. Days to anthesis and silking were significantly positively correlated with ASI and protein content, and significantly negatively correlated with number of ears per plant. The association of ASI with ear height and ear position was significant and positive and was significant and negative with number of ears per plant, tryptophan content and protein quality index. Significant and positive correlations were observed between plant and ear height, ear height and ear position, and number of ears per plant and tryptophan content. Plant and ear height correlated significantly and negatively with tryptophan content and protein quality index. Protein quality index also correlated significantly and negatively with total protein content and significantly and positively with tryptophan content.

6.3.1.3 Across random drought stress conditions

Grain yield was significantly and negatively correlated with total protein and Fe content (Table 6.3). Both days to anthesis and silking showed significant positive associations with plant and ear height, but these traits were significantly and negatively correlated with number of ears per plant. The association of ASI with protein content was significant and positive, while it was significant and negative with plant height and protein quality index. A significant and positive correlation was observed between total protein content and both and Fe and Zn; while it showed negative and significant association with molar ratio of Fe to phytic acid. Highly significant positive correlations also were observed between Fe and Zn, and between the two molar ratios (molar ratio of Fe to phytic acid and molar ratio of Zn to phytic acid).

Table 6.1 Phenotypic correlations of grain yield, agronomic and nutritional quality traits for 40 new QPM hybrids, two QPM and two normal maize checks across optimum conditions

Trait	GY	DA	DS	ASI	PH	EH	EPO	EPP	Protein	Trypt	QI	PA	Fe	Zn	MRFe
DA	0.09														
DS	-0.01	0.89**													
ASI	-0.20	-0.14	0.32*												
PH	0.09	0.44**	0.42**	0.01											
EH	0.14	0.43	0.38*	-0.09	0.85**										
EPO	0.14	0.32*	0.23	-0.17	0.49**	0.87**									
EPP	0.26	-0.24	-0.31*	-0.17	-0.12	0.08	0.25								
Protein	-0.20	0.29*	0.38*	0.22	0.50**	0.32*	0.07	-0.44**							
Trypt	0.26	-0.13	-0.17	-0.12	-0.36*	-0.33*	-0.22	0.22	-0.47*						
QI	0.30*	-0.21	-0.30*	-0.20	-0.47**	-0.38**	-0.20	0.38*	NA	NA					
PA	-0.03	0.27	0.24	-0.04	0.18	0.02	-0.16	-0.37*	0.42*	0.16	-0.07				
Fe	-0.04	-0.04	-0.02	0.05	0.15	-0.05	-0.21	-0.17	0.18	-0.10	-0.13	0.24			
Zn	0.27	0.18	0.24	0.15	0.25	0.11	-0.06	-0.34*	0.25	-0.01	-0.13	0.27	-0.19		
MRFe	0.12	0.00	-0.07	-0.14	-0.23	-0.15	-0.04	0.07	-0.15	0.20	0.20	0.05	NA	0.23	
MRZn	-0.22	-0.06	-0.11	-0.11	-0.20	-0.09	0.05	0.30*	-0.12	0.04	0.10	0.00	0.28	NA	-0.23

*P≤0.05,** P≤0.01; GY = grain yield; DA = days to anthesis; DS = days to silking; ASI = anthesis silking interval; PH = plant height; EH = ear height; EPO = ear position; EPP = number of ears per plant; Trypt = tryptophan content; QI = protein quality index; PA = phytic acid content; Fe = iron; Zn = zinc; MRFe = molar ratio of Fe to phytic acid; MRZn = molar ratio of Zn to phytic acid, NA = not applicable

Table 6.2 Phenotypic correlations of grain yield, agronomic and nutritional quality traits for 40 new QPM hybrids, two QPM and two normal maize checks across low N stress conditions

Trait	GY	DA	DS	ASI	PH	EH	EPO	EPP	Protein	Trypt	QI	PA	Fe	Zn	MRFe
DA	-0.33*														
DS	-0.41**	0.93**													
ASI	-0.39**	0.37*	0.69**												
PH	0.02	0.21	0.21	0.13											
EH	-0.10	0.14	0.27	0.40**	0.56**										
EPO	-0.17	-0.03	0.11	0.33*	-0.23	0.66**									
EPP	0.50**	-0.49**	-0.55**	-0.42**	-0.17	-0.24	-0.17								
Protein	-0.03	0.31*	0.30*	0.15	0.28	0.33*	0.16	-0.35*							
Trypt	0.23	0.06	-0.14	-0.47**	-0.07	-0.25	-0.23	0.30*	-0.07						
QI	0.22	-0.12	-0.25	-0.40**	-0.21	-0.36*	-0.26	0.43**	NA	NA					
PA	-0.11	0.19	0.13	-0.04	0.07	-0.03	-0.07	-0.23	0.24	0.17	0.00				
Fe	0.08	0.22	0.13	-0.11	-0.19	-0.19	-0.01	-0.07	-0.07	0.05	0.05	0.08			
Zn	0.10	0.20	0.12	-0.08	0.11	0.22	0.17	-0.12	0.18	0.09	-0.04	0.25	0.32*		
MRFe	-0.02	-0.21	-0.14	0.05	0.15	0.16	0.03	0.00	0.17	-0.05	-0.10	0.08	NA	-0.18	
MRZn	-0.12	-0.09	-0.04	0.07	0.00	-0.10	-0.14	0.06	-0.02	0.01	0.01	0.11	-0.31*	NA	0.22

*P≤0.05,** P≤0.01; GY = grain yield; DA = days to anthesis; DS = days to silking; ASI = anthesis silking interval; PH = plant height; EH = ear height; EPO = ear position; EPP = number of ears per plant; Trypt = tryptophan content; QI = protein quality index; PA = phytic acid content; Fe = iron; Zn = zinc; MRFe = molar ratio of Fe to phytic acid; MRZn = molar ratio of Zn to phytic acid, NA = not applicable

Table 6.3 Phenotypic correlations of grain yield, agronomic and nutritional quality traits for 40 new QPM hybrids, two QPM and two normal checks evaluated under random drought stress conditions

Trait	GY	DA	DS	ASI	PH	EH	EPO	EPP	Protein	Trypt	QI	PA	Fe	Zn	MRFe
DA	0.04														
DS	0.00	0.96**													
ASI	-0.13	0.03	0.30*												
PH	0.16	0.46**	0.49**	0.16											
EH	0.14	0.47**	0.50**	0.20	0.83*										
EPO	0.02	0.27	0.32*	0.22	0.20	0.70*									
EPP	-0.09	-0.29*	-0.29*	-0.04	-0.21	-0.25	-0.19								
Protein	-0.41**	0.25	0.33*	0.32*	0.17	0.17	0.12	-0.43*							
Trypt	0.22	0.03	0.01	-0.05	-0.21	-0.21	-0.12	-0.06	-0.02						
QI	0.38*	-0.07	-0.12	-0.19	-0.25	-0.24	-0.14	0.14	NA	NA					
PA	-0.15	-0.01	0.02	0.10	-0.05	0.11	0.25	0.06	0.19	0.06	-0.03				
Fe	-0.45**	0.09	0.17	0.31*	-0.01	0.02	0.05	-0.38*	0.67**	0.13	-0.18	0.16			
Zn	0.02	0.03	0.07	0.17	0.10	-0.07	-0.29	-0.25	0.31*	0.41**	0.21	0.05	0.48**		
MRFe	0.27	-0.07	-0.11	-0.18	-0.02	0.07	0.14	0.36*	-0.42**	-0.08	0.11	0.57**	NA	-0.35*	
MRZn	-0.07	-0.05	-0.06	-0.04	-0.11	0.13	0.38	0.24	-0.13	-0.26	-0.17	0.67**	-0.26	NA	0.69**

*P≤0.05,** P≤0.01; GY = grain yield; DA = days to anthesis; DS = days to silking; ASI = anthesis silking interval; PH = plant height; EH = ear height; EPO = ear position; EPP = number of ears per plant; Pro = total protein content in the grain; Trp = tryptophan content; QI = protein quality index; PA = phytic acid content; Fe = iron; Zn = zinc; MRFe = molar ratio of Fe to phytic acid; MRZn = molar ratio of Zn to phytic acid

Table 6.4 Phenotypic correlations of grain yield, total protein, tryptophan, phytic acid, Fe, and Zn contents under optimum, low N and random drought stress conditions

Grain yield			Total protein			Tryptophan		
	Opt	LN		Opt	LN		Opt	LN
LN	0.37*	-	LN	0.39**	-	LN	0.61**	-
RS	0.70**	0.31*	RS	0.64**	0.13	RS	0.69**	0.56**
Phytic acid			Iron			Zinc		
	Opt	LN		Opt	LN		Opt	LN
LN	0.12	-	LN	0.18	-	LN	0.44**	-
RS	0.13	-0.08	RS	0.07	0.39**	RS	0.54**	0.33*

*P≤0.05, ** P≤0.01; Opt = optimum; LN = low nitrogen; RS = random drought stress

6.3.1.4 Relationship of traits between different management conditions

Highly significant (P≤0.01) positive correlation was observed between grain yield of optimum and random drought stress conditions (Table 6.4). There was also significant and positive correlation between grain yield of optimum and low N, and low N and random drought stress conditions. For nutritional traits, highly significant positive correlations were observed between total protein, tryptophan, and Zn content of optimum with low N and random drought stress conditions (Table 6.4). There were also significant positive correlations between low N and random drought stress conditions for tryptophan, Fe and Zn contents. The amount of phytic acid for the different growing conditions showed no similarity. Fe content under low N and random drought stress conditions was significantly correlated (Table 6.4).

6.3.2 Principal component analysis

6.3.2.1 Principal component analysis across optimum management condition

For optimum conditions, six significant principal components (PC) that had eigenvalues of more than one, explained 83.16% of the variation in the dataset. Among these, PC1 and PC2 contributed 48.46% of the variation as shown in Table 6.5 and Figure 6.1. The first PC had an eigenvalue of 4.07 and accounted 26.96% of the total variation. Days to anthesis and silking, plant and ear height, total protein and tryptophan contents, and protein quality index had a loading of more than ±0.3 on this PC (Table 6.4). Grain yield, Fe, Zn and phytic acid to Fe and Zn molar ratios contributed highly to PC2 which had an eigenvalue of 2.48 and explained 15.49% of the total variation. Ear height, ear position and number of ears per plant made the largest contribution to PC3, which accounted for 15.22% of the variation with an eigenvalue of 2.44. With respect to PC4, days to silking, tryptophan content, protein quality index, phytic acid and Fe traits made the largest contribution and explained 10.22% of total variation.

Strong positive relationships were observed between plant height, ear height, ear position, days to anthesis and phytic acid, as they had small angles between them (Figure 6.1). Entries 33, 23, 24, 15 and 3 grouped together with high values for these traits. Grain yield was closely associated with molar ratio of Fe to phytic acid and Zn, and these traits were negatively associated with molar ratio of Zn to phytic acid and Fe. Entries, 14, 8, 9 and 13 had the highest values for tryptophan and protein quality index, which were highly correlated, and these traits were also negatively correlated with ASI and total protein content.

6.3.2.2 Principal component analysis across low N stress conditions

Across low N conditions, six principal components with eigenvalues of more than one was identified which explained 80.19% of the total variation in the dataset (Table 6.6). The first two PCs contributed 42.12% of the total variation (Table 6.6 and Figure 6.2). The first PC had an eigenvalue of 2.48 and contributed 25.75% of the total variation in the dataset. Days to anthesis and silking, ASI, ear height, number of ears per plant and total protein were the main contributors to PC1. Fe, Zn and phytic acid to Fe and Zn molar ration contributed the most to PC2 which had an eigenvalue of 2.63 and explained 16.4% of the variation. Days to anthesis, days to silking, ear height, ear position, Zn, and molar ratio of Zn to phytic acid were the main contributors to PC3, which accounted for 12.21% of the variation with an eigenvalue of 1.96. Plant height, tryptophan content, phytic acid and molar ratio of Fe to phytic acid traits were the main contributors to PC4, which explained 10.81% of the total variation. PC5 explained 7.83% of the total variation, with ear position, total protein, tryptophan content and protein quality index being the main contributors. (Table 6.5).

Table 6.5 Principal component analyses showing eigenvectors and eigenvalues for the 16 measured characteristics in maize hybrids across optimum environments

Trait	PC1	PC2	PC3	PC4	PC5	PC6
Grain yield	-0.05	0.34	0.16	0.18	0.40	0.07
Days to anthesis	0.30	0.18	0.10	0.35	-0.37	0.07
Days to silking	0.33	0.13	-0.02	0.26	-0.37	0.39
Anthesis silking interval	0.09	-0.09	-0.25	-0.16	-0.03	0.71
Plant height	0.38	0.06	0.18	0.06	0.23	-0.03
Ear height	0.34	0.10	0.40	-0.02	0.13	-0.02
Ear position	0.21	0.10	0.49	-0.09	0.00	-0.01
Number of ears per plant	-0.21	0.03	0.42	-0.07	0.11	0.13
Protein	0.37	-0.09	-0.21	-0.05	0.02	-0.20
Tryptophan	-0.30	0.25	-0.01	0.41	0.00	0.10
Protein quality index	-0.38	0.21	0.09	0.33	0.01	0.14
Phytic acid	0.14	0.08	-0.27	0.43	-0.04	-0.39
Ion	0.07	-0.39	-0.10	0.40	0.40	0.01
Zinc	0.16	0.43	-0.29	-0.09	0.27	0.05
Molar ratio of Fe to phytic acid	-0.11	0.40	-0.05	-0.24	-0.38	-0.28
Molar ratio of Zn to phytic acid	-0.09	-0.42	0.27	0.21	-0.33	-0.08
Eigenvalues	4.31	2.48	2.44	1.64	1.34	1.11
Percentage variation	26.96	15.49	15.22	10.22	8.37	6.90
Cumulative variation	26.96	42.45	57.67	67.89	76.26	83.16

PC = Principal component

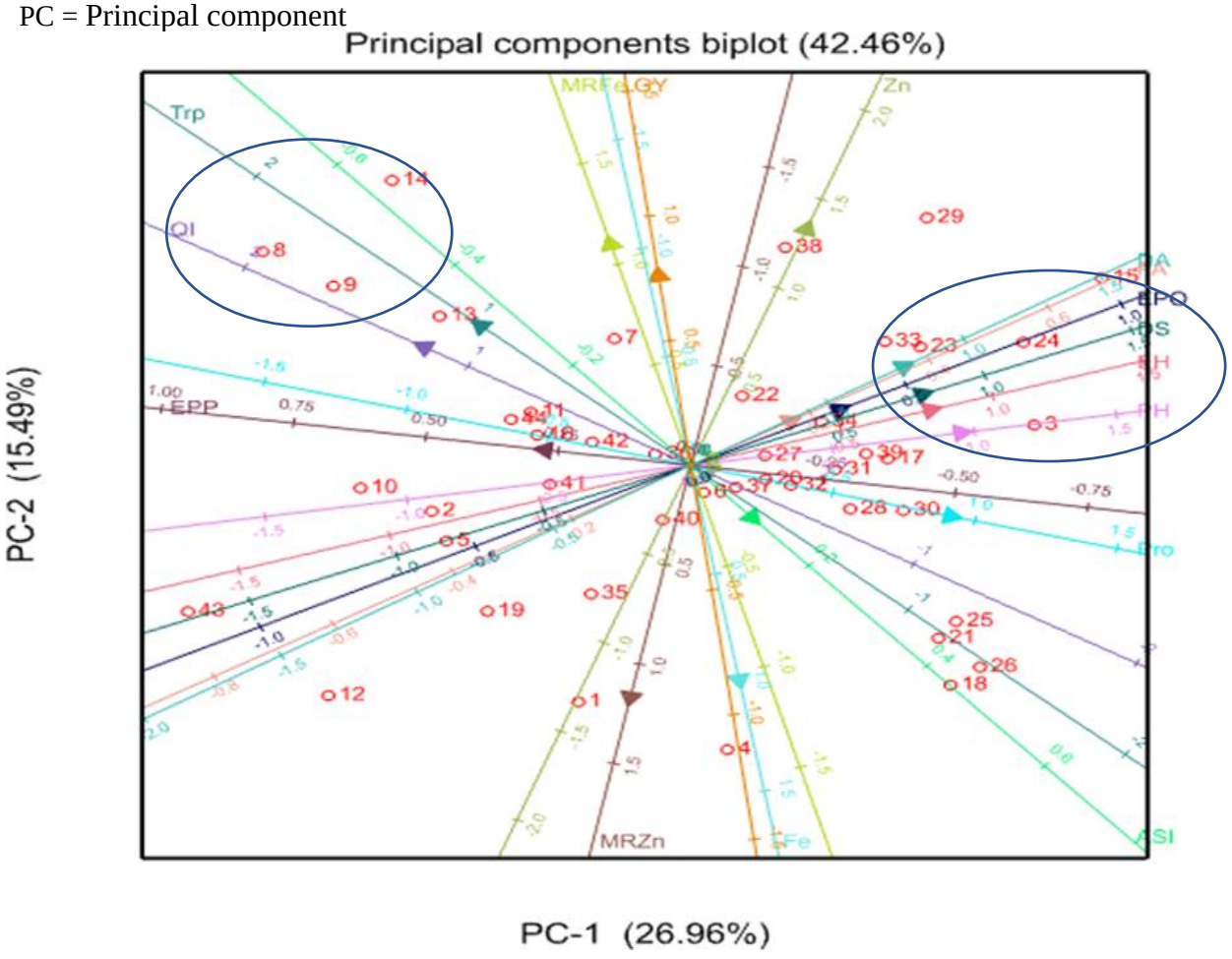

Figure 6.1 Principal component biplot showing the distribution of 44 QPM hybrids for grain yield, agronomic and nutrition traits across optimum management conditions

GY = grain yield; DA = days to anthesis; DS = days to silking; ASI = anthesis silking interval; PH = plant height; EH = ear height; EPO = ear position; EPP = number of ears per plant; Pro = total protein content in the grain; Trp = tryptophan content; QI = protein quality index; PA = phytic acid content; Fe = iron; Zn = zinc; MRFe = molar ratio of Fe to phytic acid; MRZn = molar ratio of Zn to phytic acid

Table 6.6 Principal component analyses showing eigenvectors and eigenvalues for the 16 measured characteristics in maize hybrids across low N stress conditions

Traits	PC1	PC2	PC3	PC4	PC5	PC6
Grain yield	-0.25	0.05	0.29	0.15	-0.18	0.28
Days to anthesis	0.31	0.26	-0.32	0.18	0.11	0.14
Days to silking	0.39	0.16	-0.30	0.06	0.18	0.16
Anthesis silking interval	0.36	-0.10	-0.12	-0.20	0.22	0.13
Plant height	0.18	-0.09	0.10	0.41	-0.14	0.55
Ear height	0.30	-0.12	0.36	0.10	0.29	0.21
Ear position	0.20	-0.05	0.34	-0.26	0.43	-0.29
Number of ears per plant	-0.36	-0.06	0.15	-0.01	0.08	0.21
Protein content	0.27	-0.04	0.13	0.29	-0.35	-0.15
Tryptophan content	-0.25	0.19	-0.15	0.42	0.34	-0.05
Protein quality index	-0.34	0.16	-0.19	0.18	0.49	0.06
Phytic acid	0.08	0.10	-0.14	0.38	-0.16	-0.53
Iron	-0.01	0.52	-0.03	-0.25	-0.22	0.05
Zinc	0.10	0.40	0.40	0.22	0.06	-0.16
Molar ratio of Fe to phytic acid	0.02	-0.47	0.09	0.32	0.14	-0.21
Molar ratio of Zn to phytic acid	-0.05	-0.38	-0.42	-0.02	-0.10	0.01
Eigenvalues	4.12	2.63	1.96	1.73	1.25	1.15
Percentage variation	25.75	16.4	12.21	10.81	7.83	7.19
Cumulative variation	25.75	42.15	54.36	65.17	73.00	80.19

PC = principal component

High correlations were observed between Fe and Zn, days to anthesis and days to silking, tryptophan content and protein quality index, grain yield and number of ears per plant, molar ratio of Fe to phytic acid and molar ratio of Zn to phytic acid (Figure 6.2). Entries 28, 15 and 25 grouped together with high values for days to anthesis and silking and phytic acid, entries 17, 23, 24, 26, 31, 37 and 38 grouped together with high values for protein content, plant and ear height and ear position. Entries 10, 13 and 14 grouped together with high values for tryptophan, protein quality index, grain yield and number of ears per plant.

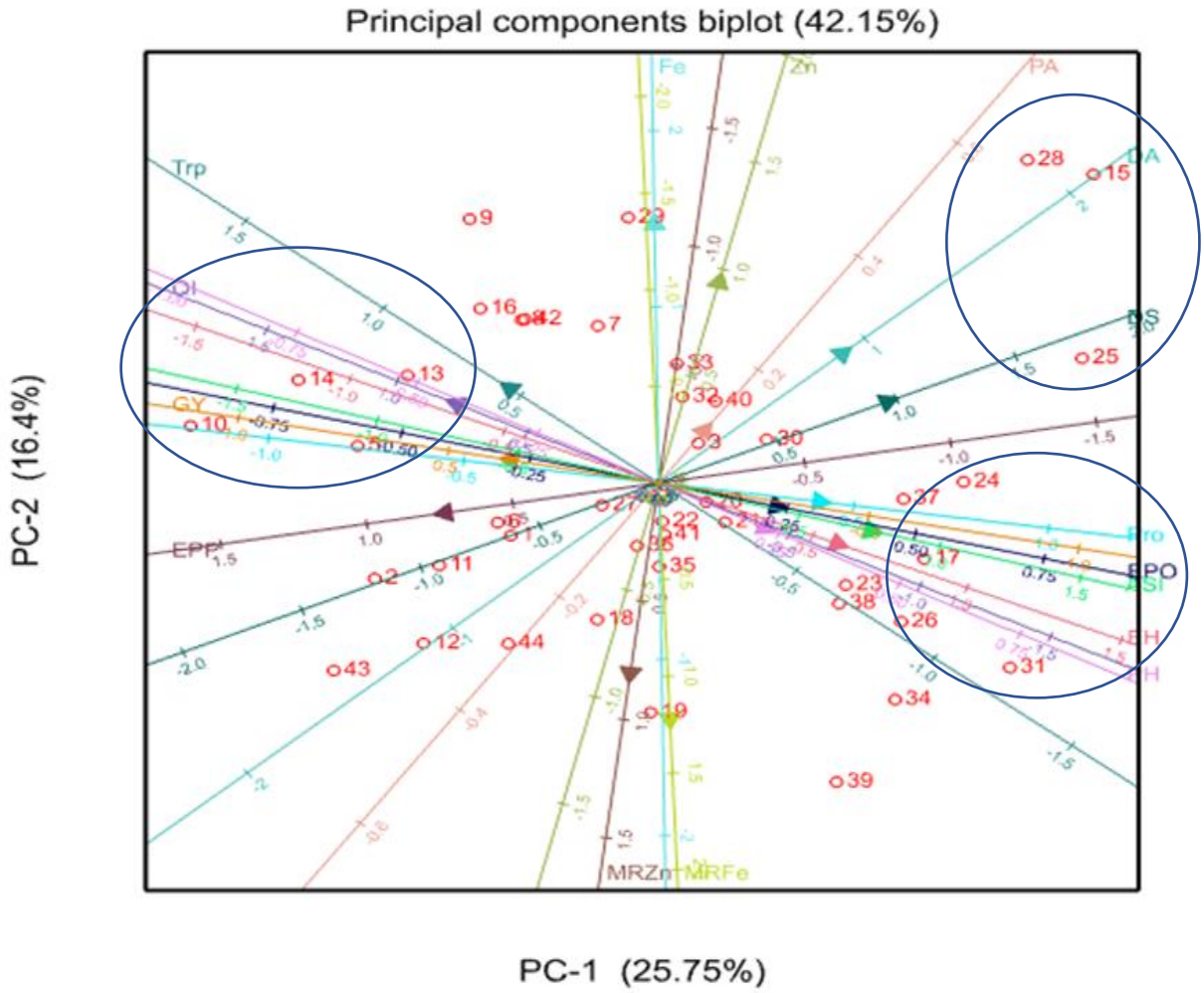

Figure 6.2 Principal component biplot showing the distribution of 44 hybrids for grain yield, agronomic and nutritional quality traits across low N stress conditions

GY = grain yield; DA = days to anthesis; DS = days to silking; ASI = anthesis silking interval; PH = plant height; EH = ear height; EPO = ear position; EPP = number of ears per plant; Pro = total protein content in the grain; Trp = tryptophan content; QI = protein quality index; PA = phytic acid content; Fe = iron; Zn = zinc; MRFe = molar ratio of Fe to phytic acid; MRZn = molar ratio of Zn to phytic acid

6.3.2.3 Principal component analysis across random drought stress conditions

Across random drought stress conditions, 77.30% of the total variation in the genotypes were observed from five PCs, which had eigenvalues of more than one. The first two PCs contributed 44.92% of the total variation (Table 6.7 and Figure 6.3). The first PC had an eigenvalue of 3.91 and accounted for 24.42% of the total variation in the dataset. Days to anthesis and silking, plant and ear height, total protein and Fe were main contributors to PC1. Ear height, ear position, Zn, molar ratio of Fe to phytic acid and molar ratio of Zn to phytic acid were the main contributors to PC2 which had an eigenvalue of 3.28 and explained 20.50% of the variation. For PC3, grain yield, tryptophan and total protein contents contributed the most and accounted for 14.60% of the variation with an eigenvalue of 2.34. Tryptophan content, protein quality

index and phytic acid traits made the largest contribution to PC4 and explained 11.15% total variation in the genotypes. PC5 explained 6.63% of the total variation, contributed by grain yield, days to anthesis and silking, ASI and ear height (Table 6.7). Under random drought stress conditions, a strong positive relationship was observed between grain yield and number of ears per plant with entries 19, 34 and 37 having high values. Protein content was highly negatively correlated with these traits (very large angle between them). Ear and plant height, and days to anthesis and silking were all highly related (Figure 6.3). Most entries grouped in the central part of the biplot, however entries like 16, 14, 8, 4 and 7 had better performance for tryptophan and Zn concentration, which were highly correlated.

Table 6.7 Principal component analyses showing eigenvectors and eigenvalues for the 16 measured characteristics in maize hybrids in maize hybrids across random drought stress conditions

Traits	PC1	PC2	PC3	PC4	PC5
Grain yield	-0.12	0.06	0.46	-0.08	0.31
Days to anthesis	0.32	0.15	0.29	0.01	-0.54
Days to silking	0.37	0.14	0.24	0.05	-0.42
Anthesis silking interval	0.22	0.01	-0.11	0.14	0.35
Plant height	0.30	0.19	0.23	-0.20	0.28
Ear height	0.31	0.32	0.20	-0.05	0.32
Ear position	0.19	0.33	0.04	0.16	0.18
Number of ears per plant	-0.29	0.09	-0.08	-0.02	-0.08
Protein content	0.37	-0.10	-0.26	0.17	-0.05
Tryptophan content	-0.07	-0.26	0.35	0.45	-0.06
Protein quality index	-0.22	-0.18	0.43	0.32	-0.04
Phytic acid	-0.01	0.23	-0.17	0.59	0.08
Iron	0.31	-0.26	-0.24	0.25	0.02
Zinc	0.16	-0.36	0.13	0.20	0.27
Molar ratio of Fe to phytic acid	-0.26	0.38	0.09	0.21	0.04
Molar ratio of Zn to phytic acid	-0.14	0.44	-0.2	0.27	-0.11
Eigenvalues	3.91	3.28	2.34	1.78	1.06
Percentage variation	24.42	20.50	14.6	11.15	6.63
Cumulative variation	24.42	44.92	59.52	70.67	77.3

PC = principal component

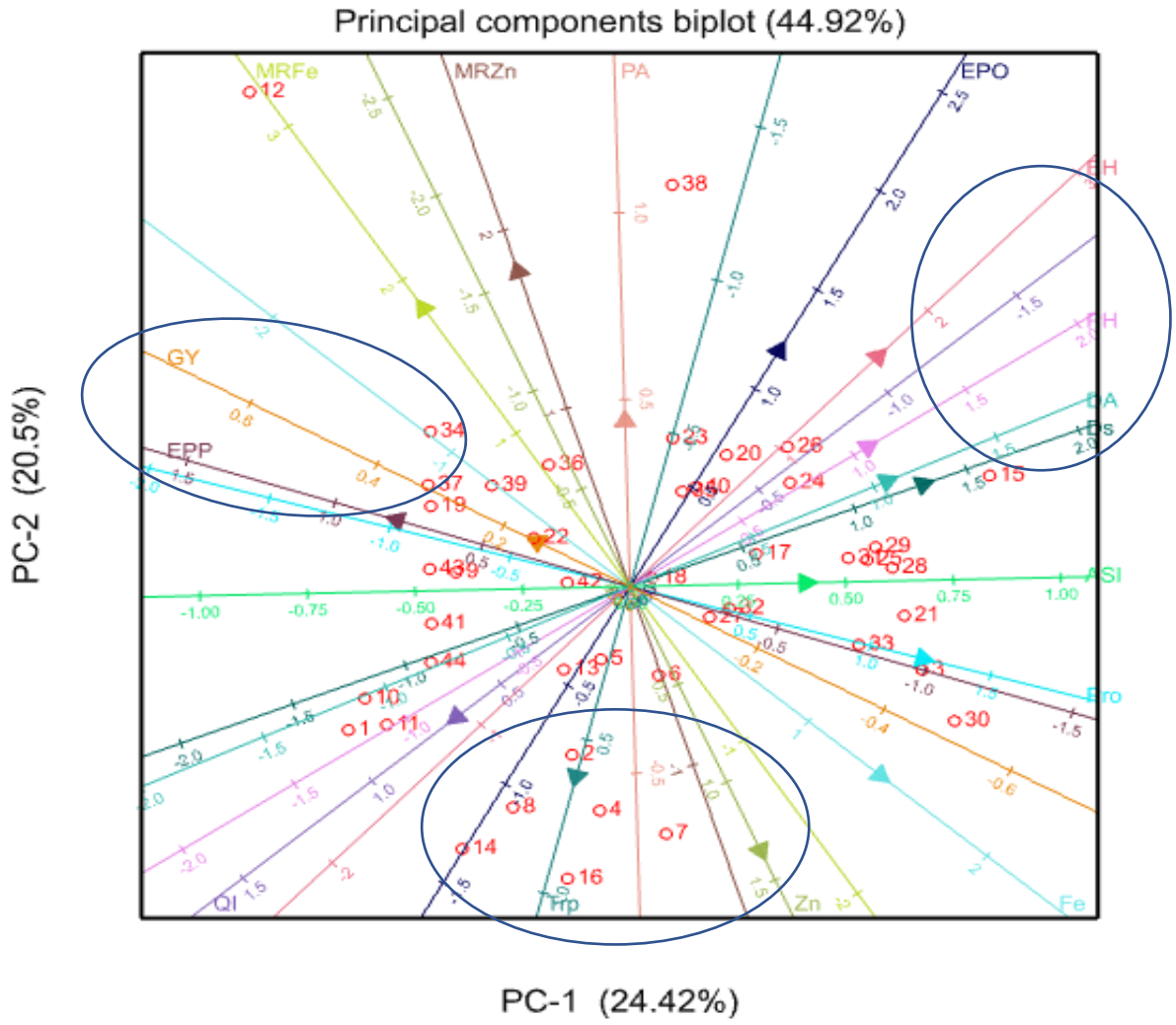

Figure 6.3 Principal component biplot showing the distribution of 44 QPM hybrids for grain yield, agronomic and nutritional traits across random drought stress conditions

GY = grain yield; DA = days to anthesis; DS = days to silking; ASI = anthesis silking interval; PH = plant height; EH = ear height; EPO = ear position; EPP = number of ears per plant; Pro = total protein content in the grain; Trp = tryptophan content; QI = protein quality index; PA = phytic acid content; Fe = iron; Zn = zinc; MRFe = molar ratio of Fe to phytic acid; MRZn = molar ratio of Zn to phytic acid

6.4 Discussion

Understanding the relationships between grain yield, agronomic and nutritional traits recorded from different management conditions would indicate the possibility of selection gains through correlated traits. Grain yield is the most important and complex trait and identification of secondary traits that have strong association with it provides a guide to specific mechanisms that contribute to improved grain yield under stress and non-stressed environments (Campos et al., 2004). However, in this study, grain yield did not show significant correlation, either positively or negatively, with most of the measured agronomic traits under optimum and random drought stress conditions. This suggested that environmental influence is responsible

for low estimates of correlation coefficient (Bankole et al., 2019). This is in disagreement with several other studies which reported significant association between grain yield and secondary traits like days to anthesis and silking, ASI, plant and ear height, number of ears per plant under optimum and drought conditions (Badu-Apraku et al., 2011; Nzuve et al., 2014; Oyekunle and Badu-Apraku, 2018). Under low N conditions the picture was different, where grain yield showed significant negative correlations with days to anthesis and silking, and ASI. This suggested that the improvement of grain yield is associated with a reduction of these traits. Positive and significant correlation was observed between grain yield and number of ears per plant under low N conditions, suggesting that this trait can be used for indirect selection for grain yield improvement under low N stress conditions. This agrees with the results of other studies which reported a strong association between grain yield and number of ears per plant, and it is one of the most important secondary traits for improving grain yield under stressed environments (Badu-Apraku et al., 2011; Oyekunle and Badu-Apraku, 2018).

Improving the nutritional quality of staple crops like maize through biofortification is an important strategy to meet the nutritional requirements of rural populations who have limited access to diversified diets, dietary supplements, and fortified foods (Prasanna et al., 2020). Studying the relationships of grain yield and nutritional quality traits are important to develop selection criteria to breed high yielding and nutritionally enriched maize genotypes (Chakraborti et al., 2009). In this study correlation of grain yield with total protein was non-significant and negative across optimum and random drought stress conditions, and significant and negative across low N stress conditions. This indicated that breeding to improve these traits simultaneously would be difficult. Similar results were reported by Ngaboyisonga et al. (2012). Shawa (2019) also stated the relationship of grain yield and protein content is a cause-effect, meaning that when protein content increases, the accumulation of starch decreases, resulting in yield decrease. Furthermore, total protein content showed positive correlations with other agronomic traits, such as days to anthesis and silking, and plant and ear height under all management conditions. This suggested that factors responsible for an increase in days to anthesis and silking, plant and ear height had an effect on improving total protein content. Contrary, Amegbor et al. (2022) reported that the total protein concentration correlated negatively with days to anthesis and ASI, and low positive corelation with plant and ear height. Álvarez-Iglesias et al. (2021) also reported protein content had significant negative correlation with plant height. On the other hand, total protein was correlated significantly and negatively

with number of ears per plant, which was highly significantly correlated with grain yield, under all management conditions.

According to Ngaboyisonga et al. (2012) the correlation of tryptophan content with grain yield was non-significant under optimum and random drought stress but significant and negative under low N stress conditions, which is similar to findings of the present study under optimum and random drought stress conditions, however it is contrary to the results under low N stress conditions. Correlation between total protein and tryptophan contents was significant and negative under optimum conditions, indicating that factors involved in increasing protein content were responsible for reduced tryptophan content. This contradicts findings of Ngaboyisonga et al. (2012) who reported significant and positive correlation between total protein and tryptophan concentrations in the grain. Moreover, non-significant and weak negative correlation was also observed between protein and tryptophan content under low N and random drought stress conditions, indicating factors responsible for the increase in these traits under these stress conditions were dissimilar with results reported by Ngaboyisonga et al. (2012).

Total protein content was not significantly correlated with Fe and Zn under optimum and low N conditions. However, the correlation coefficient increased as N level increased. Similar results were reported by Shawa (2019) who found non-significant association between protein, and Fe and Zn concentrations under optimum and low N stress conditions. Protein content was significantly positively correlated with Zn under random drought stress (but not with Fe), indicating the presence of pleiotropic effects increasing the total protein and Zn contents (but not Fe) under these stress conditions. There was also no relationship between tryptophan content and Fe and Zn, meaning they are inherited independently under optimum and low N stress conditions. However, tryptophan content was significantly and positively correlated with Zn (but not with Fe) content. This confirms that micro nutrients such as Zn plays an important role in tryptophan biosynthesis, which is increased in QPM (Hindu et al., 2018; Prasanna et al., 2020).

Fe and Zn deficiency are the 5^{th} and 6^{th} of the top 10 risk factors accountable for illnesses and diseases in developing countries (Simic et al., 2009; Akhtar et al., 2018) and more than two billion people in developing countries suffer from one or more micronutrient deficiencies (Chakraborti et al., 2011; Rautiainen et al., 2016; Garg et al., 2018; Prasanna et al., 2020). Fe

and Zn enrichment of major staple food crops like maize, through breeding, is one of the most important strategies for stable and cost-effective production of more nutritious food (Garg et al., 2018). In this study grain yield was not significantly correlated with Fe and Zn under optimum, low N and random drought stress conditions, although there was a significant and negative correlation with Fe under random drought stress conditions. A similar observation of non-significant correlation of grain yield with Fe and Zn concentration under optimum conditions was reported by Chakraborti et al. (2011) and Shawa (2019). Other studies showed a reduction in grain yield with an increase in concentration of Fe and Zn (Bänziger and Long, 2000). Fe and Zn were not significantly correlated under optimum conditions, suggesting that factors responsible for accumulation of kernel Fe and Zn concentrations are different, and genetic improvement for these two traits could be undertaken independently, which is similar to findings of Prasanna et al. (2011). On the other hand, there was significant and positive correlation between Fe and Zn under low N and random drought stress conditions, indicating that these traits can be improved simultaneously under these stress conditions. Oikeh et al. (2003), Menkir (2008) and Chakraborti et al. (2011) reported significant and positive correlation between Fe and Zn concentrations under optimum conditions.

Increased Fe and Zn concentrations in staple food crops may not necessarily translate into a proportional increase in absorbed levels in humans and other monogastric animals, as bioavailability is reduced by phytic acid, that has a strong tendency to chelate positively charged mineral ions like Fe and Zn to form insoluble salts (Oikeh et al., 2003; Simic et al., 2009; Pixley et al., 2011; Queiroz et al., 2011; Gupta et al., 2015). It is necessary to measure the Fe and Zn concentration and bioavailability of newly developed Fe and Zn enriched maize genotypes. The association of phytic acid with other nutritional traits could be used to develop selection criteria for improving mineral bioavailability. The association of phytic acid with total protein, tryptophan and protein quality index were non-significant under all management conditions except for a positive and significant correlation with total protein under optimum conditions. Under optimum environments, similar results were reported by Shawa (2019), who found positive association between total protein and phytic acid content.

Understanding the relationship of traits recorded in different growing conditions could contribute to improving the performance of genotypes under all these conditions. In this study, grain yield of optimum, low N and random drought stress conditions were highly significantly correlated, which is similar with the findings of Bänziger et al. (1997) and Ertiro et al. (2017)

who reported positive correlation of grain yield under optimum conditions with grain yield under both drought stress and low N conditions. In addition, highly significant positive correlations were also observed between total protein, tryptophan, and Zn content of optimum with low N and random drought stress conditions. This indicated that selection of genotypes for or one of these traits would indirectly improve the other traits under these given conditions. This will assist breeders to develop genotypes which perform well under all growing conditions.

The PCA showed the visual relationship of traits, which can help to identify the best genotypes that perform better for traits having a strong correlation. In the current study, 83.6, 80.18 and 77.30% of the total variation of studied traits were observed from six principal components across optimum and low N conditions, and five PCs for random drought stress conditions. This variation offers ample opportunities for the genetic improvement of the QPM genotypes through selection based on the studied traits. These results are in line with that of Shawa (2019) who studied QPM and normal maize checks for grain yield and nutritional traits across optimum and low N stress conditions. Based on the loading plot, grain yield showed the strongest relationship with number of ears per plant under low N and random drought stress conditions. This indicated that grain yield increased when number of ears per plant increased. Bänziger and Lafitte (1997), Bänziger et al. (2000), Chakraborti et al. (2009) and Tapera (2017) also reported secondary traits which are highly correlated with grain yield, and found that number of ears per plant is the most effective trait to identify high yielding genotypes under stressed and non-stressed environments. Among the nutritional traits, strong correlations were observed of Fe and Zn with tryptophan under low N and random drought stress conditions. According to Pixley et al. (2011) biofortification of maize for more than one nutrient can cause synergistic interactions enhancing the nutritional value of the genotypes. Although most of genotypes showed varying performance for measured traits under different management conditions. Entry 14 had consistently high tryptophan content under all management conditions. Several researchers reported that genotypes behaved differently under different environmental conditions for grain yield and yield related traits (Wegary et al., 2014; Ertiro et al., 2017; Setimela et al., 2017; Sserumaga et al., 2016; 2018; Mebratu et al., 2019) and for total protein, tryptophan and protein quality index (Wegary et al., 2011; Ngaboyisonga et al., 2012; Ngaboyisonga and Kiarie, 2014) and minerals (Oikeh et al., 2004; Prasanna et al., 2011).

6.5 Conclusions

Improving grain yield and nutritional quality of staple crops like maize through breeding, is an important option to improve food security and malnutrition related diseases in developing countries. The positive correlation values of secondary traits like number of ears per plant, days to anthesis and silking with grain yield indicated the possibility of developing high yielding, early maturing genotypes, especially for stressed environments. On the other hand, grain yield mostly did not significantly correlate with nutritional quality traits under all management conditions, except for protein quality index, which was significantly positively correlated with yield under optimum and random drought stress conditions, and total protein and Fe content which correlated significantly negatively with yield. Moreover, the relationship of grain yield and nutritional traits under optimum conditions showed significant positive correlations with traits recorded under low N and random drought stress conditions, indicating that selection for these traits can be done under optimum conditions for low N and random drought stress conditions. PCA confirmed the correlations between measured traits and identified QPM hybrids such as 10, 13 and 14 grouped together with high values for tryptophan, protein quality index, grain yield and number of ears per plant traits under stressed conditions. Although most of genotypes showed varying performance under the different management conditions for all traits, entry 14 was identified as a promising genotype for developing high tryptophan content under all management conditions.

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CHAPTER 7

General conclusions and recommendations

Maize is one of the most important staple crops feeding millions of people in the SSA countries, providing daily energy, protein, vitamins, and minerals to consumers. However, commonly consumed maize is naturally low in some of the essential amino acids such as lysine and tryptophan, as well as minerals like Fe and Zn, with a high content of phytic acid that impedes bioavailability of Fe and Zn in the human body. Therefore, millions of people in the world are vulnerable to malnutrition related diseases. Developing high yielding and nutritionally enriched maize cultivars through breeding is a sustainable and cost-effective approach to improve food insecurity and malnutrition problems in the developing countries.

Since maize cultivation in SSA is affected by drought and low N stress, germplasm developed for this region should be screened under stressed environments before release for commercial production. Therefore, in this study 40 new QPM hybrids, including two QPM, two non-QPM standard checks and one local check, were evaluated with the objectives of 1) determining genotypic variability and hybrid performance of QPM hybrids for grain yield and agronomic traits; 2) to determine tryptophan, Zn and Fe concentrations, and the bioavailability of Zn and Fe in QPM hybrids grown under stressed and non-stressed environments; 3) to analyse genotype by environmental interaction and grain yield stability of QPM hybrids and 4) to determine correlations between grain yield, yield related traits, tryptophan content and bioavailability of Zn and Fe in QPM hybrids evaluated under stressed and non-stressed environments.

The results of this study showed significant variation among hybrids for all studied traits across all environments, which indicated the possibility of selecting QPM hybrids that perform well across drought and low N stress conditions. Determining the relative magnitude of genotypic and phenotypic variation is useful for breeders for selection. Broad sense heritability of grain yield was higher than 0.6 across all environments except under managed drought conditions where heritability was very low (0.09), this usually showed lower selection precision when severity increase. Among agronomic traits, ASI had moderate heritability, high GCV value and genetic advance as percent of the mean across trials under almost all environmental conditions,

indicating its potential for improving synchronization problems under drought and low N stress conditions.

Grain yield was reduced by 46.9% under random drought stress, 67.1% under managed drought and 70.7% under low N environments, while ASI increased by 16.0% under random drought, 216.9% under managed drought and 187.2% under low N environments, indicating that managed drought and low N stress effects were more severe than random drought stress condition in terms of yield reduction and ASI increase. In addition, the reduced grain yield under these three conditions confirmed that drought and low N stresses are the potential threats of maize production in SSA. Protein and tryptophan concentration in the grain also decreased by 36.0% and 20.6%, respectively, while protein quality increased by 12.3% under low N conditions. Under random drought stress, protein content in the grain decreased by 14.0% while tryptophan and protein quality index increased by 5.3% and 24.7%, respectively. This indicating that the effect of low N on protein and tryptophan concentrations were more sever in QPM hybrids.

This study revealed some QPM hybrids that were superior in terms of grain yield and tryptophan concentration under optimum, random drought stress and low N conditions as compared to the best QPM and normal maize checks. Entries 14 (CZH15142Q), 10 (CZH142238Q) and 9 (CZH142237Q) were among the best hybrids for grain yield and tryptophan content across all environments, indicating the genetic gains in the CIMMYT QPM breeding programme and recommended for use in hybrid programmes aimed at reducing food insecurity and malnutrition problems in ESA region.

The concentration of Fe and Zn also decreased by 48.2% and 36.4% under low N stress and 62.6% and 8.77% under random drought stress, respectively. Large variability was observed among evaluated hybrids for Fe and Zn concentrations. Some QPM hybrids showed good performance for Fe and Zn concentration compared with the best QPM and normal checks across optimum, low N, random drought stress and across all environments. For example, in combined analysis, entries 28 (CZH17197Q) and 12 (CZH15099Q) for Fe and entries 15 (CZH16006Q), 14 (CZH15142Q), 29 (CZH17198Q) and 30 (CZH17199Q) for Zn were a few of the top performing hybrids. Increasing concentration of Fe and Zn in maize genotypes is not enough, as maize genotypes should have low concentrations of anti-nutrients such as phytic

acid. In this study, low phytic acid content and molar ratios with Fe and Zn were observed in some QPM genotypes across all environments, suggesting high Fe and Zn bioavailability.

Significant environment and G × E interaction effects indicated the inconsistent grain yield performance of hybrids, which changed in ranking from location to location. Because of this, multi-location and multi-season trials are vital to reduce the negative impact of G × E on the selection of potential hybrids. The AMMI and GGE models are currently the most important tools for quantifying and explaining G × E effects in maize breeding. Entries 10 (CZH142238Q) and 14 (CZH15142Q) were performed better than all QPM and non-QPM commercial checks under stressed and non-stressed environments and identified by AMMI and GGE biplot analysis as the best yielding and stable hybrids and recommend these QPM hybrids should be advanced into variety verification for commercialization to improve food and nutritional security of smallholder farmers in SSA who depend on maize. GGE biplot analysis was effective in defining mega-environments for the different management conditions. The polygon showed four mega environments under optimum, three mega environments under random drought, two mega environments under managed drought and three mega environments under low N conditions. Environments KW, BIN and BK2 were identified as the most discriminating and representative for optimum, random drought stress and low N conditions, respectively, hence these sites are ideal environments for selection in the respective management conditions.

Knowing the relationship of traits recorded in different environment conditions (especially drought and lown) could contribute to improving the performance of genotypes under stress non-stress environment conditions. In this study, grain yield showed significant and positive association with number of ears per plant, and negative and significant association with days to anthesis and silking, and ASI under low N stress, which confirmed their importance in developing high yielding and early maturing genotypes. Almost none of the nutritional traits correlated significantly with yield under any of the management conditions, indicating a lack of common genes for simultaneous improvement of grain and nutritional traits. Fe and Zn were highly significantly correlated under low N and random stress conditions. In general, the results of this study showed the possibility of identifying QPM hybrids that have excellent performance for grain yield and nutritional traits under optimal and stress conditions.

LIST OF APPENDICES

Appendix 1 Grain yield and agronomic performance of QPM hybrids and commercial checks evaluated under optimum conditions during 2018 to 2020 in ESA

Entry	Genotype name	GY t ha^{-1}	DA days	DS days	ASI days	PH cm	EH cm	EPO ratio	EPP #
1	ZS261	7.3	70.11	71.39	1.29	250.39	124.46	0.50	1.06
2	Mama MQ623	7.8	70.29	71.71	1.43	243.96	122.71	0.50	1.10
3	PAN53	8.8	72.11	73.14	1.04	256.36	129.25	0.51	0.98
4	SC533	7.6	70.07	70.96	0.89	247.89	121.64	0.49	0.98
5	CZH04032	7.8	70.46	71.64	1.18	244.96	118.64	0.49	1.13
6	CZH132044Q	8.7	72.39	73.14	0.75	258.93	134.57	0.52	1.21
7	CZH132018Q	8.2	72.21	73.25	1.04	244.11	123.32	0.51	1.08
8	CZH142236Q	8.4	71.11	72.07	0.96	235.86	118.04	0.50	1.23
9	CZH142237Q	8.8	71.68	72.04	0.36	243.96	121.04	0.50	1.29
10	CZH142238Q	8.7	71.00	71.82	0.82	243.89	118.68	0.49	1.20
11	CZH15098Q	8.6	69.75	70.54	0.79	245.07	122.93	0.50	1.27
12	CZH15099Q	8.9	70.46	71.14	0.68	245.96	125.21	0.51	1.23
13	CZH15117Q	8.5	70.96	71.96	1.00	248.29	122.96	0.50	1.18
14	CZH15142Q	8.7	70.64	71.64	1.00	250.18	120.14	0.48	1.23
15	CZH16006Q	8.5	73.68	75.50	1.82	259.50	135.50	0.52	1.14
16	CZH16022Q	8.4	72.86	74.68	1.82	236.21	117.47	0.50	1.00
17	CZH16021Q	8.8	71.75	73.11	1.36	257.21	129.71	0.50	1.02
18	CZH17187Q	8.1	72.36	73.82	1.46	260.75	137.54	0.53	1.25
19	CZH17188Q	8.1	69.68	70.93	1.25	243.46	124.86	0.51	1.17
20	CZH17189Q	8.7	70.96	71.68	0.71	264.71	140.07	0.53	1.32
21	CZH17190Q	7.9	71.86	72.79	0.93	261.21	133.07	0.51	1.01
22	CZH17191Q	8.4	71.54	71.79	0.25	249.07	132.79	0.53	1.09
23	CZH17192Q	8.6	72.25	73.89	1.64	266.93	135.93	0.51	1.11
24	CZH17193Q	8.6	72.46	73.36	0.89	268.82	140.86	0.52	1.16
25	CZH17194Q	7.9	72.54	73.79	1.25	253.46	128.32	0.51	1.19
26	CZH17195Q	7.7	73.29	74.64	1.36	254.64	131.39	0.52	1.18
27	CZH17196Q	8.2	71.11	72.04	0.93	260.68	132.61	0.51	1.07
28	CZH17197Q	8.3	72.68	73.43	0.75	260.00	130.18	0.50	1.03
29	CZH17198Q	8.4	71.89	72.82	0.93	267.64	145.14	0.54	1.10
30	CZH17199Q	8.1	71.36	73.11	1.75	258.50	127.84	0.50	1.13
31	CZH17200Q	8.0	71.68	72.68	1.00	257.39	133.07	0.52	1.01
32	CZH17201Q	7.8	73.54	74.32	0.79	257.82	123.86	0.48	1.01
33	CZH17202Q	8.3	72.14	74.04	1.89	257.61	134.61	0.52	1.23
34	CZH17203Q	8.5	72.57	73.75	1.18	258.00	138.21	0.54	1.16
35	CZH17204Q	8.1	70.39	71.61	1.21	255.89	132.29	0.52	1.28
36	CZH17205Q	8.1	70.93	72.61	1.68	251.68	133.18	0.53	1.25
37	CZH17206Q	8.5	71.82	72.86	1.04	254.75	129.32	0.51	1.27
38	CZH17207Q	8.7	74.04	74.71	0.68	259.29	143.96	0.56	1.29
39	CZH17208Q	7.8	73.21	74.21	1.00	257.57	139.39	0.54	1.23
40	CZH17209Q	7.8	70.21	71.50	1.29	252.93	133.54	0.53	1.30
41	CZH17210Q	8.4	69.79	70.71	0.93	251.14	130.46	0.52	1.28
42	MH1633	7.9	71.46	72.25	0.79	249.46	120.68	0.48	1.05
43	MH1634	8.3	70.68	71.14	0.46	244.71	121.68	0.50	1.20
44	QS7646	8.9	70.18	72.29	2.11	253.11	129.21	0.51	1.27
	Mean	8.28	71.55	72.65	1.10	253.27	129.33	0.51	1.16
	LSD	0.45	0.78	0.75	0.59	7.24	6.64	0.02	0.08

GY = grain yield; DA = days to anthesis; DS = days to silking; ASI = anthesis silking interval; PH = plant height; EH = ear height; EPO = ear position; EPP = number of ears per plant; LSD = least significant difference

Appendix 2 Grain yield and agronomic performance of QPM hybrids and commercial checks evaluated under random stress conditions during 2018 to 2020 in ESA

Entry	Genotype	GY t ha^{-1}	DA days	DS days	ASI days	PH cm	EH cm	EPO ratio	EPP #
1	ZS261	3.19	65.44	67.39	1.94	191.65	85.92	0.44	1.00
2	Mama MQ623	4.09	65.28	67.67	2.39	182.67	90.87	0.49	0.97
3	PAN53	4.94	66.50	68.78	2.28	212.54	103.76	0.48	0.86
4	SC533	3.54	64.72	66.44	1.72	196.46	89.06	0.44	0.95
5	CZH04032	3.71	65.78	67.83	2.06	196.28	94.51	0.48	0.98
6	CZH132044Q	4.50	67.61	69.50	1.89	209.92	98.56	0.46	1.04
7	CZH132018Q	4.11	66.67	68.61	1.94	188.87	93.53	0.48	0.93
8	CZH142236Q	4.74	66.89	68.56	1.67	193.71	90.43	0.46	1.14
9	CZH142237Q	4.76	66.50	68.06	1.56	201.03	98.69	0.48	1.09
10	CZH142238Q	4.82	65.22	67.06	1.83	192.04	92.80	0.47	1.11
11	CZH15098Q	4.36	64.83	66.89	2.06	189.69	88.64	0.46	1.14
12	CZH15099Q	4.53	65.17	67.22	2.06	195.69	97.13	0.49	1.18
13	CZH15117Q	4.45	65.94	67.94	2.00	197.94	92.44	0.46	1.02
14	CZH15142Q	5.33	65.22	67.00	1.78	202.46	93.08	0.45	1.05
15	CZH16006Q	4.21	68.33	70.72	2.39	207.85	103.30	0.49	0.91
16	CZH16022Q	4.58	67.44	69.06	1.61	188.81	87.78	0.46	0.96
17	CZH16021Q	4.50	67.61	69.06	1.44	215.53	102.44	0.47	0.93
18	CZH17187Q	4.63	66.39	68.56	2.17	215.46	101.24	0.46	1.12
19	CZH17188Q	4.56	65.00	67.22	2.22	202.31	98.22	0.48	1.08
20	CZH17189Q	4.00	65.56	67.33	1.78	208.72	109.36	0.52	1.06
21	CZH17190Q	3.78	68.06	69.83	1.78	213.03	100.54	0.47	1.02
22	CZH17191Q	4.29	66.00	67.72	1.72	202.11	93.70	0.46	0.98
23	CZH17192Q	4.89	67.33	69.11	1.78	211.16	102.29	0.48	0.98
24	CZH17193Q	4.39	67.44	69.33	1.89	213.57	103.92	0.48	0.98
25	CZH17194Q	3.94	67.22	69.50	2.28	199.24	98.04	0.49	0.92
26	CZH17195Q	4.12	67.50	69.89	2.39	199.82	96.18	0.48	1.07
27	CZH17196Q	3.78	67.39	69.56	2.17	200.43	95.64	0.47	1.00
28	CZH17197Q	4.18	68.17	70.06	1.89	202.27	100.00	0.49	0.98
29	CZH17198Q	4.31	67.11	69.33	2.22	212.02	105.28	0.49	1.00
30	CZH17199Q	4.21	66.39	69.39	3.00	215.19	102.01	0.47	1.02
31	CZH17200Q	4.13	67.11	69.06	1.94	209.57	101.08	0.47	0.99
32	CZH17201Q	4.20	67.39	69.50	2.11	215.09	102.27	0.47	0.97
33	CZH17202Q	3.79	67.11	69.56	2.44	202.46	97.86	0.48	1.10
34	CZH17203Q	4.74	66.67	68.56	1.89	202.16	100.43	0.49	1.13
35	CZH17204Q	4.20	66.11	68.39	2.28	213.89	105.83	0.49	1.12
36	CZH17205Q	4.52	66.50	68.44	1.94	204.74	96.86	0.47	1.04
37	CZH17206Q	4.51	66.72	68.50	1.78	204.58	96.88	0.46	1.22
38	CZH17207Q	4.68	69.00	70.89	1.89	206.67	105.99	0.51	1.16
39	CZH17208Q	4.57	66.67	68.67	2.00	197.22	93.75	0.47	1.06
40	CZH17209Q	4.06	65.89	67.67	1.78	199.41	101.06	0.50	1.07
41	CZH17210Q	3.97	64.94	66.50	1.56	195.62	93.74	0.47	1.06
42	MH1633	4.35	66.94	68.67	1.72	197.33	93.52	0.46	0.98
43	MH1634	3.99	66.39	67.89	1.50	198.62	93.31	0.46	1.03
44	QS7646	4.89	65.56	67.83	2.28	197.57	91.19	0.46	1.12
	Mean	4.32	66.54	68.52	1.98	202.35	97.34	0.47	1.03
	LSD	0.54	1.04	1.13	0.52	12.25	7.32	0.03	0.10

GY = grain yield; DA = days to anthesis; DS = days to silking; ASI = anthesis silking interval; PH = plant height; EH = ear height; EPO = ear position; EPP = number of ears per plant; LSD = least significant difference

Appendix 3 Grain yield and agronomic performance of QPM hybrids and commercial checks evaluated under managed drought conditions evaluated during 2018 to 2020 in ESA

Entry	Genotype	GY t ha^{-1}	DA days	DS days	ASI days	PH cm	EH cm	EPO ratio	EPP #
1	ZS261	2.6	61.0	65.5	4.5	186.4	88.5	0.47	0.9
2	Mama MQ623	2.8	62.3	65.5	3.2	172.7	88.0	0.49	0.9
3	PAN53	3.0	63.8	66.3	2.5	183.8	92.4	0.50	0.9
4	SC533	2.1	61.0	64.5	3.5	168.2	80.8	0.47	0.7
5	CZH04032	2.6	63.3	67.3	4.0	179.1	91.0	0.49	0.9
6	CZH132044Q	2.3	66.2	70.0	3.8	185.1	93.5	0.49	0.9
7	CZH132018Q	3.0	64.7	68.3	3.7	156.9	81.7	0.51	1.0
8	CZH142236Q	3.1	63.0	64.7	1.7	190.1	86.8	0.46	0.9
9	CZH142237Q	3.6	64.2	66.0	1.8	165.9	87.5	0.52	0.9
10	CZH142238Q	3.5	62.5	64.7	2.2	181.4	85.0	0.46	0.9
11	CZH15098Q	2.6	60.5	63.7	3.2	167.8	79.8	0.47	1.0
12	CZH15099Q	3.2	62.0	63.8	1.8	172.8	95.2	0.54	1.0
13	CZH15117Q	3.0	62.8	66.0	3.2	174.7	83.0	0.47	0.9
14	CZH15142Q	3.2	62.5	64.2	1.7	180.3	83.3	0.46	1.0
15	CZH16006Q	2.8	65.2	69.8	4.7	191.6	107.9	0.55	0.8
16	CZH16022Q	2.3	64.3	69.3	5.0	168.0	83.5	0.48	0.8
17	CZH16021Q	2.5	63.2	67.7	4.5	184.0	97.9	0.52	0.9
18	CZH17187Q	2.3	63.5	68.5	5.0	193.9	95.9	0.48	0.8
19	CZH17188Q	3.0	63.3	66.7	3.3	169.8	89.6	0.51	0.9
20	CZH17189Q	2.7	62.8	65.5	2.7	177.0	96.4	0.53	0.8
21	CZH17190Q	2.5	65.2	70.7	5.5	184.0	92.5	0.49	0.8
22	CZH17191Q	3.1	63.8	65.2	1.3	176.9	94.4	0.52	0.9
23	CZH17192Q	3.3	64.5	67.5	3.0	194.6	106.9	0.53	0.8
24	CZH17193Q	2.2	63.3	68.0	4.7	185.2	95.5	0.50	0.7
25	CZH17194Q	1.8	64.8	69.3	4.5	168.5	87.7	0.50	0.8
26	CZH17195Q	1.9	64.0	68.8	4.8	168.1	89.2	0.52	0.9
27	CZH17196Q	1.9	64.0	67.7	3.7	174.8	96.1	0.54	0.9
28	CZH17197Q	2.8	65.3	69.2	3.8	191.1	108.9	0.55	0.8
29	CZH17198Q	2.7	63.7	67.5	3.8	187.7	103.4	0.53	0.8
30	CZH17199Q	2.5	64.7	69.7	5.0	183.6	94.8	0.50	0.9
31	CZH17200Q	2.3	65.3	68.5	3.2	173.2	98.3	0.56	1.0
32	CZH17201Q	2.3	64.8	68.2	3.3	175.8	83.6	0.47	0.9
33	CZH17202Q	2.5	65.3	69.2	3.8	170.1	84.0	0.48	1.0
34	CZH17203Q	2.3	65.0	70.2	5.2	172.1	96.1	0.54	0.9
35	CZH17204Q	3.1	62.0	64.3	2.3	184.5	92.9	0.49	1.0
36	CZH17205Q	2.4	62.8	67.0	4.2	171.0	87.9	0.49	0.9
37	CZH17206Q	2.4	62.0	66.0	4.0	172.9	86.2	0.48	0.9
38	CZH17207Q	2.8	66.2	71.0	4.8	176.0	99.3	0.55	0.9
39	CZH17208Q	2.4	62.7	67.3	4.7	171.7	90.0	0.51	0.9
40	CZH17209Q	3.3	62.5	64.7	2.2	178.2	101.6	0.55	1.0
41	CZH17210Q	2.7	61.5	63.2	1.7	173.7	92.1	0.52	1.0
42	MH1633	2.7	64.3	67.0	2.7	169.9	80.4	0.46	0.8
43	MH1634	2.6	62.0	63.8	1.8	169.0	78.5	0.46	0.8
44	QS7646	2.8	63.2	67.3	4.2	181.3	91.6	0.49	0.8
Mean		2.67	63.53	67.03	3.50	177.34	91.35	0.50	0.88
LSD		0.63	1.20	1.73	1.47	12.35	7.20	0.03	0.13

GY = grain yield; DA = days to anthesis; DS = days to silking; ASI = anthesis silking interval; PH = plant height; EH = ear height; EPO = ear position; EPP = number of ears per plant; LSD = least significant difference

Appendix 4 Grain yield and agronomic performance of QPM hybrids and commercial checks evaluated under low N stress conditions evaluated during 2018 to 2020 in ESA

Entry	Genotype	GY t ha^{-1}	DA days	DS days	ASI days	PH cm	EH cm	EPO ratio	EPP #	Sen 0 -10
1	ZS261	2.24	79.56	82.19	2.63	186.13	86.44	0.46	0.85	3.43
2	MamaMQ623	2.77	78.63	81.31	2.69	188.25	93.19	0.50	0.98	3.00
3	PAN53	2.83	80.56	83.94	3.38	214.63	96.63	0.45	0.89	2.83
4	SC533	2.10	80.13	82.69	2.56	186.81	87.25	0.47	0.91	3.18
5	CZH04032	2.44	79.19	81.63	2.44	185.56	90.56	0.48	0.96	2.73
6	CZH132044Q	2.60	82.13	84.38	2.25	205.38	99.19	0.48	0.90	3.21
7	CZH132018Q	2.86	81.69	84.75	3.06	187.56	90.19	0.48	0.91	2.53
8	CZH142236Q	2.21	82.25	85.06	2.81	181.75	88.63	0.48	0.98	3.15
9	CZH142237Q	2.60	81.63	83.94	2.31	192.63	91.13	0.47	0.97	3.11
10	CZH142238Q	2.59	80.31	82.63	2.31	188.88	83.50	0.44	1.12	3.28
11	CZH15098Q	2.96	78.94	80.88	1.94	200.38	98.06	0.49	1.00	3.08
12	CZH15099Q	2.63	79.44	82.25	2.81	187.56	95.63	0.52	0.99	3.19
13	CZH15117Q	2.59	81.25	83.94	2.69	197.31	92.63	0.47	0.94	2.84
14	CZH15142Q	3.19	79.56	82.06	2.50	196.00	94.00	0.48	1.02	3.00
15	CZH16006Q	2.32	84.88	89.69	4.81	199.69	97.81	0.49	0.89	2.58
16	CZH16022Q	2.57	82.63	86.13	3.50	191.69	85.63	0.44	0.91	2.76
17	CZH16021Q	2.51	82.50	86.88	4.38	199.25	96.00	0.48	0.89	3.01
18	CZH17187Q	2.12	80.94	84.50	3.56	205.56	99.13	0.48	0.99	3.35
19	CZH17188Q	2.37	78.75	83.69	4.94	187.13	90.25	0.48	0.90	3.38
20	CZH17189Q	2.39	80.50	83.38	2.88	204.44	103.06	0.50	1.01	4.09
21	CZH17190Q	2.06	82.56	85.88	3.31	202.13	90.75	0.44	0.82	3.40
22	CZH17191Q	2.26	81.69	84.31	2.63	197.13	94.06	0.47	0.92	2.78
23	CZH17192Q	2.24	81.75	85.56	3.81	205.88	103.19	0.50	0.89	2.96
24	CZH17193Q	2.03	82.19	86.06	3.88	206.19	99.25	0.48	0.82	2.93
25	CZH17194Q	2.48	82.63	87.31	4.69	193.13	97.00	0.50	0.83	3.19
26	CZH17195Q	2.07	83.25	87.94	4.69	189.56	93.81	0.49	0.90	3.46
27	CZH17196Q	2.83	80.19	83.38	3.19	202.50	98.88	0.49	0.96	2.66
28	CZH17197Q	1.93	83.38	86.94	3.56	191.63	93.56	0.49	0.82	3.63
29	CZH17198Q	2.60	81.56	84.56	3.00	200.06	96.38	0.48	0.88	3.00
30	CZH17199Q	2.00	81.06	85.31	4.25	198.94	92.69	0.46	0.88	3.63
31	CZH17200Q	2.08	80.94	84.94	4.00	209.50	102.06	0.49	0.86	3.88
32	CZH17201Q	2.36	82.69	85.50	2.81	198.50	88.88	0.44	0.92	3.43
33	CZH17202Q	2.21	81.44	85.06	3.63	202.06	93.81	0.46	0.94	3.59
34	CZH17203Q	2.97	81.75	85.56	3.81	199.13	102.75	0.51	0.90	2.98
35	CZH17204Q	2.47	79.88	82.44	2.56	202.31	100.50	0.50	0.97	3.93
36	CZH17205Q	1.92	79.81	83.19	3.38	193.44	93.69	0.48	0.92	4.11
37	CZH17206Q	2.15	81.31	85.44	4.13	196.50	94.38	0.48	0.82	4.00
38	CZH17207Q	2.21	84.50	88.44	3.94	197.50	96.69	0.49	0.90	2.71
39	CZH17208Q	2.02	80.44	84.25	3.81	201.44	97.31	0.48	0.83	3.60
40	CZH17209Q	2.37	80.19	83.81	3.63	192.44	96.25	0.50	0.89	3.41
41	CZH17210Q	2.00	81.00	84.75	3.75	188.56	89.19	0.47	0.90	3.09
42	MH1633	2.44	80.44	83.13	2.69	190.31	89.31	0.47	0.88	3.55
43	MH1634	2.65	78.50	80.81	2.31	188.69	86.06	0.46	1.03	3.09
44	QS7646	2.55	78.94	83.50	4.56	196.81	92.88	0.47	1.07	2.94
Mean		2.40	81.08	84.41	3.33	196.16	94.14	0.48	0.92	3.22
LSD		0.32	1.44	1.56	1.03	9.63	7.75	0.03	0.10	0.53

GY = grain yield; DA = days to anthesis; DS = days to silking; ASI = anthesis silking interval; PH = plant height; EH = ear height; EPO = ear position; EPP = number of ears per plant; sen = leaf senescence; LSD = least significant difference

Appendix 5 Mean values for nutritional traits of QPM hybrids and commercial checks evaluated under optimum conditions at Bako during 2018 to 2020

Entry	Genotype	Protein	Tryptophan	QI	Fe	Zn	PA	MRFe	MRZn
1	ZS261	8.29	0.081	0.98	34.96	19.22	5.57	13.89	29.16
2	Mama MQ623	7.58	0.085	1.13	21.98	13.25	5.66	22.18	46.09
3	PAN53	8.97	0.062	0.69	27.70	23.37	6.16	20.04	28.95
4	SC533	8.61	0.060	0.69	41.16	14.35	5.54	12.26	39.25
5	CZH04032	8.22	0.090	1.11	25.76	17.24	5.82	19.83	33.60
6	CZH132044Q	7.40	0.090	1.23	36.15	14.08	5.44	15.97	41.54
7	CZH132018Q	8.80	0.107	1.21	25.30	23.31	6.16	26.27	28.26
8	CZH142236Q	7.48	0.096	1.29	28.76	21.76	5.38	20.79	26.80
9	CZH142237Q	6.91	0.102	1.47	22.94	18.59	5.18	20.01	33.00
10	CZH142238Q	8.10	0.098	1.21	31.29	21.96	6.10	17.54	33.83
11	CZH15098Q	8.12	0.077	0.94	29.68	22.32	5.49	17.17	30.41
12	CZH15099Q	6.93	0.081	1.20	36.05	10.86	4.97	14.06	51.00
13	CZH15117Q	7.52	0.083	1.12	27.05	21.63	5.08	17.31	24.49
14	CZH15142Q	7.67	0.101	1.32	24.95	31.44	5.71	23.28	18.16
15	CZH16006Q	8.37	0.072	0.85	31.95	24.05	6.08	16.99	25.97
16	CZH16022Q	8.13	0.101	1.33	25.43	22.39	6.00	21.14	28.93
17	CZH16021Q	9.38	0.063	0.68	37.19	19.80	5.83	18.38	31.48
18	CZH17187Q	8.52	0.065	0.80	29.35	21.19	5.88	17.89	31.49
19	CZH17188Q	8.21	0.075	0.94	27.00	12.18	4.99	16.65	42.62
20	CZH17189Q	7.71	0.075	0.98	31.86	14.93	5.78	15.69	38.28
21	CZH17190Q	8.85	0.065	0.75	33.01	19.86	5.64	14.60	30.63
22	CZH17191Q	8.25	0.072	0.88	27.56	25.83	5.10	16.72	19.49
23	CZH17192Q	8.21	0.081	0.98	31.18	25.74	5.93	17.53	23.34
24	CZH17193Q	9.48	0.103	1.11	22.59	20.18	6.09	23.72	30.25
25	CZH17194Q	9.17	0.073	0.81	33.48	18.91	5.81	17.49	30.56
26	CZH17195Q	8.59	0.062	0.73	34.15	16.32	5.74	14.44	35.50
27	CZH17196Q	7.91	0.085	1.10	30.66	23.30	5.99	17.14	27.18
28	CZH17197Q	8.05	0.076	0.96	29.20	16.87	5.74	16.90	34.03
29	CZH17198Q	7.86	0.102	1.30	28.86	26.81	5.73	18.14	21.56
30	CZH17199Q	9.05	0.068	0.75	27.76	25.57	4.99	15.81	21.26
31	CZH17200Q	8.18	0.074	0.91	27.76	16.32	6.11	18.85	40.06
32	CZH17201Q	8.24	0.075	0.94	19.58	16.68	5.87	25.59	36.19
33	CZH17202Q	8.13	0.077	0.97	28.61	20.08	5.40	26.31	27.44
34	CZH17203Q	8.14	0.085	1.03	29.79	22.84	5.64	16.23	28.46
35	CZH17204Q	7.93	0.075	0.98	26.65	17.32	5.54	17.87	34.01
36	CZH17205Q	6.71	0.070	1.04	21.62	19.39	5.14	21.67	29.01
37	CZH17206Q	9.48	0.087	0.93	36.88	19.15	6.49	16.39	37.51
38	CZH17207Q	7.58	0.085	1.13	20.70	17.68	5.39	25.52	35.07
39	CZH17208Q	8.04	0.070	0.89	22.58	18.82	5.13	19.36	32.11
40	CZH17209Q	7.81	0.075	0.96	29.80	20.42	5.90	17.69	31.53
41	CZH17210Q	7.76	0.079	1.05	28.70	17.74	5.78	17.37	34.13
42	MH1633	8.94	0.093	1.05	28.18	24.44	6.13	18.60	24.77
43	MH1634	7.07	0.098	1.40	27.19	13.06	5.53	19.39	42.71
44	QS7646	7.60	0.082	1.12	24.99	22.07	4.66	16.97	22.69
	Mean	8.13	0.08	1.02	28.82	19.85	5.64	18.58	31.65
	LSD	0.86	0.016	0.23	5.85	3.82	0.44	4.95	8.33

QI = protein quality index; Fe = iron; Zn = zinc; PA = phytic acid; MRFe = phytic acid to Fe molar ratio; MRZn = phytic acid to zinc molar ratio; LSD = least significant difference

Appendix 6 Mean values for nutritional traits of QPM hybrids and commercial checks evaluated under low N stress conditions at Bako during 2018 to 2020

Entry	Genotype	Protein	Tryptophan	QI	Fe	Zn	PA	MRFe	MRZn
1	ZS261	5.26	0.044	0.83	23.39	11.49	4.41	15.95	37.74
2	MamaMQ623	4.28	0.068	1.58	16.71	13.52	4.35	22.71	32.56
3	PAN53	5.00	0.051	1.03	20.36	10.63	4.62	20.51	43.30
4	SC533	5.11	0.044	0.82	17.53	12.25	5.31	26.56	43.25
5	CZH04032	5.51	0.076	1.39	15.01	14.94	5.07	30.59	33.43
6	CZH132044Q	5.01	0.074	1.47	13.03	12.51	4.58	30.04	36.48
7	CZH132018Q	5.80	0.068	1.19	19.48	11.91	5.01	21.91	42.55
8	CZH142236Q	5.93	0.084	1.40	26.39	12.17	4.59	15.02	43.62
9	CZH142237Q	4.49	0.060	1.32	28.81	19.95	5.12	15.23	25.30
10	CZH142238Q	5.96	0.077	1.30	17.61	9.11	5.09	25.14	61.04
11	CZH15098Q	5.23	0.049	0.99	20.16	14.53	4.58	19.28	31.24
12	CZH15099Q	3.78	0.062	1.64	18.12	8.79	4.11	19.57	46.12
13	CZH15117Q	5.47	0.084	1.53	11.75	11.63	3.84	28.94	34.10
14	CZH15142Q	5.47	0.068	1.27	23.93	8.38	4.73	17.14	61.47
15	CZH16006Q	6.25	0.054	0.88	22.25	16.93	4.68	18.61	27.54
16	CZH16022Q	3.85	0.065	1.69	16.79	12.38	4.78	25.22	38.01
17	CZH16021Q	6.11	0.055	0.90	14.04	9.25	4.66	28.53	49.70
18	CZH17187Q	4.58	0.072	1.60	18.71	7.86	4.03	18.36	57.55
19	CZH17188Q	4.72	0.065	1.38	18.86	10.70	4.86	21.94	45.04
20	CZH17189Q	5.77	0.053	0.90	18.11	13.36	4.39	20.73	32.43
21	CZH17190Q	6.87	0.076	1.10	16.24	8.83	5.05	28.92	56.29
22	CZH17191Q	5.17	0.064	1.24	8.53	12.76	4.39	48.19	33.81
23	CZH17192Q	6.18	0.073	1.18	15.73	8.57	5.60	33.69	68.56
24	CZH17193Q	4.97	0.058	1.21	18.70	16.12	4.38	19.96	26.81
25	CZH17194Q	5.48	0.059	1.09	21.16	12.08	4.88	20.89	40.03
26	CZH17195Q	4.31	0.047	1.16	16.11	11.30	4.28	23.33	37.46
27	CZH17196Q	5.43	0.074	1.39	16.53	13.40	5.00	25.84	37.27
28	CZH17197Q	6.10	0.061	1.00	22.39	23.47	4.43	16.93	19.10
29	CZH17198Q	5.24	0.094	1.83	16.63	13.47	4.65	24.99	34.04
30	CZH17199Q	5.22	0.065	1.27	16.57	17.10	5.04	26.26	31.14
31	CZH17200Q	6.04	0.057	0.97	15.97	10.19	4.98	26.44	49.70
32	CZH17201Q	6.70	0.097	1.43	15.72	9.25	4.55	24.62	48.45
33	CZH17202Q	4.66	0.060	1.29	16.77	12.64	4.28	22.55	34.35
34	CZH17203Q	7.14	0.062	0.86	11.48	12.71	4.97	36.87	38.47
35	CZH17204Q	6.18	0.047	0.75	16.19	9.26	4.90	26.09	52.01
36	CZH17205Q	4.25	0.058	1.38	13.04	11.62	5.45	35.44	46.26
37	CZH17206Q	5.92	0.052	0.87	26.30	14.26	5.14	17.16	42.28
38	CZH17207Q	5.16	0.069	1.34	11.55	9.81	4.60	34.11	46.34
39	CZH17208Q	4.98	0.055	1.11	17.87	8.59	4.17	20.28	48.99
40	CZH17209Q	5.41	0.059	1.08	19.49	12.43	4.72	20.91	37.68
41	CZH17210Q	4.99	0.065	1.32	18.99	9.77	4.99	23.10	50.87
42	MH1633	5.41	0.077	1.46	22.79	13.98	5.52	20.62	39.44
43	MH1634	5.83	0.062	1.10	21.93	10.38	4.23	16.84	43.15
44	QS7646	4.52	0.058	1.30	12.37	10.28	3.91	28.04	37.46
	Mean	5.356	0.064	1.221	17.954	12.146	4.700	24.181	41.419
	LSD	1.35	0.023	0.56	7.43	5.05	0.67	12.36	21.89

QI = protein quality index; Fe = iron; Zn = zinc; PA = phytic acid; MRFe = phytic acid to Fe molar ratio; MRZn = phytic acid to zinc molar ratio; LSD = least significant difference

Appendix 7 Mean values for nutritional traits of QPM hybrids and commercial checks evaluated under optimum conditions at Ambo during 2018 to 2020

Entry	Genotype	Protein	Tryptophan	QI	Fe	Zn	PA	MRFe	MRZn
1	ZS261	8.28	0.094	1.13	35.37	17.45	6.95	22.72	40.53
2	Mama MQ623	8.04	0.091	1.14	19.32	17.46	6.29	28.51	38.30
3	PAN53	9.08	0.055	0.62	28.50	26.41	6.84	21.88	26.87
4	SC533	8.97	0.062	0.70	24.40	23.31	5.66	24.29	24.63
5	CZH04032	7.52	0.092	1.22	31.87	22.68	5.99	21.43	29.83
6	CZH132044Q	7.81	0.094	1.20	30.80	17.71	6.29	17.72	35.95
7	CZH132018Q	7.45	0.091	1.23	29.25	20.59	6.57	23.77	31.49
8	CZH142236Q	7.28	0.101	1.39	13.92	22.23	5.78	36.05	25.84
9	CZH142237Q	7.36	0.096	1.32	27.62	24.28	6.66	22.24	27.29
10	CZH142238Q	6.88	0.103	1.49	37.65	19.98	6.55	15.30	32.27
11	CZH15098Q	9.14	0.081	0.90	18.06	22.25	5.86	28.30	28.47
12	CZH15099Q	6.72	0.096	1.43	42.37	16.03	5.75	15.00	36.64
13	CZH15117Q	7.17	0.084	1.19	19.93	16.49	5.71	45.47	37.31
14	CZH15142Q	8.02	0.101	1.26	24.30	21.95	6.81	43.62	31.51
15	CZH16006Q	7.86	0.070	0.90	26.72	33.88	6.14	24.79	18.73
16	CZH16022Q	8.30	0.115	1.40	30.14	14.54	6.56	19.44	47.82
17	CZH16021Q	8.48	0.086	1.03	26.40	20.97	6.93	30.49	33.14
18	CZH17187Q	8.42	0.081	0.98	35.62	12.53	6.40	16.04	70.12
19	CZH17188Q	7.64	0.059	0.78	26.23	17.49	5.71	37.61	32.15
20	CZH17189Q	8.47	0.091	1.08	29.46	20.16	6.83	20.10	34.73
21	CZH17190Q	8.40	0.074	0.89	33.12	22.93	5.68	16.00	26.23
22	CZH17191Q	6.99	0.065	0.96	28.81	23.03	6.42	25.92	30.55
23	CZH17192Q	7.57	0.075	1.01	23.41	24.76	6.06	25.46	24.91
24	CZH17193Q	9.68	0.088	0.91	37.41	24.57	6.36	15.04	25.83
25	CZH17194Q	8.96	0.063	0.71	30.72	19.18	6.36	17.91	33.34
26	CZH17195Q	8.67	0.067	0.79	21.05	13.52	5.95	27.86	50.67
27	CZH17196Q	7.80	0.064	0.82	26.35	21.31	6.19	20.30	30.45
28	CZH17197Q	7.88	0.085	1.07	33.60	20.31	5.92	18.91	29.03
29	CZH17198Q	9.30	0.087	0.95	22.65	23.28	5.82	28.90	25.25
30	CZH17199Q	8.77	0.072	0.82	27.09	22.87	5.35	17.35	23.46
31	CZH17200Q	8.93	0.080	0.90	16.14	20.16	5.63	29.66	28.13
32	CZH17201Q	8.14	0.085	1.07	35.69	21.65	6.06	19.65	28.31
33	CZH17202Q	8.08	0.069	0.85	24.81	25.65	6.19	22.89	23.98
34	CZH17203Q	7.82	0.084	1.07	22.69	16.15	5.52	22.52	34.13
35	CZH17204Q	7.42	0.082	1.14	34.32	15.39	5.98	17.34	39.90
36	CZH17205Q	8.55	0.102	1.19	30.98	20.75	6.21	19.07	30.00
37	CZH17206Q	8.10	0.082	1.02	23.67	17.49	5.62	20.66	32.89
38	CZH17207Q	7.67	0.079	1.04	23.25	20.68	5.94	27.46	28.59
39	CZH17208Q	7.66	0.084	1.10	22.03	15.76	6.15	24.79	38.75
40	CZH17209Q	8.21	0.086	1.06	23.13	18.68	5.69	22.02	30.53
41	CZH17210Q	8.71	0.080	0.92	21.74	21.69	5.51	25.20	25.24
42	MH1633	7.77	0.099	1.27	28.18	21.37	6.62	21.88	30.65
43	MH1634	6.89	0.085	1.25	30.13	12.31	5.19	21.15	51.15
44	QS7646	7.34	0.085	1.17	25.88	19.48	5.35	21.01	29.88
	Mean	8.05	0.08	1.05	27.38	20.26	6.09	23.72	32.62
	LSD	1.11	0.020	0.25	6.11	4.42	0.53	7.35	9.36

QI = protein quality index; Fe = iron; Zn = zinc; PA = phytic acid; MRFe = phytic acid to Fe molar ratio; MRZn = phytic acid to zinc molar ratio; LSD = least significant difference

Appendix 8 Mean values for nutritional traits of QPM hybrids and commercial checks evaluated under low N conditions at Ambo during 2018 to 2020

Entry	Genotype	Protein	Tryptophan	QI	Fe	Zn	PA	MRFe	MRZn
1	ZS261	5.7	0.064	1.12	17.38	8.71	4.24	20.68	48.73
2	MamaMQ623	7.8	0.088	1.15	8.65	17.56	5.49	56.55	31.42
3	PAN53	6.5	0.059	0.92	27.07	15.94	5.04	16.23	33.64
4	SC533	7.4	0.059	0.80	24.79	13.98	5.31	18.51	38.07
5	CZH04032	6.0	0.084	1.40	23.42	11.65	4.98	18.05	42.28
6	CZH132044Q	6.4	0.092	1.43	8.50	18.63	5.47	55.80	29.55
7	CZH132018Q	5.6	0.059	1.04	29.23	16.04	5.08	14.91	31.27
8	CZH142236Q	5.7	0.074	1.32	11.36	22.17	4.72	35.51	21.13
9	CZH142237Q	6.9	0.084	1.25	16.39	17.45	5.21	27.13	29.33
10	CZH142238Q	5.6	0.086	1.54	25.54	14.79	5.10	17.04	34.51
11	CZH15098Q	7.6	0.059	0.79	7.37	21.45	4.75	55.99	21.86
12	CZH15099Q	7.3	0.077	1.08	8.49	14.19	4.54	45.35	31.67
13	CZH15117Q	6.0	0.080	1.38	24.93	15.83	4.60	15.66	28.69
14	CZH15142Q	6.3	0.092	1.46	18.63	23.51	4.89	22.29	20.75
15	CZH16006Q	7.2	0.073	1.02	30.85	19.38	4.54	12.60	23.34
16	CZH16022Q	6.6	0.089	1.34	23.35	14.90	5.33	19.74	35.25
17	CZH16021Q	7.7	0.061	0.79	12.08	21.22	5.13	36.05	23.84
18	CZH17187Q	7.0	0.073	1.04	16.41	10.81	5.40	28.51	49.15
19	CZH17188Q	7.1	0.057	0.79	7.34	12.18	4.37	52.63	36.01
20	CZH17189Q	7.4	0.082	1.12	14.95	17.03	4.01	24.32	24.11
21	CZH17190Q	8.0	0.085	1.06	19.11	11.24	5.36	23.78	47.64
22	CZH17191Q	8.1	0.066	0.82	28.12	12.41	5.06	15.38	40.24
23	CZH17192Q	5.9	0.063	1.10	12.25	23.03	5.27	36.45	22.51
24	CZH17193Q	8.8	0.066	0.74	9.98	17.91	5.22	44.37	28.83
25	CZH17194Q	9.1	0.046	0.50	26.56	18.82	5.32	17.04	28.01
26	CZH17195Q	7.9	0.058	0.74	11.36	9.97	5.16	38.66	51.32
27	CZH17196Q	7.2	0.056	0.77	14.83	17.02	4.60	28.27	26.82
28	CZH17197Q	7.2	0.064	0.88	27.97	19.12	5.40	16.71	28.91
29	CZH17198Q	6.9	0.070	1.05	31.70	22.69	5.41	14.66	23.85
30	CZH17199Q	6.2	0.067	1.10	14.66	16.80	5.20	30.41	30.94
31	CZH17200Q	8.2	0.063	0.79	8.73	22.02	5.70	56.88	25.52
32	CZH17201Q	8.9	0.077	0.87	18.88	20.21	6.07	27.40	30.86
33	CZH17202Q	7.1	0.079	1.16	22.45	19.34	5.15	19.52	26.28
34	CZH17203Q	8.7	0.075	0.86	10.65	14.21	5.08	40.48	51.83
35	CZH17204Q	6.3	0.082	1.29	13.95	22.13	5.02	30.58	22.34
36	CZH17205Q	6.6	0.070	1.05	17.54	17.65	5.26	25.52	29.95
37	CZH17206Q	7.6	0.084	1.10	8.98	15.87	4.81	45.38	29.88
38	CZH17207Q	5.8	0.071	1.22	12.53	11.06	5.17	35.13	46.42
39	CZH17208Q	7.5	0.061	0.81	8.19	11.13	4.95	51.34	52.45
40	CZH17209Q	6.9	0.072	1.04	22.41	18.50	4.88	18.59	26.73
41	CZH17210Q	6.2	0.060	0.96	11.20	17.32	4.42	34.01	25.06
42	MH1633	7.1	0.082	1.17	19.21	18.19	5.13	22.83	28.02
43	MH1634	7.1	0.076	1.08	7.67	14.50	5.25	62.98	37.41
44	QS7646	6.5	0.067	1.03	17.84	12.26	5.27	25.33	44.82
	Mean	7.0	0.071	1.04	17.12	16.61	5.05	30.80	32.75
	LSD	1.5	0.0234	0.3763	6.11	4.42	0.53	7.35	9.36

QI = protein quality index; Fe = iron; Zn = zinc; PA = phytic acid; MRFe = phytic acid to Fe molar ratio; MRZn = phytic acid to zinc molar ratio; LSD = least significant difference

Appendix 9 Mean values for nutritional traits of QPM hybrids and commercial checks evaluated under optimum conditions at Harare during 2018/19

Entry	Genotype	Protein	Tryptophan	QI	Fe	Zn	PA	MRFe	MRZn
1	ZS261	8.6	0.074	0.86	15.31	15.78	4.74	26.27	29.64
2	MamaMQ623	9.9	0.090	0.91	17.43	17.51	4.82	23.46	27.11
3	PAN53	10.9	0.063	0.58	15.17	17.83	4.42	24.70	24.34
4	SC533	10.1	0.060	0.60	17.75	19.91	4.64	22.25	22.99
5	CZH04032	10.0	0.091	0.91	16.60	17.53	4.42	22.61	24.81
6	CZH132044Q	10.8	0.085	0.80	16.71	21.33	4.36	22.13	20.24
7	CZH132018Q	9.9	0.083	0.84	17.81	20.88	5.02	23.94	23.64
8	CZH142236Q	8.2	0.091	1.12	17.83	18.63	4.26	20.39	22.50
9	CZH142237Q	8.4	0.087	1.04	14.00	18.06	3.89	23.60	21.21
10	CZH142238Q	9.1	0.110	1.21	16.67	18.74	4.92	25.29	25.87
11	CZH15098Q	11.5	0.088	0.77	17.75	19.36	4.72	22.68	24.26
12	CZH15099Q	9.7	0.096	1.00	15.76	16.25	4.56	24.54	27.64
13	CZH15117Q	10.8	0.107	0.99	18.70	22.12	4.80	21.84	21.54
14	CZH15142Q	9.1	0.077	0.87	14.61	18.04	4.73	27.44	25.99
15	CZH16006Q	11.4	0.071	0.63	16.26	19.55	4.39	22.92	22.08
16	CZH16022Q	8.4	0.089	1.05	15.07	17.08	3.85	21.70	22.24
17	CZH16021Q	11.5	0.087	0.76	19.02	20.99	4.63	20.76	21.73
18	CZH17187Q	11.8	0.071	0.61	15.77	19.63	3.97	21.35	20.41
19	CZH17188Q	10.3	0.080	0.78	16.37	17.15	4.20	21.72	24.10
20	CZH17189Q	10.7	0.088	0.82	17.03	17.44	5.17	25.76	29.22
21	CZH17190Q	10.6	0.075	0.71	17.42	16.56	4.60	22.42	27.39
22	CZH17191Q	9.4	0.063	0.67	14.46	18.05	4.46	26.14	24.31
23	CZH17192Q	10.3	0.081	0.79	17.36	18.38	4.52	22.08	24.23
24	CZH17193Q	11.9	0.082	0.69	16.48	19.98	5.72	29.64	28.93
25	CZH17194Q	11.3	0.057	0.51	16.27	15.56	4.50	23.58	28.51
26	CZH17195Q	12.0	0.060	0.51	18.45	17.19	4.73	21.96	27.12
27	CZH17196Q	8.8	0.080	0.91	16.39	19.19	4.17	21.78	21.53
28	CZH17197Q	11.4	0.073	0.64	19.84	17.96	4.92	21.31	27.23
29	CZH17198Q	10.7	0.104	0.98	16.95	20.84	4.44	22.24	21.15
30	CZH17199Q	11.0	0.076	0.69	24.02	21.48	4.73	16.76	21.69
31	CZH17200Q	10.9	0.073	0.67	19.24	18.66	4.82	21.51	25.55
32	CZH17201Q	9.8	0.076	0.80	15.72	18.05	4.13	22.30	22.52
33	CZH17202Q	11.0	0.084	0.77	14.93	20.20	4.75	26.98	23.13
34	CZH17203Q	9.2	0.068	0.74	14.30	16.17	4.09	24.25	24.92
35	CZH17204Q	9.6	0.098	1.02	15.87	16.73	4.77	25.61	28.32
36	CZH17205Q	10.2	0.069	0.68	14.08	17.06	4.30	25.93	24.83
37	CZH17206Q	10.1	0.082	0.81	13.56	19.58	4.59	28.70	23.12
38	CZH17207Q	9.7	0.070	0.73	13.85	15.68	4.47	27.34	28.04
39	CZH17208Q	10.7	0.079	0.74	15.08	20.39	4.78	26.87	23.02
40	CZH17209Q	10.2	0.076	0.75	18.78	19.58	4.69	21.30	23.75
41	CZH17210Q	9.2	0.082	0.89	18.42	18.81	4.23	19.49	22.28
42	MH1633	10.3	0.084	0.81	17.48	19.24	4.45	21.78	22.79
43	MH1634	9.1	0.083	0.92	14.57	15.44	4.05	23.72	25.77
44	QS7646	8.1	0.069	0.86	13.72	17.08	3.72	22.99	21.40
	Mean	10.1	0.08	0.80	16.56	18.44	4.52	23.45	24.39
	LSD	0.99	0.019	0.23	2.91	3.25	0.55	4.76	5.13

QI = protein quality index; Fe = iron; Zn = zinc; PA = phytic acid; MRFe = phytic acid to Fe molar ratio; MRZn = phytic acid to zinc molar ratio; LSD = least significant difference

Appendix 10 Mean values for nutritional traits of QPM hybrids and commercial checks evaluated under random stress conditions at Gwumbi during 2018/19

Entry	Genotype	Protein	Tryptophan	QI	Fe	Zn	PA	MRFe	MRZn
1	ZS261	9.9	0.079	0.81	16.53	17.88	4.87	25.01	26.82
2	MamaMQ623	10.1	0.099	0.97	18.22	18.89	5.13	23.86	26.88
3	PAN53	11.0	0.070	0.63	17.54	19.85	4.41	21.36	21.87
4	SC533	10.4	0.053	0.51	18.28	19.94	4.83	22.43	24.17
5	CZH04032	10.1	0.086	0.85	16.40	18.19	4.86	25.22	26.34
6	CZH132044Q	9.0	0.096	1.07	16.22	19.74	4.78	25.05	23.84
7	CZH132018Q	9.8	0.094	0.97	17.28	21.80	4.74	23.58	21.41
8	CZH142236Q	9.7	0.101	1.04	16.84	20.12	4.72	23.83	23.12
9	CZH142237Q	8.8	0.087	0.99	14.55	18.04	4.66	27.11	25.39
10	CZH142238Q	9.9	0.103	1.04	14.64	17.35	4.55	26.53	25.75
11	CZH15098Q	9.6	0.075	0.79	15.39	18.21	4.58	25.24	24.74
12	CZH15099Q	8.9	0.070	0.79	14.28	14.92	6.53	38.67	43.00
13	CZH15117Q	9.4	0.065	0.69	15.59	17.04	4.22	22.97	24.39
14	CZH15142Q	9.7	0.101	1.04	16.16	22.76	4.96	26.05	21.67
15	CZH16006Q	11.3	0.067	0.59	16.52	19.50	5.10	26.25	25.77
16	CZH16022Q	9.9	0.110	1.10	16.95	19.44	4.43	22.49	22.54
17	CZH16021Q	10.3	0.079	0.77	15.51	18.25	4.63	25.31	24.97
18	CZH17187Q	9.4	0.063	0.67	13.67	20.15	4.36	27.05	21.44
19	CZH17188Q	9.1	0.078	0.86	13.83	15.62	4.44	27.21	27.97
20	CZH17189Q	10.0	0.073	0.73	17.02	18.40	5.00	25.35	27.03
21	CZH17190Q	10.7	0.070	0.65	17.88	18.31	4.54	21.50	24.46
22	CZH17191Q	9.5	0.059	0.62	15.55	18.15	5.12	27.85	27.68
23	CZH17192Q	9.5	0.069	0.75	14.97	15.40	4.36	25.07	27.94
24	CZH17193Q	11.0	0.094	0.85	16.06	18.97	5.43	28.67	28.14
25	CZH17194Q	11.6	0.076	0.66	17.05	17.80	4.92	24.54	27.23
26	CZH17195Q	11.3	0.065	0.57	16.50	15.74	4.88	25.24	30.59
27	CZH17196Q	9.7	0.072	0.74	14.98	18.05	4.36	24.69	23.86
28	CZH17197Q	10.6	0.073	0.69	18.66	18.21	4.90	22.28	26.57
29	CZH17198Q	10.8	0.082	0.77	17.13	19.62	5.04	25.06	25.37
30	CZH17199Q	10.7	0.082	0.76	19.24	20.99	4.86	21.40	22.83
31	CZH17200Q	10.7	0.063	0.59	18.46	17.52	4.86	22.36	27.32
32	CZH17201Q	9.9	0.109	1.10	16.13	20.06	5.26	27.67	25.82
33	CZH17202Q	11.0	0.080	0.73	18.66	20.52	5.27	24.04	25.45
34	CZH17203Q	7.2	0.070	1.01	14.43	15.99	4.57	26.85	28.05
35	CZH17204Q	9.6	0.079	0.83	15.12	16.85	4.41	24.78	26.23
36	CZH17205Q	9.8	0.068	0.72	13.70	18.07	4.96	30.76	27.04
37	CZH17206Q	9.2	0.073	0.80	12.76	19.16	4.90	32.60	25.29
38	CZH17207Q	8.9	0.066	0.75	13.18	16.11	4.92	31.68	30.43
39	CZH17208Q	9.8	0.075	0.76	13.57	17.34	4.85	30.39	28.26
40	CZH17209Q	10.8	0.063	0.58	17.23	17.12	4.97	24.49	28.69
41	CZH17210Q	9.5	0.070	0.73	15.55	18.09	4.87	26.56	26.50
42	MH1633	10.0	0.086	0.86	16.41	18.17	5.37	27.85	29.14
43	MH1634	9.4	0.080	0.85	14.12	14.86	4.31	25.86	28.53
44	QS7646	8.7	0.072	0.82	14.21	17.63	4.23	25.28	23.66
	Mean	9.9	0.1	0.8	16.0	18.3	4.8	25.9	26.2
	LSD	1.3	0.025	0.27	2.66	2.66	0.70	4.30	4.35

QI = protein quality index; Fe = iron; Zn = zinc; PA = phytic acid; MRFe = phytic acid to Fe molar ratio; MRZn = phytic acid to zinc molar ratio; LSD = least significant difference

Appendix 11 Means of nutritional traits of quality protein QPM hybrids and commercial checks evaluated across optimum environments during 2018 to 2020

Entry	Genotype	Protein	Tryptophan	QI	Fe	Zn	PA	MRFe	MRZn
1	ZS261	8.3	0.085	1.02	31.19	17.82	5.95	19.89	33.80
2	MamaMQ623	8.2	0.088	1.09	20.01	15.78	5.74	24.97	39.17
3	PAN53	9.4	0.059	0.64	25.51	23.47	6.09	21.71	27.19
4	SC533	9.1	0.061	0.67	29.77	19.05	5.41	19.07	30.15
5	CZH04032	8.3	0.091	1.11	26.37	19.47	5.61	21.02	30.33
6	CZH132044Q	8.2	0.090	1.13	30.12	16.98	5.56	17.90	35.04
7	CZH132018Q	8.5	0.096	1.14	25.38	21.74	6.09	24.80	28.63
8	CZH142236Q	7.5	0.097	1.30	20.64	21.32	5.31	26.81	25.55
9	CZH142237Q	7.4	0.096	1.32	23.02	20.76	5.51	21.62	28.36
10	CZH142238Q	7.8	0.102	1.32	30.91	20.52	6.05	18.19	31.62
11	CZH15098Q	9.2	0.081	0.89	22.64	21.70	5.48	22.72	28.40
12	CZH15099Q	7.4	0.090	1.25	34.52	14.01	5.20	16.53	40.58
13	CZH15117Q	8.0	0.088	1.12	22.53	19.67	5.27	29.48	29.03
14	CZH15142Q	8.1	0.096	1.21	22.62	24.96	5.95	32.25	25.07
15	CZH16006Q	8.8	0.071	0.82	26.72	27.08	5.76	21.29	22.30
16	CZH16022Q	8.3	0.104	1.20	25.24	18.19	5.79	20.57	35.15
17	CZH16021Q	9.4	0.077	0.84	29.24	20.51	6.03	23.70	30.20
18	CZH17187Q	9.1	0.072	0.83	29.14	17.41	5.70	17.84	44.73
19	CZH17188Q	8.4	0.069	0.84	24.57	15.30	5.12	26.05	34.73
20	CZH17189Q	8.6	0.084	0.99	27.93	17.52	6.08	19.47	35.05
21	CZH17190Q	9.0	0.070	0.79	29.93	20.43	5.45	16.72	28.22
22	CZH17191Q	8.0	0.068	0.87	25.44	23.15	5.50	22.28	24.88
23	CZH17192Q	8.4	0.079	0.95	25.31	23.87	5.70	21.61	24.15
24	CZH17193Q	10.0	0.093	0.94	27.30	21.89	6.12	21.43	28.22
25	CZH17194Q	9.5	0.066	0.71	28.93	18.35	5.77	18.88	31.26
26	CZH17195Q	9.3	0.064	0.71	25.77	15.37	5.62	21.31	39.89
27	CZH17196Q	8.0	0.075	0.95	26.08	21.68	5.70	19.33	27.35
28	CZH17197Q	8.7	0.079	0.94	29.09	18.46	5.65	18.59	30.67
29	CZH17198Q	9.0	0.096	1.09	24.00	24.21	5.51	23.27	22.95
30	CZH17199Q	9.3	0.071	0.77	26.74	23.67	5.08	16.61	22.22
31	CZH17200Q	9.0	0.076	0.86	21.41	18.32	5.66	23.71	32.38
32	CZH17201Q	8.5	0.079	0.96	25.25	18.94	5.59	22.55	30.30
33	CZH17202Q	8.7	0.075	0.88	24.35	22.33	5.59	25.08	25.19
34	CZH17203Q	8.2	0.081	0.99	23.85	18.83	5.28	20.35	30.02
35	CZH17204Q	8.1	0.082	1.05	27.56	16.43	5.56	19.20	35.23
36	CZH17205Q	8.1	0.082	1.03	23.86	19.47	5.40	21.48	28.57
37	CZH17206Q	9.0	0.084	0.94	26.93	18.57	5.76	20.56	32.78
38	CZH17207Q	8.0	0.079	1.01	20.35	18.48	5.42	26.66	31.07
39	CZH17208Q	8.4	0.078	0.94	20.86	17.91	5.47	23.03	32.95
40	CZH17209Q	8.4	0.080	0.96	24.93	19.56	5.57	20.14	29.57
41	CZH17210Q	8.4	0.080	0.96	23.86	19.53	5.36	20.92	28.20
42	MH1633	8.7	0.093	1.09	26.04	22.17	5.99	20.55	26.72
43	MH1634	7.4	0.090	1.24	25.84	13.23	5.10	20.96	42.70
44	QS7646	7.6	0.081	1.09	23.09	20.04	4.74	19.79	25.31
	Mean	8.500	0.082	0.99	25.71	19.80	5.58	21.63	30.39
	LSD	0.59	0.011	0.14	3.30	2.37	0.30	3.48	4.96

QI = protein quality index; Fe = iron; Zn = zinc; PA = phytic acid; MRFe = phytic acid to Fe molar ratio; MRZn = phytic acid to zinc molar ratio; LSD = least significant difference

Appendix 12 Means of nutritional traits of quality protein QPM hybrids and commercial checks evaluated across low N environments during 2018 to 2020

Entry	Genotype	Protein	Tryptophan	QI	Fe	Zn	PA	MRFe	MRZn
1	ZS261	5.5	0.054	0.97	20.38	10.10	4.32	18.31	43.23
2	MamaMQ623	6.0	0.078	1.37	12.68	15.54	4.92	39.63	31.99
3	PAN53	5.8	0.055	0.97	23.71	13.28	4.83	18.37	38.47
4	SC533	6.3	0.051	0.81	21.16	13.12	5.31	22.53	40.66
5	CZH04032	5.8	0.080	1.40	19.21	13.29	5.02	24.32	37.85
6	CZH132044Q	5.7	0.083	1.45	10.77	15.57	5.02	42.92	33.01
7	CZH132018Q	5.7	0.063	1.11	24.35	13.97	5.05	18.41	36.91
8	CZH142236Q	5.8	0.079	1.36	18.87	17.17	4.65	25.26	32.37
9	CZH142237Q	5.7	0.072	1.29	22.60	18.70	5.16	21.18	27.32
10	CZH142238Q	5.8	0.081	1.42	21.57	11.95	5.09	21.09	47.77
11	CZH15098Q	6.4	0.054	0.89	13.77	17.99	4.66	37.63	26.55
12	CZH15099Q	5.5	0.069	1.36	13.30	11.49	4.33	32.46	38.89
13	CZH15117Q	5.7	0.082	1.45	18.34	13.73	4.22	22.30	31.39
14	CZH15142Q	5.9	0.080	1.36	21.28	15.95	4.81	19.71	41.11
15	CZH16006Q	6.7	0.063	0.95	26.55	18.15	4.61	15.60	25.44
16	CZH16022Q	5.2	0.077	1.48	20.07	13.64	5.05	22.48	36.63
17	CZH16021Q	6.9	0.058	0.84	13.06	15.23	4.90	32.29	36.77
18	CZH17187Q	5.8	0.072	1.32	17.56	9.33	4.71	23.44	53.35
19	CZH17188Q	5.9	0.061	1.09	13.10	11.44	4.61	37.28	40.53
20	CZH17189Q	6.6	0.067	1.01	16.53	15.19	4.20	22.52	28.27
21	CZH17190Q	7.4	0.080	1.08	17.67	10.03	5.20	26.35	51.96
22	CZH17191Q	6.6	0.065	1.03	18.32	12.58	4.72	31.78	37.02
23	CZH17192Q	6.1	0.068	1.14	13.99	15.80	5.43	35.07	45.53
24	CZH17193Q	6.9	0.062	0.97	14.34	17.01	4.80	32.16	27.82
25	CZH17194Q	7.3	0.052	0.80	23.86	15.45	5.10	18.96	34.02
26	CZH17195Q	6.1	0.052	0.95	13.74	10.63	4.72	30.99	44.39
27	CZH17196Q	6.3	0.065	1.08	15.68	15.21	4.80	27.05	32.04
28	CZH17197Q	6.7	0.062	0.94	25.18	21.30	4.91	16.82	24.00
29	CZH17198Q	6.1	0.082	1.44	24.16	18.08	5.03	19.83	28.94
30	CZH17199Q	5.7	0.066	1.18	15.62	16.95	5.12	28.33	31.04
31	CZH17200Q	7.1	0.060	0.88	12.35	16.10	5.34	41.66	37.61
32	CZH17201Q	7.8	0.087	1.15	17.30	14.73	5.31	26.01	39.65
33	CZH17202Q	5.9	0.069	1.22	19.61	15.99	4.72	21.03	30.32
34	CZH17203Q	7.9	0.069	0.86	11.06	13.46	5.02	38.67	45.15
35	CZH17204Q	6.2	0.064	1.02	15.07	15.70	4.96	28.33	37.18
36	CZH17205Q	5.4	0.064	1.21	15.29	14.64	5.35	30.48	38.10
37	CZH17206Q	6.8	0.068	0.98	17.64	15.06	4.97	31.27	36.08
38	CZH17207Q	5.5	0.070	1.28	12.04	10.43	4.88	34.62	46.38
39	CZH17208Q	6.3	0.058	0.96	13.03	9.86	4.56	35.81	50.72
40	CZH17209Q	6.2	0.066	1.06	20.95	15.46	4.80	19.75	32.20
41	CZH17210Q	5.6	0.062	1.14	15.09	13.54	4.70	28.55	37.97
42	MH1633	6.3	0.079	1.31	21.00	16.08	5.32	21.72	33.73
43	MH1634	6.5	0.069	1.09	14.80	12.44	4.74	39.91	40.28
44	QS7646	5.5	0.062	1.17	15.10	11.27	4.59	26.68	41.14
Mean		6.20	0.07	1.13	17.34	14.51	4.88	27.77	36.93
LSD		0.97	0.016	0.33	4.44	3.68	0.46	9.07	13.80

QI = protein quality index; Fe = iron; Zn = zinc; PA = phytic acid; MRFe = phytic acid to Fe molar ratio; MRZn = phytic acid to zinc molar ratio; LSD = least significant difference