Evaluation of Maize Hybrids for Low-Nitrogen Stress Tolerance, Yield Stability and Genetic Purity

by

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GENERAL ABSTRACT

Nitrogen stress is among the major abiotic constraints that impede maize production in Africa. Therefore, development of maize varieties that are tolerant to low N stress conditions and stable across environments is needed. Assessment of genetic purity of inbred lines and their F1 hybrids is among the quality control measures in hybrid breeding, seed production, variety release as well as intellectual property protection (IP). The objectives of this research were, therefore: a) to assess the grain yield performance, genetic parameter estimates, correlations and conduct path coefficient analysis for grain yield and related traits under low N and optimum conditions, b) to assess the magnitude of genotype by environmental interaction (GEI) and hybrid yield stability under low N and optimum conditions and c) to assess the genetic purity of maize parental lines and their F1 hybrids. To achieve these objectives, 170 single cross maize hybrids were evaluated across low N and optimum environments at three locations in South Africa (SA) during 2017/18 summer season *viz*. Potchefstroom, Vaalharts and Cedara. The experimental setup comprised of five-production conditions across these three locations. The collected data was subjected to analyses using Genstat software 18th edition, SPSS version 25 and SAS version 9.3. For genetic purity analysis, 158 single-cross maize hybrids along with 30 elite parental inbred lines were genotyped using 92 SNPs markers and the molecular data was analysed using GenAlex software.

Results revealed that variance due to environment, genotype and GEI were highly significant ($P<0.001$) for all the traits under low N and optimum conditions. Lower heritability values were observed for grain yield (0.29) compared to secondary traits including days to anthesis, plant height, ear height and anthesis-silking interval, which had heritability estimates of 0.85, 0.43, 0.38 and 0.52, respectively. Higher phenotypic coefficient of variation (PCV) as compared to genotypic coefficient of variation (GCV) were observed under low N and optimum environments, respectively. Under low N, grain yield was positively correlated with field weight, plant height and ear height, but negatively correlated with days to silking, anthesis-silking interval and leaf senescence. Under optimum environment, grain yield was positively correlated with field weight and ears per plant, and negatively correlated with days to anthesis, days to silking, anthesis- silking interval, plant height and ear height. Highest positive direct effect on grain yield was observed for days to silking and field weight under low N, while under optimum, field weight and days to anthesis exhibited the highest direct effects. AMMI and GGE biplot analyses revealed high yielding hybrids in each specific environment and high yielding and stable hybrids across the environments. Five high yielding and stable hybrids across environments; G134 (I-42/CKDHL0295), G12 (CB399/CML442), G24 (CK21/CML216), G33 (CKDHL0089/CML442) and G102 (CML544/I-42) are recommended for further evaluation and

release. Using SNP markers, 66.7% of maize parental lines genotyped were considered pure with residual heterozygosity of <5%, while the remaining 33.3% had residual heterozygosity levels of > 5% hence not pure. Cluster analysis effectively discriminated the parental lines into three distinct genetic clusters. Parent-offspring test conducted on 158 hybrids resulted to the elimination of 38% of the hybrids due to genetic contamination of their parental inbred lines. Of the 68 hybrids that passed the parent-offspring test, seven hybrids, including SCHP29, SCHP95, SCHP94, SCHP134, SCHP44, SCHP114 and SCHP126, were selected as potential candidates for further evaluation and possible release in South Africa due to their outstanding yield performance.

DECLARATION

I, Chimwemwe Josia, declare that:

i) The research reported in this dissertation, except where otherwise indicated or acknowledged, is my original work;

ii) This dissertation has not been submitted in full or in part for any degree or examination to any other university;

iii) This dissertation does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons;

iv) This dissertation does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers.

   a) Where other written sources have been quoted, then their words have been re-written but the general information attributed to them has been referenced;
   b) Where their exact words have been used, their writing has been placed inside quotation marks, and referenced;

v) This dissertation does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the dissertation and in the References sections.

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DEDICATION

I would like to dedicate this dissertation to my beloved parents Mr Arnold Josiya and Mrs. Dorothy Josiya for all their effort and sacrifice to make sure that I get educated.

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LIST OF ABBREVIATIONS

AFLP = Amplified fragment length polymorphism

AMMI = Additive main effect and multiplicative interaction

ANOVA = Analysis of variance

ARC-GCI = Agricultural Research Council-Grain Crops Institute

ASV = Ammi stability value

CV = Coefficient of variation

DA = Days to 50% anthesis

DAFF = Department of Agriculture, Forestry and Fisheries

DS = Days to 50% silking

ED = Ear diameter

EL = Ear length

GA = Genetic advance

GAM = Genetic advance as percentage mean

GCV = Genotypic coefficient of variation

GD = Genetic distance

GEI = Genotype-by-environment interaction

GGE = Genotype plus genotype-by-environment interaction

GV = Genotypic variance

GY = Grain yield

He = Expected heterozygosity

Ho = Observed heterozygosity

IPCA = Interaction principal component axis

KASP = Kompetitive allele specific polymerase chain reaction

KPE = Number of kernels per ear

LAN = Limestone ammonium nitrate

LSD = Least significant difference

MS = Mean square

Na = Total number of alleles per locus

Ne = Number of effective alleles per locus

PC = Principal component

PCR = Polymerase chain reaction

PCV = Phenotypic coefficient of variation

PIC = Polymorphic information content

PV = Phenotypic variance

RFLP = Restriction fragment length polymorphism

RPE = Number of kernel rows per ear

SE = Standard error

SNP = Single nucleotide polymorphism

SA = South Africa

SS = Sum of squares

SSA = Sub-Saharan Africa

SSR = Simple sequence repeat

CHAPTER 1
INTRODUCTION TO DISSERTATION

1.1 Importance of maize globally

Maize is a fundamental crop in Africa and Asia considered as a staple crop and feeding more than 300 million of the continent's most vulnerable people (Prasanna, 2015). It is an important nutritional source of carbohydrate, protein, iron, vitamin B, and minerals (IITA, 2014) and accounts for about 15% of the caloric intake of the population (Badu-Apraku and Akinwale, 2011). The crop has wide adaptation to different agro-ecological zones ranging from sea level to an altitude of about 3000 m above sea-level (Kang'ethe, 2011). At global scale, maize is the third most important cereal after wheat and rice (FAO, 2011). The demand for maize is expected to increase even more by 2020 (Mkumbwa, 2011) as it is becoming more important in the livelihoods of many poor farmers in sub-Saharan Africa (SSA).

About 43% of maize produced in South Africa is white and primarily for human consumption and the remaining 57% is yellow maize used for animal feed production (DAFF, 2017). In terms of gross value towards agricultural production in SA, maize has been the largest contributor of all the field crops with gross value of 46.2% followed by sugarcane (12.9%), soybeans (10.3%), wheat (9.9%) and sunflower seed (6.2%) (DAFF, 2017). Thus, maize plays a significant role both at household level and towards the economy of the country.

1.2 Maize production overview in South Africa

South Africa is leading in maize production across the African continent (Baloyi, 2011), producing more than 10 million metric tonnes of maize per year (FAOSTAT, 2014). The country has the largest area under maize production and yield output per hectare has always been the highest within the African continent. For example, overall maize production for 2016/17 reached a record high of about 17.5 million tonnes more than double the level of 2015/16 and well above the previous five-year average (FAO, 2017). The significant production upsurge reflects a 35% increase in plantings, spurred by higher prices, and bumper yields, mostly owing to good rainfall season following the dry weather conditions experienced in the previous season. Maize production estimate for 2017/18 season indicated a decline by 6% as compared to the previous year due to lower planting of white maize (FAO, 2017). Furthermore, the decline has further been attributed to lower profitability of maize reflected by the low price for the commodity in 2017. However, though the cereal output is expected to drop in 2018 from the record 2017 level, preliminary forecast suggest it will remain above average.

The main maize-producing regions in SA include Free State (FS), North-West (NW), Gauteng, KwaZulu-Natal (KZN), Mpumalanga (MP) and the Northern Cape provinces (USDA, 2017). Figure 1.1 shows 2016/17 maize production contribution by each province within SA.

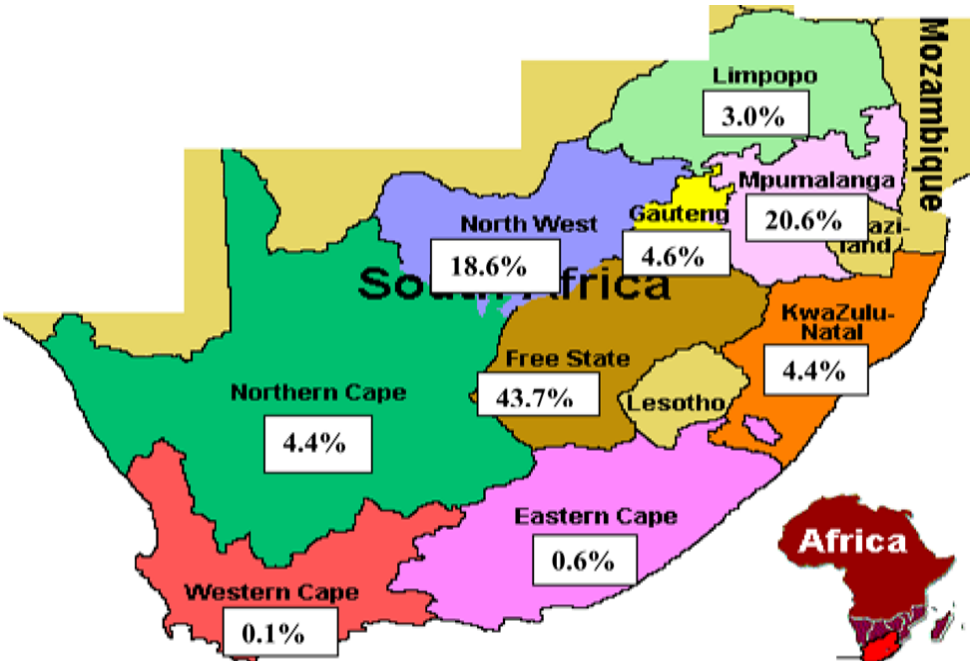

Figure 1.1 Maize production estimates in each province in SA (USDA, 2017)

1.3 Constraints to maize production in sub-Saharan Africa (SSA)

Maize yield per hectare realised in Africa is 40% of the productivity registered in developed countries (Agriorbit, 2017). Such deficit in SSA is due to several factors categorised as biotic, abiotic and social-economic.

1.3.1 Abiotic factors

Among the abiotic production constraints, drought and low soil fertility especially low N are the main limiting factors to maize production and the latter is the most challenging constraint (Banziger and Lafitte, 1997; Banziger and Cooper, 2001, Sanchez 2010, Gage *et al.,* 2012). Nutrient deterioration leading to soil fertility decline is a common phenomenon among smallholder farming systems, as many farmers cannot afford inorganic fertilizers.

Continuous farming without adequate use of fertiliser has also resulted in the depletion of essential soil nutrients in SSA (Sanchez, 2010). Soil fertility deterioration is further caused by increased population pressure, especially in areas with fragile ecosystems, such as SSA (Dao, 2013).

Since fertiliser application rates are low, annual net loss of major soil nutrients such as; nitrogen, potassium and phosphorous may reach >4 metric tonnes and these losses equal to 30 to 60 kg NPK ha^{-1} yr^{-1} on 45% of cropped land, and exceed 60 kg NPK ha^{-1} yr^{-1} on a further 40% (Henao and Baanante, 2006; Craswell and Vlek, 2013). Low rates of NPK addition are associated with sharp reduction in the length of the traditional fallow period between crops, hence restricting natural soil nutrients recovery. Furthermore, there has been a steady loss of soil organic carbon because low yielding crops return very small amount of residues and the remnant residues are usually, burned or grazed. This altogether results in soil fertility decline.

Drought, on the other hand, is a widespread phenomenon in most of the farming areas within SSA. An estimated 22% of mid-altitude/subtropical and 25% of lowland tropical maize growing regions are annually stricken by inadequate water supply (Heisey and Edmeades, 1999). Drought occurs due to inadequate or poor distribution of rainfall resulting in decrease of maize output across the SSA region. SSA is the most severely affected region in the world where almost half of the land surface is exposed to a high risk of meteorological drought (Ribaut *et al.,* 2009) such that all the agro-ecological zones in SSA may be affected by mid- and late season drought. Drought affects maize yields by limiting season length and through random stress that can occur at any time during the cropping cycle (Dao, 2013). For instance, drought incidences in 1992 and 2002 reduced maize production in the southern Africa region by 50% (Semagn *et al.,* 2014). The negative impact of drought may grow as the threat of climate change becomes a reality and drought has been reported as the most important challenge of maize production in the region on several times (Kassie *et al.,* 2012).

1.3.2 Biotic factors

Pests, diseases and parasitic weeds are among the biotic factors leading to low maize productivity in SSA. Pests and diseases pose significant threats to maize grain production in SSA, with insect pests alone capable of destroying 1.5% of the world's total crop production annually (Mohamed, 2013). Sibiya *et al.* (2013a) reported that stalk borer and cutworms were the most prevalent insect pests affecting maize production in SA. As of late, a new insect pest (fall armyworm) is causing significant yield damage to maize in southern Africa. For instance, preliminary assessments, conducted between mid-February and the end of April 2017 revealed that approximately 356,000 hectares of crops were affected by the fall armyworm infestation in seven Southern Africa Development Community (SADC) member states: Botswana, Democratic Republic of the Congo, Malawi, Namibia, South Africa, Swaziland and Zambia (FAO, 2017).

According to Sibiya *et al.,* (2013a), maize diseases such as cob rots, grey leaf spots (GLS), maize streak virus (MSV), northern leaf bright (NLB), phaeosphaeria leaf spot (PLS) and common rust are among the important diseases causing economic maize yield loss in SA.

Parasitic weeds cause major maize yield losses in SSA. Most African smallholder farmers often respond to problems of low soil fertility by planting on large areas. This tactic often exceeds their weed management capacity due to limited use of herbicides and thus make weeding very labour intensive. This habit aggravates the problem of weeds in their field. Striga Asiatic is one of the most important weeds limiting maize grain yield in SSA. Yield losses attributable to this weed may range from 10 to 100% depending on the genotype grown, climatic conditions, soil fertility status and levels of infestation (Akaogu *et al.,* 2013). Weed incidences are closely associated with a decline in soil fertility and addition of nitrogen fertiliser will often allow crops to offset the effects of the parasitic weeds.

1.3.3 Social-economic factors

Social-economic factors that impede maize productivity include public policies and investments that do not work to the advantage of poor smallholder farmers and consumers, women and less-favoured areas; poor and inadequate infrastructure; inequitable access to land and other critical resources; poorly functioning and marketing systems; and lack of access to credit and technical assistance. Furthermore, fewer facilities and service providers are available to women than men although women produce about 75% of the domestically grown food in SSA (Dao, 2013). Other important factors contributing to low yield include the low adoption of improved varieties by farmers (Sibiya *et al.*, 2013b) and the non-use of appropriate farming techniques (Etoundi and Dia, 2008). These challenges if not properly checked will eventually lead to higher global price, malnutrition, poverty and hunger.

1.4 Problem statement and justification

Maize remains the staple crop for the majority of households within sub-Saharan Africa (SSA). However, maize productivity in SSA countries is severely constrained by several factors. Studies have reported low maize yields on smallholder farmers' fields ranging from 1.8 t ha^{-1} to 3.5 t ha^{-1} (Baloyi *et al.,* 2011; Fanadzo *et al.,* 2009) confirming the negative impact of these production constraints. The most important constraints include increasing incidences of soil infertility mainly nitrogen deficiency and drought incidences aggravated by climate change (Edmeades *et al*., 2006; Meseka *et al.*, 2008). In addition, the presence of genotype by environment interaction (GEI) and low stability of genotypes across locations also impedes maize production within the region (Zelke and Lalise, 2015).

Hence conducting GEI studies and yield stability tests for potential varieties across locations becomes a requirement before variety release to ascertain whether the variety is suitable for broad or specific adaptation (Bernardo, 2002).

Tarekegne and Das (2015) also indicated that low N plus drought and heat are the major abiotic stresses in SSA that reduce maize yield. Bänziger *et al.* (2006) reported yield losses of up to 80% due to low nitrogen (N) and drought, while Logrono and Lothrop (1996) reported a yield loss of 10% to 50% per year due to low N. The soils within the region are low in organic matter and nitrogen content arising from high leaching rate, denitrification, decomposition, erosive action of rainfall, continuous cropping, removal of crop residues and use of low inputs (Adekayode and Ogunkoya, 2010). Thus, adequate nitrogen for optimum growing conditions for the maize plant should be provided through inorganic fertilizer application. Chemical fertilizers are expensive and, hence, unaffordable to the vast majority of smallholder farmers in many African countries (Banziger and Diallo, 2004). However, only a few maize varieties are available that can tolerate low N and drought stress in tropical Africa (Nyombayire *et al.*, 2011) and at the same time, tropical maize continues to be exposed to these stresses. Hence, there is an urgent need for high quality low N tolerant maize varieties that will perform consistently across the environments. To address the need, this study was conducted to evaluate recently developed single-cross maize hybrids for low N stress tolerance and yield stability. The evaluation process was aided with the use of secondary traits, since consideration of secondary traits improves selection efficiency under low nitrogen stress conditions (Bänziger and Lafitte 1997). In addition, single-cross hybrids are high yielding with better adaptability to a new set of cropping systems and management practices (Dass *et al.,* 2009). Genetic purity of the developed hybrids was assessed using SNP markers. Quality control information generated will help the Agricultural Research Council-Grain Crop Institute (ARC-GCI) maize breeding programme to improve on quality delivery and ensure development of pure and high yielding hybrids.

1.5 Research goal and objectives

The overall research goal was to evaluate the yield stability of ARC-GCI‘s newly developed single-cross maize hybrids under low N and optimum conditions and conduct SNP-based quality control analysis on the maize parental lines and the resultant F1 hybrids.

Specific objectives of the study

The specific objectives of the study were to:

a) Assess grain yield performance, genetic parameter estimates, conduct correlations, and path coefficient analysis for grain yield and related traits under low N and optimum conditions.
b) Assess the magnitude of genotype-by-environmental interaction and stability of grain yield of single-cross maize hybrids under low N and optimum conditions; and
c) Assess the genetic purity of maize parental inbred lines and their F1 hybrids using SNP markers.

1.6 Research Hypothesis

a) There are significant differences in grain yield performance and genetic parameter estimates among the single-cross maize hybrids and that the relationship between grain yield and related traits does exist.
b) Grain yield performance and stability of maize hybrids under low N and optimum conditions are affected by genotype by environmental interactions.
c) There is genetic purity among the maize parental inbred lines and their F1 hybrids based on SNP markers.

1.7 Structure of Dissertation

The objectives were addressed in each chapter that constitutes the dissertation. Each chapter is an independent, potential manuscript for journal publication and thus, there may be some overlaps of content and references with other chapters. The chapters are as follows:

Chapter One:	General introduction.
Chapter Two:	Literature review.
Chapter Three:	Grain yield performance of maize hybrids, estimation of genetic parameters, path and correlation analysis across low N and optimum environments.
Chapter Four:	Genotype by environment interaction and stability analysis among single-cross maize hybrids across low N and optimum environments.
Chapter Five:	SNP-based assessment of genetic purity in maize breeding and seed production.
Chapter Six:	General overview of the research findings.

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1.8 References

Adekayode, F. and M. Ogunkoya. 2010. Effect of quantity and placement distances of inorganic 15-15-15 fertilizer in improving soil fertility status and the performance and yield of maize in a tropical rain forest zone of Nigeria. Journal of Soil Science and Environmental Management 1: 155-163.

Agriorbit. 2017. How to Increase Maize Production in Africa.https://www.agriorbit.com/increase-maize-production-africa/: (Accessed on 15th October 2018)

Akaogu, I., B. Badu-Apraku, V. Adetimirin, I. Vroh-Bi, M. Oyekunle and R. Akinwale. 2013. Genetic diversity assessment of extra-early maturing yellow maize inbreds and hybrid performance in Striga-infested and Striga-free environments. The Journal of Agricultural Science 151: 519-537.

Badu-Apraku, B. and R. Akinwale. 2011. Cultivar evaluation and trait analysis of tropical early maturing maize under Striga-infested and Striga-free environments. Field Crops Research 121: 186-194.

Badu-Apraku, B., R. Akinwale, S. Ajala, A. Menkir, M. Fakorede and M. Oyekunle. 2011. Relationships among traits of tropical early maize cultivars in contrasting environments. Agronomy Journal 103: 717-729.

Badu-Apraku, B., M. Fakorede, A. Menkir and D. Sanogo. 2012. Conduct and management of maize field trials. International Institute of Tropical Agriculture.

Baloyi, R.T. 2011. Technical effeciency in maize production by small-scale farmers in Ga-Mothiba, Limpopo Province, South Africa. University of Limpopo (Turfloop Campus).

Baloyi, R.T., Belete, A., Hlongwane, J.J. and M.B. Masuku. 2011. Technical efficiency in maize production by small-scale farmers in GaMothiba, Limpopo Province, South Africa. African Journal of Agricultural Research, 7: 5478-5482

Bänziger, M. and H. Lafitte. 1997. Breeding tropical maize for low N environments. II. The values of secondary traits for improving selection gains under low N. Crop Science 37: 1110-1117.

Bänziger, M. and M. Cooper. 2001. Breeding for low input conditions and consequences for participatory plant breeding examples from tropical maize and wheat. Euphytica 122: 503-519.

Banziger, M and Diallo A. 2004. Progress in developing drought and N stress tolerant maize cultivars for eastern and southern Africa. In Progress in developing drought and N stress tolerant maize cultivars for eastern and southern Africa, Integrated approaches to higher maize productivity in the new millennium. Proceedings of the 7th Eastern and Southern Africa Regional Maize Conference, CIMMYT/KARI, Nairobi, Kenya, February 2002. 189-194.

Bänziger, M., P.S. Setimela, D. Hodson and B. Vivek. 2006. Breeding for improved abiotic stress tolerance in maize adapted to southern Africa. Agricultural Water Management 80: 212-224.

Bernardo, R. 2002. Breeding for quantitative traits in plants. Stemma Press.

Craswell, E.T. and P.L. Vlek. 2013. 15 Mining of Nutrients in African Soils Due to Agricultural Intensification..

DAFF. 2017. Trend in the agricultural sector. https://www.daff.gov.za/ : (Accessed on 3rd October 2017)

Dass, S., J. Kaul, A. Manivannan and G. Chikkappa. 2009. Single cross hybrid maize-A viable solution in the changing climate scenario. Indian Journal of Genetics and Plant Breeding 69: 331-334.

Dao, A. 2013. Genetic Characterization, Association Mapping and Evaluation of Feterotic Patterns of Tropical Maize (*Zea mays* L.) Inbred Lines Under Drought and Non-Drought Conditions. PhD dessertation, University of Ghana.

Du Plessis, J. 2003. Maize production. Department of Agriculture in cooperation with ARC-Grain Crop Institute. Republic of South Africa. http://www.arc.agric.za/arc-John

Edmeades, G., Bänziger, M., Campos, H. and Schussler, J. 2006. Improving tolerance to abiotic stresses in staple crops: a random or planned Process? Plant Breeding: The Arnel R. Hallauer International Symposium, Wiley Online Library.

Etoundi, S.M.n and K. B. Dia. 2008. Determinants of the adoption of improved varieties of Maize in Cameroon: case of CMS 8704.

Fanadzo, M., C. Chiduza and P. Mnkeni. 2009. Investigation of agronomic factors constraining productivity of grain maize (*Zea mays* L.) at Zanyokwe irrigation scheme, Eastern Cape, South Africa. Journal of Applied Biosciences 17: 948-958.

Food and Agricultural Organization Statistical Databases . 2017. GIEWS-Global Information and Early Warning Systems. http://www.fao.org/giews/en/: (Accessed on 7th October 2017)

Food and Agricultural Organization Statistical Databases 2014. Food and agricultural commodities production. Rome, Italy. http://faostat.fao.org/site/339/default.aspx. (Accessed 3 July 2018)

Food and Agricultural Organization (FAO) Statistical Databases (2016). Production. http://faostat3.fao.org/browse/Q/*/E (Accessed 20 December 2017).

Food and Agricultural Organization (FAO) Statistical Databases. (2014). FAOSTAT: Agriculture Data. Available online: http://faostat.fao.org. (Accessed 25 December 2017).

Food and Agricultural Organization (FAO) Statistical Databases. (2011). FAOSTAT: Agriculture Data. Available online: http://faostat.fao.org. (Accessed 28 December 2017).

Gage, D., J. Bangnikon, H. Abeka-Afari, C. Hanif, J. Addaquay, V. Antwi et al. 2012. The market for maize, rice, soy, and warehousing in Northern Ghana. USAID's Enabling Agricultural Trade (EAT) Project Report. https://lib.dr.iastate.edu/cgi/viewcontent.cgi?article=1480&context=abe_eng_conf: (Accessed 12 September 2018)

Gibbon, D., J. Dixon and D. Flores Velazquez. 2007. Beyond drought tolerant maize: study of additional priorities in maize.https://www.statista.com/statistics/263977/ world-grain-production-by-type/). Statista, 2018

Henao, J. and C. Baanante. 2006. Agricultural production and soil nutrient mining in Africa: Implications for resource conservation and policy development. Muscle Shoals, AL: IFDC.

Heisey, P.W. and G.O. Edmeades. 1999. Part of CIMMYT 1997/98 world maize facts and trends--Maize production in drought-stressed environments: Technical options and research resource allocation. CIMMYT, Mexico D.F.

IITA (2014) Maize 2014, available at http://www.iita.org/maize. (Accessed on 16 September 2017)

Kang'ethe, E. 2011. Situation analysis: Improving food safety in the maize value chain in Kenya. FAO Report. College of Agriculture and Veterinary Science, University of Nairobi, Nairobi.

Kassie, G.T., O. Erenstein, W. Mwangi, P. LaRovere, P.S. Setimela and A.S. Langyintuo. 2012. Characterization of maize production in Southern Africa: Synthesis of CIMMYT/DTMA household level farming system surveys in Angola, Malawi, Mozambique, Zambia and Zimbabwe. International Maize and Wheat Improvement Center (CIMMYT).Logrono, M. and J. Lothrop. 1996. Impact of drought and low nitrogen on maize production in Asia. Drought-and Low N-Tolerant Maize: 39. https://link.springer.com/chapter/10.1007/978-94-017-2685-6_4. (Accessed 28 September 2018).

Meseka, S., A. Menkir and A. Ibrahim. 2008. Yield potential and yield stability of maize hybrids selected for drought tolerance. Journal of Applied Biosciences 3: 82-90.

Mkumbwa, S.S. 2011. Cereal food commodities in Eastern Africa: consumption-production gap trends and projections for 2020. https://mpra.ub.uni-muenchen.de/42113: (Accessed 3 September 2018)

Mohamed, N.S. 2013. Insect damage: Damage on Post-harvest. International Centre of Insect Physiology and Ecology (ICIPE), INPhO (www.icipe.org).

Moswetsi, G., M. Fanadzo and B. Ncube. 2017. Cropping systems and agronomic management practices in smallholder farms in South Africa: Constraints, challenges and opportunities. Journal of Agronomy 16: 51-64.

Nyombayire, A. 2011. Combining ability and genotype by environment interaction of selected maize inbred lines for performance under low nitrogen and drought stress. MSc dissertation, Makerere University.

Nyombayire, A., Edema, R., Asea, G. and Gibson, P., 2011. Combining ability of maize inbred lines for performance under low nitrogen and drought stresses. 10th African Crop Science Conference Proceedings, Maputo, Mozambique, 10-13 October 2011, African Crop Science Society.

Prasanna, B. 2015. Global trends, challenges and opportunities for maize: Lessons for Asia. https://repository.cimmyt.org/bitstream/handle/10883/4431/Global%20Trends%20Challenges%20and%2. (Accessed 2 October 2018)

Ribaut, J.-M., J. Betran, P. Monneveux and T. Setter. 2009. Drought tolerance in maize. Handbook of maize: its biology. Springer. p. 311-344.

Sánchez, P.A. 2010. Tripling crop yields in tropical Africa. Nature Geoscience 3: 299–300

Semagn, K., C. Magorokosho, V. Ogugo, D. Makumbi and M.L. Warburton. 2014. Genetic relationships and structure among open-pollinated maize varieties adapted to eastern and southern Africa using microsatellite markers. Molecular breeding 34: 1423-1435.

Sibiya, J., P. Tongoona, J. Derera and I. Makanda 2013a. Smallholder farmers perceptions of maize diseases, pests, and other production constraints, their implications for maize breeding and evaluation of local maize cultivars in KwaZulu-Natal, South Africa. African Journal of Agricultural Research 8: 1790-1798.

Sibiya, J., P. Tongoona, J. Derera and I. Makanda. 2013b. Farmers' desired traits and selection criteria for maize varieties and their implications for maize breeding: A case study from KwaZulu-Natal Province, South Africa. Journal of Agriculture and Rural Development in the Tropics and Subtropics (JARTS) 114: 39-49.

Tarekegne, A. and Das, B. 2015. Breeding for Low N Stress Tolerance in Maize. New Maize Breeders' Training Course, 16 Aug - 6 Sep 2015. Cresta Golf View Hotel, Lusaka, Zambia.

USDA. 2017. Global Agricultural Information Network. https://www.fas.usda.gov/databases/global-agricultural-information-network-gain.(Accessed on 2nd September 2018)

Zelke, H. and A.M. Lalise. 2015. Genotype by Environment Interaction and Yield Stability of Maize (*Zea mays* L.) Hybrids in Ethiopia. Haramaya University.

CHAPTER 2
REVIEW OF LITERATURE

2.1 Introduction

This chapter serves to review the progress made in terms of breeding for low nitrogen (N) stress tolerance in maize within Eastern and Southern Africa (ESA). Marker-assisted quality control genotyping for the maize parental lines and the resultant F1 maize hybrids forms part of the components in this review chapter. It gives strong theoretical and practical dimensions of current and previous prominent research and plant breeding work accomplished by other scientists in the context of low N stress tolerance, genotype-by-environmental interaction (GEI) and stability analyses, heritability, path and correlation analysis and marker assisted quality control genotyping.

2.2 Maize taxonomy and origin

Maize (*Zea, mays L*.) is a diploid (2n = 2x = 20) C4 plant that belongs to the grass family Poaceae. There are five species in the genus *Zea*, but *Z. mays* is the only cultivated species. Maize originated from the wild grasses commonly referred to as teosinte (*Z*. *Mexicana*) with centre of origin being Mesoamerica, primarily Mexico and the Caribbean (Verheye, 2010). It is a predominantly cross-pollinating species with exceptional genetic diversity leading to broad adaptation and responsiveness to selection pressure. Therefore, the maize plant has sufficient genetic potential for further improvement.

2.3 Maize improvement efforts in Sub-Saharan Africa

The successes of maize production in sub-Saharan Africa (SSA) are attributed to the ongoing research work conducted by International Maize and Wheat Improvement Center (CIMMYT) and International Institute of Tropical Agriculture (IITA) in collaboration with private and public research institutes. Together these research organisations are developing and implementing improved agricultural innovations for improved cultivars with enhanced tolerance to biotic and abiotic stresses for the African continent. CIMMYT and research partners' efforts towards improved maize productivity include those directed towards improving maize varieties for multiple stress tolerance. In 1997, CIMMYT initiated a product oriented breeding programme for southern Africa, targeted at improving maize for drought prone environments (Bänziger *et al.,* 2002). Since then, significant progress has been made in improving maize for stress tolerance with the formation of hybrids, inbred lines and open-pollinated varieties.

According to Mashingaidze, (2012), Improved Maize for African Soils (IMAS) project was launched in 2010 and the project ran from 2010 and ended in 2016. IMAS developed maize varieties that are better at capturing the small amount of fertilizer that African farmers can afford, and uses the nitrogen they take up more efficiently to produce grain (Grain SA, 2012).

According to Grain SA (2012), a public-private partnership project called Water Efficiency Maize for Africa (WEMA) began in 2008. The project was in response to a growing call by African farmers, leaders, and scientists to address the devastating effects of drought. WEMA products are drought tolerant white single-cross and three-way conventional and transgenic hybrids that give at least 20% yield advantage under moderate drought conditions. Work is currently in progress to further protect the yield benefits by the inclusion of Monsanto's Bt gene to confer resistance to stem borers and fall armyworm thereby enhancing yield stability. This will increase and stabilise maize production and food self-sufficiency at household level within SSA.

2.4 Low nitrogen stress

Low soil fertility is a major global problem with most effects felt strongly in the developing countries where majority of the population predominantly depend on agriculture (Tully *et al.,* 2015). The problem of low nitrogen in the African soil is one of the consequences of soil degradation that has led to decline in crop productivity, hunger and poverty. Therefore, breeding for low N tolerance becomes a priority to breeders for sustainable maize production within SSA. The following sections provide a detailed review regarding low N stress.

2.4.1 Nitrogen and its economic importance

Nitrogen (N) is among the essential elements necessary for plant growth and development. Maize growth is highly sensitive to the levels of N in the soil. Banzinger and Diallo (2014), reported that maize growth is highly sensitive to the amount of N available in the soils and that most of the tropical soils are nitrogen deficient. Hence, it is justifiable to develop more varieties that are tolerant to low N. Among several functions, N plays a key role in different metabolic pathways and participates in protein synthesis and chlorophyll biosynthesis, and critical processes for early phenological stages of plant development (Basso and Ceretta, 2000). Nitrogen is mostly abundant in plant leaves especially in photosynthetic enzymes where it may account for up to 4% of the dry weight. Since N uptake, biomass production and grain yield are strongly associated, the N requirement of maize can be related to grain yield (Banziger *et al.,* 2000). Additionally, the abundant availability of N in plant leaves confirms that N plays a significant role in leaf chlorophyll formation and this is an indicator that N is the main determinant of grain yield (Banziger *et al*., 2000).

It is therefore, essential to initiate low N breeding programme to increase maize productivity under the nitrogen deficient tropical soils.

2.4.2 Status of low N in SSA soils

There is continued low use of inorganic fertilisers among smallholder farmers leading to low levels of N in African soils below the plant requirement. Generally, an average value of 8 kg ha^{-1} is applied as a supplementary source of soil N (Heisey and Norton, 2007; Vanlauwe *et al.,* 2010), which is very low compared to the 50 kg ha^{-1} target set by the 2006 Africa Fertiliser Summit (Vanlauwe *et al*., 2010). Statistics indicate that the SSA region uses very low levels of N, at an average of 11 kg ha^{-1} yr^{-1} (Sommer *et al*., 2013), despite the range of 90 to 120 kg ha^{-1} yr^{-1} recommended for optimum production. Heisy and Mwangi (1996) reported that high cost of fertiliser is among the major reasons why fertiliser use in the region is low. According to Mosier *et al.* (2005), high cost of fertiliser in SSA is due to inadequate transportation and distribution infrastructure. However there has been slight improvement on the use of fertiliser in SSA, from 6-7 kg ha^{-1} yr^{-1} in 2008 to 11 kg ha^{-1} yr^{-1} in 2014, and 12 kg ha^{-1} yr^{-1} in 2015, though this is still far below the Abuja declaration target of 50 kg ha^{-1} yr^{-1} by 2015 (Wanzala, 2010). Larsson (2005) also reported that in the years 2000 to 2002, 53% of smallholder farmers in SSA applied 0 kg ha^{-1} of fertilizer while the other 47% actually applied inorganic fertiliser at the average rate of 14 kg ha^{-1}, which is far below the requirement of 300 to 400 kg ha^{-1} for optimal production.

Consequently, because of these practices of low fertilizer applications, the problem of low N in SSA has persisted resulting in low yields compared to those realised in the developed countries and research trials (Fakorede *et al.,* 2003). Hence, low N remains one of the major abiotic factors limiting maize production in the tropics where fertiliser is rarely used and organic matter is rapidly mineralised (Bänziger and Lafitte, 1997). Additionally, removal of crop residues as feed and fuel also contributes to the occurrence of low N condition in the African soils (Zambezi and Mwambula, 1997).

2.4.3 Relationship between low N and drought stress

Nitrogen movement in the soil depends on the availability of adequate soil moisture. During drought stress conditions, water availability and movement is highly reduced hence drought and low N stress are highly and positively associated (Derera, 2005). Fisher *et al.* (2015), reported that over 40% of Africa's maize growing regions are prone to drought stress, which frequently cause between 10-25% yield losses; and around 25% of the maize grown suffers frequent droughts, with 50% yield losses incurred.

These drought prone areas are also the same areas, which are low in soil fertility, especially nitrogen, resulting in the concurrent occurrence of low N stress and drought stress conditions.

Drought stress during plant development will reduce the rate of absorption of nutrients including nitrogen (Sanaullah *et al.,* 2012) by reducing nutrient supply through mineralization as well as the movement of nutrients in the soil (Lambers *et al.,* 2008). Enhanced mineralization occurs after availability of moisture through irrigation or natural rainfall, following a drought stress period (Austin *et al.,* 2004) due to the nutrient release from dead microbial biomass that would have accumulated during the period of stress (Borken and Matzner, 2009).

Absorption, utilization and mobilization of essential nutrients are very critical for plant growth and development. Under drought stress conditions, the nutrient use efficiency of a plant is highly reduced. According to Xu *et al.* (2012), although plant nutrient use efficiency, including nutrient uptake, transport, assimilation and remobilization depends on the genetic and environmental influences, the impact of limited soil moisture availability significantly reduces nutrient uptake processes. Various researchers have confirmed the association of these two abiotic stresses and reported similar plant responses to drought and low stress. Presterl *et al*. (2002) reported nitrogen deficit as a cause of delayed leaf area expansion and shoot growth but increased the growth of the root, which is likened to plant drought stress response. Badu-Apraku *et al.* (2013) reported that genotypes with drought tolerance capability also shows significant degree of tolerance to low soil N stress. Banziger *et al.* (1999) reported that improvement for drought tolerance using recurrent selection leads to correlated improvement for low-N tolerance. Hence, improvement of either of these two abiotic stresses has a positive contribution towards stress tolerance to the other stress. Similarly, secondary traits such as number of ears per plant, anthesis-silking interval and leaf senescence have been reported to aid selection for yield improvement under low N and drought (Banzinger *et al.,* 2000). This clearly demonstrates that there is a strong association between low N stress and drought stress tolerance that breeders can utilise in developing varieties that are tolerant to both low N and drought stress.

2.4.4 Maize response to low N Stress

Maize responds positively to any addition of N fertilisers (Lafitte, 2000), and any N stress significantly reduces grain yield. Maize plant responds to N stress conditions in various ways including reduced plant size and radiation use efficiency and remobilization of stem N to the growing sinks (Vance, 2001). Occurrence of low N stress during the early and vegetative phenological stages of plant development causes premature leaf yellowing of leaves; a deficiency symptom for insufficient N in the soils. It causes retarded growth, reduced plant size and reduced photosynthesis. All together, these will lead to reduced biomass production and

eventually low grain weight and reduced grain kernel numbers (Muchow and Davis, 1988; Vance, 2001). Photosynthesis is drastically reduced due to the reduction of leaf area development because of stress and accelerated leaf senescence. Leaves form the basic units for photosynthesis and their reduced growth, and limitations by leaf senescence will affect biomass production by photosynthesis. Leaf senescence proceeds from the bottom of the plant, as N is remobilised from older leaves to younger leaves and grain (Bänziger et al., 2000).

Unlike drought stress, there is a consistent pattern in maize growth and development response to low N stress conditions irrespective of the location (Banziger *et al*., 2000). During the early stage of growth and development, N requirement of the crop is far less than the available soil N source from mineralization and the plant does not suffer. As the growth progresses, the crop N requirement increases and exceeds the rate of mineralization of soil N leading to deficit (Banziger *et al*., 2000). This phenomenon is manifested at the vegetative phonological phase of the crop and thus the need for application of inorganic fertilizer at this stage to meet the N deficit gap that would have been created in the soil. Failure to do this may result in N stress in the plant. However, plants adjust to low N stress to some extent by remobilising N from older tissue, and depending on the timing of N stress, several yield-determining factors are disadvantaged.

Nitrogen stress before flowering reduces leaf area development, photosynthesis rate, and the number of ear spikelets (potential grains). Nitrogen stress during flowering stage results in kernel and ear abortion, whereas N stress during grain filling accelerates leaf senescence and reduces crop photosynthesis and kernel weight (Banziger *et al.,* 2000). Severe N stress typically delays silking more than anthesis, leading to extended anthesis-silking interval (SI). The delay in silk emergence results in ineffective pollination, and kernel and ear abortions. Additionally, low-N stress induces premature leaf yellowing (first stage of senescence) though this condition is reversible if N fertiliser is applied.

2.4.5 Genetic gain under low N stress

Genetic gain is defined as the difference in the mean value of the selection criterion between the original generation and the next generation, which is formed from only the selected individuals, when they are compared in the same environment (USDA/NIFA, 2018). The selection criterion is the trait(s) on which selection is based. Genetic gain studies are useful to predict the success of selection for important characteristics (Castleberry *et al.,* 1984; Duvick, 2005; Wang *et al*., 2011). Milestones in genetic gain realised in crop improvement studies have been reported in many countries such as the United States of America (USA) (Duvick *et al*., 2004); Argentina (Eyherabide and Damilano, 2001); Canada (Tollenaar, 1989) and China

(Ma *et al*., 2015), with substantial contributions to yield. On the other hand, genetic gain studies in SSA are difficult to realise due to differences in materials used under different management operations. Badu-Apraku *et al*. (2014) reported genetic gains from 2.28 to 2.61 t ha^{-1} under low N and from 3.2 to 3.65 t ha^{-1} under high N, giving the same relative gain of 30 kg ha^{-1} for the three season period of research under both management levels.

Substantial genetic gains have been achieved globally through breeding, but any significant increases above that will only be attainable with the use of improved agronomic practices, pest and disease control and fertiliser use (Duvick, 2005). Initial improvement for low N tolerance was conducted by CIMMYT on Across 8328 maize cultivar based on its superiority under low and high N conditions. This is a yellow-grained, late-maturing maize genotype that is adapted to the lowland tropical regions. Initial selection gains in Across 8328, according to Edmeades *et al*. (1994) were 84 and 120 kg ha^{-1} under low N and high N, respectively. Several lines were then selected from Across 8328 BN C5 based on higher frequency of low N tolerant top-crosses. Bello *et al*. (2014) evaluated six hybrids from 1980 through to 2000 for genetic gains under different N levels, reporting 0.67 to 4.89 t ha^{-1} gains in grain yield. Genetic gain in yield *per se* was 42% between 1980 and 2000; and 36% between 1990 and 2000 under optimal fertilization. This means the responsiveness of modern cultivars was higher than that of the hybrids used in the 1980s and 1990s. Kim (1997) estimated a yield advantage of between 20-40% in high yielding environments, especially for research stations with grain yield of over 14.7 t ha^{-1}. Adebo and Olaoye (2010) and Kamara *et al.* (2004) observed a 24% yield increase between the 1970s and 1990s and 0.41% per year between 1970 and 1999 for hybrid cultivars.

2.4.6 Breeding strategy for low N tress tolerance

Plant breeding approaches used in the development of improved maize varieties for tolerance to low N stress include selection for improved yield under high nitrogen and specific mechanisms expected to confer tolerance to low nitrogen (Lafitte and Banziger, 1997). However, breeding gains under low N are predicted to be higher when selection is conducted under both low and high N environments (Bänziger and Lafitte, 1997). Therefore, it is imperative that a breeder must have the right genetic materials and evaluate them for low N stress tolerance accurately under relevant conditions to the target environment.

The Agricultural Research Council-Grain Crops Institute adopted the use of managed low N stress breeding as a way of selecting for low N tolerant genotypes in maize as championed by CIMMYT. It is imperative that some levels of N must be available in the soil in order for significant yield differences to be realised during screening for low N tolerance. The primary goal of managed low N stress is to achieve at least a yield reduction range of 25-35% lower

than the potential yield under optimum conditions. For example, if an ideal genotype yields 7.0 t ha^{-1} under optimum conditions, that genotype must yield 1.5 - 2.5 t ha^{-1} less under low N (Bänziger *et al.,* 1997).

According to Ndhlela (2012), the soil is considered low N when it has 7 ppm N under managed nitrogen stress. Agricultural Research Council-Grain Crop Institute conducts research for low N tolerance by utilising previously N-depleted plots. Practically, the level of depletion differs among the nitrogen-depleted plots due to differences in number of years of depletion which the field has been subjected to. Plant breeders, especially at ARC-GCI, prefer using a continuously depleted N block over several seasons to manage low N stress from season to season. N stress can be increased by continuously using the same low N block, choosing a sandy soil texture field (where other factors are not limiting), growing non-leguminous crops during the rotation/off-season, reducing the time when land is fallow or removing maize stover and weeds after every harvest and hand weeding.

2.4.7 Breeding and selection for low N stress tolerance

Breeding for low N stress tolerance is easier than breeding for drought stress tolerance due to consistency pattern of the effect of nitrogen deficiency on plant growth and development over time unlike drought stress (Banziger *et al.,* 2000). Therefore, testing genotypes under one level of relatively severe low N stress suffices for low N tolerance selection for various levels of nitrogen deficiency. CIMMYT research studies have shown that genotypes selected for drought tolerance also perform better under low nitrogen stress environments.

According to Edmeades *et al.* (1995), selection for drought tolerance at flowering simultaneously improves low nitrogen stress tolerance. Vasal *et al*. (1997) reported that genes responsible for stress tolerance are present in most elite maize populations at relatively low frequency and selection under controlled low N environments is effective in breeding varieties that are tolerant to low N stress. Since yield is a quantitative trait, its improvement under nitrogen stress will depend on how the respective genes respond to stress. Hence, to maximise genetic gain under nitrogen stress, selection environment similar to the target environment is ideal (Bänziger and Lafitte 1997).

2.4.8 Secondary traits for low N tolerance selection

Breeding for low N is a complex process due to low heritability of the traits of interest, and reduction in genotypic variation between low N tolerant genotypes. In low N crop improvement programmes, breeders are primarily concerned with selection for increased grain yield but selection for grain yield under stress conditions may introduce some level of bias as heritability

that determines genetic advance under selection changes with stress pattern and severity (Banziger *et al.,* 2000). Similarly, Bolanos and Edmeades (1993) observed that the use of grain yield alone as a measure of a genotype improvement for stress tolerance is inefficient; but the use of secondary traits that are highly correlated to grain yield and with high heritability values could help to increase selection efficiency under low N.

Secondary traits are beneficial as they strengthen the ability to identify genotypes with genetic potential to tolerate low-N stress. This is possible since the heritability of some secondary traits remains high even under stressed conditions contrary to heritability for grain yield (Banziger and Lafitte, 1997). Banziger *et al*. (2000) highlighted some secondary traits that can guide selection for low-N tolerance in addition to grain yield. Some of these traits overlap for both drought and low-N stresses and these are anthesis-silking interval, leaf senescence and number of ears per plant. Badu-Apraku *et al.* (2011) also reported that the most reliable secondary traits for selection for improved grain yield under low N were plant height, days to silking, days to anthesis, number of ears per plant, anthesis-silking interval, stay green characteristics, ear aspect and plant aspect. Secondary traits are also useful in developing a low N selection index formula for improving maize genotypes under low-N stress. However, it is important to note that when selecting for grain yield under stress environments, only secondary traits having significant correlations with grain yield should be carefully considered. A good secondary trait must be genetically associated with grain yield under stress with high heritability, easy and cheap to measure (Banziger *et al.,* 2000).

2.5 Genotype by environment interaction (GEI) and stability analysis

2.5.1 Genotype x environment interaction

According to Ahmadi *et al.* (2012), Genotype -by -environment interaction (GEI) is defined as differential ranking of genotypes among locations and years. Genotype by environmental interaction consist of crossover interaction (COI) or rank changes across environments and non-COI or scale changes across the environments, with the former being the most crucial interaction in plant breeding (Baker, 1988). Genotype by environment interaction makes it difficult for breeders to select the best performing and most stable genotypes, thus GEI delays breeding progress (Smithson and Grisley, 1992). Most of the tropical regions in SSA have experienced great environmental variability lately due to climate change and grain yield being a complex trait has been greatly affected (Beyene *et al.,* 2011).

Some biotic and abiotic stresses are responsible for the occurrences of GEI. For instance, GEI in most of the growing environments stems from factors related to temperature, rainfall, seasonal length, within season drought, subsoil pH and social economic factors linked to sub-

optimal inputs application (Bänziger *et al.,* 2006). Therefore, enhancing genotypes' resistance or tolerance to different stresses might minimise GEI and that it is important to include GEI studies when breeding for low N stress tolerance. Sellah et al. (1997) reported significant GEI effects for grain yield, days to mid-silking, plant height and number of ears per plant under both high and low nitrogen, implying that the fertility level influenced genotypic expression. Hence, it is justifiable to conduct GEI analysis when breeding for low N. Gallais and Coque (2005) reported in their studies that significant genotype by N interaction has effects on grain yield. They attributed this to the existence of genotype x N interaction effects on kernel number leading to the conclusion that reducing kernel abortion just after fertilisation increased tolerance to low N. Significant genotype x N interaction effects for grain yield means that levels of N in the soil has great influence on grain yield.

Genotype-by-environment interaction is a main concern among plant breeders because it impedes progress from selection and has important repercussions for testing and cultivar release. It poses a challenge in cultivar recommendation because it is statistically difficult to deduce the main effect in control of a particular trait (Kang, 1997). The GEI reduces the relationship that exists between phenotypic ad genotypic variance resulting in best performing genotypes in one environment performing poorly in another. There are a number of methods that have been used by breeders to explore GEI. These methods are discussed below.

2.5.1.1 Analysis of variance

Once the combined ANOVA reveals that genotype (G) and environment (E) main effects and G x E interaction (GEI) are statistically significant, stability analysis is performed on the multi-environment yield data in order to measure the stability levels. Significant GEI means that selection from one environment may often perform poorly in another environment and that the variety is unstable across the environments. Therefore, the ultimate aim of stability analysis is to generate conclusions that would guide breeding direction to develop genotypes with good adaptation to fairly wide environments within seasons and across regions and cultivation conditions (Sabaghnia *et al.*, 2012, Sabaghnia *et al.*, 2013). Several stability measures have been used, including Wricke's ecovalence (Wricke, 1962), Eberhart and Russell's (1966) linear regression method, Shukla's procedure (Shukla, 1972) and cultivar performance (Lin and Binns, 1988). Though these stability analyses procedures are still useful, the use of additive main effects and multiplicative (AMMI) and genotype and genotype by environment interaction (GGE) models are the most common and effective methods in GEI studies.

The magnitude of GEI provides information concerning the likely areas of adaptation of a given genotype. Knowledge of GEI can help to minimise the cost of extensive genotype evaluation

by eliminating unnecessary testing sites and determining efficient methods for using time and resources in a breeding programme (Ceccarelli, 1989).

2.5.1.2 Additive main effect and multiplicative interactions (AMMI)

The AMMI analysis is one of the useful multivariate methods adopted for exploiting GEI. It is a useful decision-making tool employed by breeders during genotype evaluation for quantifying the magnitudes of GEI and the identification of stable and well-adapted genotypes across environments (Acciaresi and Chidichimo, 1999; Vargas *et al.,* (1999). The method is capable of handling both the main effects and GEI in multi-location yield trials more effectively and efficiently than any other statistical model (Gauch, 1993). Nzuve *et al.* (2013), using AMMI analysis reported significant effects for genotypes, environment and the genotype by environment interaction. The results suggest that different hybrids could be selected for different agro-ecological zones confirming the capacity of AMMI model to assess the existence of GEI. Other studies have also reported significant GEI for grain yield in maize genotypes (Carson et al., 2002; Makumbi, 2006; Menkir and Ayodele, 2005). The merger of analysis of variance (ANOVA) and principal component analysis (PCA) in the AMMI model alongside with prediction assessment is an important tool in understanding GEI. The GEI is partitioned into several orthogonal axes namely interaction principal component analysis (IPCA) using PCA of AMMI. IPCA1 and IPCA 2 are used in the construction of a biplot because higher IPCA axes are subjected to noise and have no predictive value (Van Eeuwijk, 1995). According to Wallace *et al.* (1993) statistical analysis of AMMI model can separate and quantify GxE interaction effects on yield and other measurable traits in a multi-location trial.

2.5.1.3 Genotype and genotype by environment interaction (GGE) biplot analysis

Genotype and genotype by environmental interaction biplot is one of the best multivariate, visual method for exploiting GEI and effective stability analysis tool for identification of stable genotypes (Kang, 2003). The biplot analysis was proposed by Gabriel (1971) and modified by Yan *et al.* (2000, 2002, and 2007) to analyse and quantify GEI across multi-environments. GGE biplot displays the genotype plus genotype x environment interaction of the multi-environment (MET) trial data, hence allowing the breeder to concentrate on the part of the MET data that is most useful for genotype selection (Kang, 2003). Yan *et al*. (2007) hinted out that the GGE biplot was the most appropriate type of biplots for mega-environment investigation, genotype evaluation and test location evaluation. With GGE biplot, the test location evaluation is conducted graphically and the test locations are evaluated by defining three parameters. According to Yan (2001), these parameters are; the ability to discriminate between genotypes (Discrimination ability), the ability to represent the target region (representativeness) and the biplot distance from an ideal location (Desirability index). The

performance of the genotype (G) and the interaction of the genotype and the environments (GEI) are important elements to consider when selecting desirable genotypes. On top of dissecting GEI, GGE biplot analysis helps to analyse genotype by trait data, genotype by marker data and diallel cross data. These aforementioned aspects make GGE biplot the most comprehensive tool in quantitative genetics and plant breeding (Kang, 2003). However, critics of GGE biplot analysis criticised it for not separating G from GE, and believed that G pertains to broad adaptations and GE pertains to narrow adaptations (Gauch, 2006). To the contrary, advocates of GGE biplot analysis believed that G and GE must be considered at the same time since G and GE are both relevant to cultivar and test location evaluation (Yan *et al.,* 2007). Yang *et al*. (2009) questioned the robustness of GGE biplot analysis in revealing crossover GEI, which was counter-criticized (Yan *et al.,* 2010). Nevertheless, GGE biplot analysis is being increasingly used by researchers, such as in evaluating test locations for a combination of breeding traits instead of a single trait (Blanche and Myers 2006; Baxevanos *et al.,* 2008).

GGE biplot analysis has been used to evaluate test location additional to mega-environment analysis and genotype evaluation. However, GGE works better for a limited number of genotypes and locations and for a small number of years of multi-location trials. Fan *et al*. (2007) used GGE biplot analysis for multi-location trials at provincial level with ten locations in two years and much emphasis was on the genotype effect for grain yield. Similarly, Yan *et al*. (2000) applied GGE biplot analysis to evaluate wheat trials composed of 10 years and seven to 14 locations as well as mega-environment investigation though it was less focused on test-location evaluation. In Nigeria, GGE biplot analysis was used to evaluate maize genotypes, but without touching test location evaluation and only four locations and 5 years multi-environmental trials were involved (Badu-Apraku and Akinwale 2011). In this study, GGE biplot analysis will be used to evaluate a large number of maize genotypes evaluated across five environments in one cropping season.

2.6 Correlation and path analysis for grain yield and yield components in maize hybrids

Plant breeding aims to improve genotypes in one or more characteristics to suit human needs, though yield is the most important objective in many breeding programmes. Direct selection for yield may be ineffective and difficult due to the complexity of yield and its variability under different environmental conditions due to GEI. Therefore, genetic gains in yield improvements can be improved through exploitation of the relationship that exists between yield and related traits (Machikowa and Laosuwan, 2011).

Correlation analysis estimates the nature of relationship that may exist between two variables, usually yield and its related traits. Correlation estimates demonstrate a cause and effect relationship such that a change in the independent variable causes a change in the dependent variable (Bello *et al.,* 2010). The relationship can be either positive or negative, strong or weak. Where variables are positively correlated, both independent and dependent variables change in the same direction whereas in negatively correlated variables, the variables change in opposite directions. Information on correlations is important in maize where selection of yield is indirect and achieved through selection of secondary traits (Bello and Olaoye, 2009). However, correlations fall short in describing the importance of each trait contributing to the final yield (Sreckov *et al.,* 2011). This inadequacy can be misleading where observed variations are due to more than one indirect cause (Bizeti *et al.,* 2004). Therefore, there is need for a more in-depth analysis of the interactions to understand the importance of each trait and rank their importance in selection. One way to achieve this is by using the path coefficient analysis (Udensi and Ikpeme, 2012).

Path coefficient analysis plays a critical role in partitioning the observed change in the dependent variable into contributory effects by each independent variable (Beiragi *et al.,* 2011). It is a useful method for examining direct and indirect relationships of complex traits. Understanding of the grain yield-secondary traits relationship will greatly improve selection methods (Rafiq *et al.,* 2010) as it helps to rank the secondary traits in order of their importance in yield improvement. The breeder will then target traits with highest contributory effects for selection.

2.7 Heritability, variance components, genetic advance for grain yield and yield components

Adequate understanding of the mode of inheritance of quantitative characters is an essential component of plant breeding. Heritability estimates indicate the extent to which given characters would be transmitted to the next generation (Acquaah, 2007). The knowledge of heritability of a character helps plant breeders to predict the advance from selection. The higher the heritability, the simpler the selection process and the greater the response from selection (Padmaja *et al.,* 2008). Scientists have defined heritability as the measure of phenotypic variance among individuals in a population due to genetic causes and it has predictive function in plant breeding (Nyquist and Baker, 1991).

Heritability estimates exist in two folds, thus broad-sense and narrow-sense heritability. Broad-sense heritability is the ratio of genetic variance to phenotypic variance and it captures the proportion of phenotypic variation due to genetic values that include additive, dominant and

epistatic effects. In other ways, all the gene actions are involved. Narrow-sense heritability is defined as the ratio of additive variance to phenotypic variance and it takes into account only that proportion of genetic variation that is due to additive genetic effects (Falconer and Mackay 1995). Heritability in narrow sense is the most important aspect in plant selection programs. It determines the breeding value of a population since response to artificial and natural selection depends on additive genetic variance (Hill *et al.,* 2008). Ramanujam and Thirumalachar (1967) reported on the limitation of estimating heritability in broad sense as inclusion of both additive and epistatic gene effects simultaneously thus, heritability estimates in broad sense would be reliable if accompanied by high genetic advances.

Estimation of heritability in a population depends on the partitioning of observed variation into components that reflect unobserved genetic and environmental factors as well as empirical data on the observed and expected resemblance between relatives (Wray and Visscher 2008). Knowledge of heritability determines the choice of selection strategy most useful to improve a given character to predict selection gains and determine the relative important of genetic effect in controlling that particular trait (Kashiani *et al.,* 2010; Laghari *et al.,* 2010). Therefore, characters with high heritability values can be easily fixed with simple selection, translating into quick progress. Najeeb *et al.* (2009) reported that heritability alone has no practical importance without genetic advance. Genetic advance shows the degree of gain achieved in a character under a given selection pressure. High genetic advances coupled with high heritability estimates offer the most suitable condition for selection. Several researchers (Rafique *et al.,* 2004; Akbar *et al.,* 2008; Rafiq *et al.,* 2010) have reported high heritability and high genetic advances for different yield controlling traits in maize. Hence, adequate knowledge of these genetic parameters is key for effective crop improvement programmes.

2.8 Marker-assisted genetic purity assessment of inbred lines, diversity and hybrids parent-offspring test

Genetic purity assessment of maize inbred lines, diversity and parental-offspring test are important quality control genotyping procedures for successful hybrid breeding programmes and seed production. It is key in deploying high quality hybrid seeds with increased crop yield potential (heterosis) as well as intellectual property (IP) protection. Quality controls prevent genetically impure lines to be part of the breeding program and identify possible errors that might compromise quality along the seed production value chain. Knowledge of genetic diversity among the parental inbred lines is very important for successful crop improvement programmes (Choukan, 2011).

2.8.1 Methods used in genetic purity assessment, diversity and parent-offspring test

Genetic purity, diversity and parent-offspring test can be estimated using a number of methods including phenotypic pedigree data and molecular markers (Semagn *et al*., 2012). Phenotypic pedigree data uses observable descriptors while molecular markers genotype identification is based directly at the DNA level. Various types of molecular markers can be used to estimate these quality parameters (diversity, purity and parent-offspring test) and these include, restriction fragment length polymorphisms (RFLP), random amplified polymorphism DNA (RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeats (SSRs) and single nucleotide polymorphism (SNP). With the advancement of technology, currently there is a shift towards the use of SNP markers due to their low cost per data point, high genomic abundance, co-dominance, potential for high throughput analysis and lower genotyping error (Foster *et al.,* 2010; Semagn *et al.,* 2012). Parida *et al*. (2012) further complemented that SNP markers have gained significant importance in plant breeding because of their excellent genetic attributes and suitability for genetic diversity analysis and evolutionary relationships, understanding of population substructure, detection of genome-wide linkage disequilibrium, and association mapping of genes controlling complex phenotypic traits.

According to Semagn *et al.* (2012), SNP markers have emerged as a powerful biotechnology tool for many molecular genetic studies including diversity and marker-assisted breeding. Lu *et al.* (2011) studied the genetic diversity using SNP markers between tropical and temperate lines and reported that tropical germplasm had substantially higher genetic distances (0.238-0.548) than temperate germplasm (0.224-0.473). Similarly, Semagn *et al*. (2012) carried a study on quality control genotyping for assessment of genetic purity and identity in diverse tropical maize inbred lines using SNPs and reported that genetic purity varied from 68.7% to 100% with 71.3% of the lines considered pure. This background information demonstrates the effectiveness of SNP markers in diversity studies and quality control genotyping.

2.8.2 Selection of SNPs and quality control genotyping approach

Based on several data sets obtained from a wide range of sample sizes and genetic background, CIMMYT global maize breeding programme recommended a subset of 50 to 100 SNPs for low cost quality control genotyping (Semagn *et al*. (2012). These SNPs were validated in an array of tropical and subtropical maize inbred lines developed by CIMMYT and widely used by maize improvement programs across SSA, Latin America and Asia. Based on this background, Semagn *et al*. (2012) used 28 maize inbred lines to study genetic identity among different seed sources by genotyping them with 53n and 1065 ANPs using KASPar and Golden Gate platform, respectively.

Additional set of 554 inbred lines was also used for studying genetic purity. In the end, a quality control genotyping protocol that could minimise errors in genetic analysis was developed. Based on the same set of SNPs, Chen *et al*. (2016) further outlined two approaches that can be used in quality control (QC) genotyping based on the number of markers used thus "broad QC" and "rapid QC". "Broad QC" genotype analyses genotype genetic identity using 50 –100 carefully selected SNPs recommended by CIMMYT. This approach reveals the identity and purity of the founding parents and evaluates the levels of residual heterozygosity within a particular parental inbred line. On the other hand, "Rapid QC" uses a smaller sub-set of 10 - 15 selected markers, and can help quickly assess with high accuracy the possibility of mislabelling of entries across a panel of inbred lines or seed lots.

2.8.3 Inbred lines genetic diversity and maize hybrid breeding

Hybrid breeding is an important breeding approach for increased production taking advantage of heterosis/hybrid vigour manifestation. Heterosis, or hybrid vigour, is the increased performance of hybrid progeny compared to their inbred parents (East, 1908). Heterosis is manifested in increased size, growth rate, and other parameters in the resultant F_1 hybrid generation after crossing two inbred lines (Melchinger and Gumber, 1998; Tollenaar *et al.,* 2004). Exploitation of heterosis was chiefly responsible for the tremendous increase in maize yield in the United States of America (USA) between the1930s and the 1970s (Duvick, 2001). Information on genetic diversity helps in grouping germplasm into appropriate heterotic groups for successful breeding programmes since genetically divergent genotypes are expected to have high hybrid vigour (Dandolini *et al,.* 2008). Knowledge on diversity is crucuial for plant breeders to know the extent of already existing genetic variation within the plant breeding materials and identfy areas for further improvement (Cholastova *et al.,* 2011). Therefore, use of divergent genotypes is ideal for realisation of higher yield in a breeding programme. Dandolini *et al.* (2008) and Hallauer and Miranda (1988) further agree that divergent population improvement usually targets to achieve yield increase through exploitation of heterosis.

2.8.4 Genetic purity of maize parental inbred lines

According to Gowda *et al*. (2017), maize inbred lines for hybrid production are considered genetically pure when the proportion of heterozygous loci does not exceed 5%. Any inbred line showing more than 5% but less than 15% heterozygous loci is not genetically pure hence requires purification by performing ear-to-row selection. The worst scenario occurs when inbred lines exhibit >15% heterozygosity and such level of contamination is due to unrelated genetic materials that warrant the lines to be discarded or extensively reselected for the original genotype. Daniel *et al.* (2012) reported on the use of SSR markers in genetic purity

analysis where genetic purity level of the inbred lines ranged between 91.3% and 98.7% while the hybrids ranged between 81.3% and 95%. In a similar study, Elçi and Hançer (2015) reported on genetic purity of more than 98% for maize hybrids and thir parental inbred lines using 50 SSR markers. Ertiro *et al.* (2017) using 220878 SNP markers on 265 maize inbred lines found that only 22% of the inbred lines were pure with residual heterozygosity levels of < 5% while the other 78 % were consired not pure due to their residual heterozygosity levels between 5.1% and 31.5%. Overall, maize inbred lines with genetic purity of >95% are acceptable for use in maize hybrid breeding and seed production (Gowda *et al.*, 2017).

2.8.5 Parent-offspring test and crossing validation

Parent-offspring test for F1 hybrids confirms whether a specific hybrid is indeed derived from the original parental inbred lines without any foreign pollen contamination (Gowda *et al.*, 2017.) This test helps the breeder or commercial seed producer to validate the crossing process during hybrid seed production at the production site. In SSA, low-cost three-way maize hybrids are widely commercialized as compared to single-cross hybrids. In the process of developing three-way hybrids, and during the formation of single-cross parents of such hybrids, there is a possibility of contamination. Therefore, parent-offspring tests before cultivar commercialisation are necessary to increase the confidence of seed producers and ensure production of genuine high-quality hybrid seed for commercialisation.

2.9 Conclusion

Low nitrogen stress is among the major threats to sustainable maize production in SSA. The most vulnerable are the smallholder farmers who happen to dominate the farming sector in most of the SSA countries. Literature has also revealed that there is untapped potential for breeders to develop maize varieties that are tolerant to low N. Hence, breeding maize hybrids tolerant to low nitrogen is feasible. Genotype-by-environment interaction is of great concern to plant breeders as it causes variability in the performance of varieties in different locations. Cross-over interaction (COI) GEI impedes progress from selection and poses important repercussions for testing and cultivar release. This suggest the need to evaluate hybrids in different locations and years to identify the high yielding and stables ones before recommending them for release. Literature review has also revealed that quality control genotyping for assessment of genetic purity, diversity and parent-offspring test is a critical component for successful hybrid breeding programmes and seed production. Single nucleotide polymorphism SNP markers are the most preferred biotechnology tool for quality control genotyping.

2.10 References

Acciaresi, H.A. and H.O. Chidichimo. 1999. Genotype-environment interaction in avena sativa l: employing AMMI and factorial correspondence models. Pesquisa Agropecuária Brasileira 34: 1823-1830.

Acquaah, G. 2007. Principles of Plant Genetics and Breeding Blackwell. Malden, UA. Pp509-518.

Adebo, F.A. and G. Olaoye. 2010. Growth indices and grain yield attributes in six maize cultivars representing two era of maize breeding in Nigeria. Journal of Agricultural Science 2: 218.

Adebo, F. and G. Olaoye. 2015. Growth indices and grain yield attributes in six maize cultivars representing two era of maize breeding in Nigeria. Journal of Agricultural Research and Development 14: 11-25.

Ahmadi, J., A. Mohammadi and T. Najafi Mirak. 2012. Targeting promising bread wheat (*Triticum aestivum L*.) lines for cold climate growing environments using AMMI and SREG GGE Biplot analyses. Journal of Agricultural Science and Technology 14: 645-657.

Akbar, M., M.S. Shakoor, A. Hussain and M. Sarwar. 2008. Evaluation of maize 3-way crosses through genetic variability, broad sense heritability, characters association and path analysis. Journal of Agricultural Research (Pakistan). 46(1): 39-45.

Alumira J., Rusike J. 2005. The green revolution in Zimbabwe. Electronic Journal of Agricultural and Development Economics 2:50-66.

Austin, A.T., L. Yahdjian, J.M. Stark, J. Belnap, A. Porporato, U. Norton et al. 2004. Water pulses and biogeochemical cycles in arid and semiarid ecosystems. Oecologia 141: 221-235.

Badu-Apraku, B., M. Fakorede and M. Oyekunle. 2014. Agronomic traits associated with genetic gains in maize yield during three breeding eras in West Africa. Maydica 59:49–57.

Badu-Apraku, B., M. Oyekunle, A. Menkir, K. Obeng-Antwi, C. Yallou, I. Usman et al. 2013. Comparative performance of early-maturing maize cultivars developed in three eras under drought stress and well-watered environments in West Africa. Crop Science 53: 1298-1311.

Badu-Apraku, B. and R. O Akinwale. 2011. Cultivar evaluation and trait analysis of tropical early maturing maize under Striga-infested and Striga-free environments. Field Crops Research 121: 186-194.

Badu-Apraku, B., M. Oyekunle, R.O Akinwale and A.F. Lum. 2011. Combining Ability of Early-maturing White Maize Inbreds under Stress and Nonstress Environments All rights reserved. No part of this periodical may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopying, recording, or any information storage and retrieval system, without permission in writing from the publisher. Agronomy Journal 103: 544-557.

Baker, R. 1988. Tests for crossover genotype-environmental interactions. Canadian Journal of Plant Science 68: 405-410.

Bänziger, M., Betrán, F. J. and Lafitte, H. R. 1997. Efficiency of high-nitrogen selection environments for improving maize for low-nitrogen target environments. Crop Science 37:1103-1109.

Bänziger, M., Edmeades, G. O. and Lafitte, H. R. 2002. Physiological mechanisms contributing to the increased N stress tolerance of tropical maize selected for drought tolerance. Field Crops Research 75:223-233.

Bänziger, M. and H. Lafitte. 1997. Breeding tropical maize for low N environments. II. The values of secondary traits for improving selection gains under low N. Crop Science 37: 1110-1117.

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

Bänziger, M., G. Edmeades and H. Lafitte. 1999. Selection for drought tolerance increases maize yields across a range of nitrogen levels. Crop Science 39: 1035-1040.

Banziger, M., G. Edmeades, D. Beck and M. Bellon. 2000. Breeding for drought nitrogen stress tolerance in maize: from theoryto practice. Centro Internacional de Mejoramiento de Maiz y Trigo, Mexico (Mexico).

Bänziger, M. and J. de Meyer. 2002. Collaborative maize variety development for stress-prone environments in southern Africa. Farmers, scientists, and plant breeding: Integrating knowledge and practice. CABI, Oxon, United Kingdom: 269-296.

Bänziger, M., P.S. Setimela, D. Hodson and B. Vivek. 2006. Breeding for improved abiotic stress tolerance in maize adapted to southern Africa. Agricultural Water Management 80: 212-224.

Basso, C. and C. Ceretta. 2000. Manejo do nitrogênio no milho em sucessão a plantas de cobertura de solo, sob plantio direto. Revista Brasileira de Ciência do Solo 24(4):905-915

Blanche, S.B. and G.O. Myers. 2006. Identifying discriminating locations for cultivar selection in Louisiana. Crop Science 46: 946-949.

Baxevanos, D., C. Goulas, J. Rossi and E. Braojos. 2008. Separation of cotton cultivar testing sites based on representativeness and discriminating ability using GGE biplots. Agronomy Journal 100: 1230-1236.

Beiragi, M.A., M. Ebrahimi, K. Mostafavi, M. Golbashy and S.K. Khorasani. 2011. A study of morphological basis of corn (*Zea mays L*.) yield under drought stress condition using correlation and path coefficient analysis. Journal of Cereals and Oilseeds 2: 32-37.

Bello, O. and G. Olaoye. 2009. Combining ability for maize grain yield and other agronomic characters in a typical southern guinea savanna ecology of Nigeria. African Journal of Biotechnology 8 (11): 2518-2522.

Bello, O., S. Abdulmaliq, M. Afolabi and S. Ige. 2010. Correlation and path coefficient analysis of yield and agronomic characters among open pollinated maize varieties and their F 1 hybrids in a diallel cross. African Journal of Biotechnology 9: 2633-2639.

Bello, O.B., O.J. OLAWUYT, S.Y. Abdulmaliq, S.A. Ige, J. MAHAMOOD, M.A. AZEEZ et al. 2014. Yield performance and adaptation of early and intermediate drought-tolerant maize genotypes in Guinea Savanna of Nigeria. Sarhad Journal of Agriculture 30(1): 53-66

Beyene, Y., S. Mugo, C. Mutinda, T. Tefera, H. Karaya, S. Ajanga et al. 2011. Genotype by environment interactions and yield stability of stem borer resistant maize hybrids in Kenya. African Journal of Biotechnology 10: 4752-4758.

Bizeti, H.S., C.G.P.d. Carvalho, J.R.P.d. Souza and D. Destro. 2004. Path analysis under multicollinearity in soybean. Brazilian archives of biology and technology 47: 669-676.

Bolaños, J., G. Edmeades and L. Martinez. 1993. Eight cycles of selection for drought tolerance in lowland tropical maize. III. Responses in drought-adaptive physiological and morphological traits. Field Crops Research 31: 269-286.

Borken, W. and E. Matzner. 2009. Reappraisal of drying and wetting effects on C and N mineralization and fluxes in soils. Global change biology 15: 808-824.

Carson, M., M. Goodman and S. Williamson. 2002. Variation in aggressiveness among isolates of Cercospora from maize as a potential cause of genotype-environment interaction in gray leaf spot trials. Plant Disease 86: 1089-1093.

Castleberry, R., C. Crum and C. Krull. 1984. Genetic Yield Improvement of US Maize Cultivars under Varying Fertility and Climatic Environments 1. Crop science 24: 33-36.

Ceccarelli, S. 1989. Wide adaptation: How wide? Euphytica 40: 197-205.

Chen, J., C. Zavala, N. Ortega, C. Petroli, J. Franco, J. Burgueño et al. 2016. The development of quality control genotyping approaches: a case study using elite maize lines. PloS one 11: e0157236.

Cholastova, T., M. Soldanova and R. Pokorny. 2011. Random amplified polymorphic DNA (RAPD) and simple sequence repeat (SSR) marker efficacy for maize hybrid identification. African Journal of Biotechnology 10: 4794-4801.

Choukan, R. 2011. Genotype, environment and genotype× environment interaction effects on the performance of maize (*Zea mays L.)* inbred lines.

Crossa, J. 1990. Statistical analyses of multilocation trials. Advances in agronomy. Elsevier. Page 55-85.

Dandolini, T.S., C.A. Scapim, A.T. do Amaral Júnior, C.A. Mangolin, M.d.F.P. da Silva, A. de Souza Mott et al. 2008. Genetic divergence in popcorn lines detected by microsatellite markers. Crop Breeding and Applied Biotechnology v. 8, n. 4, p. 313-320, 2008

Daniel, I., J. Adetumbi, O. Oyelakin, S. Olakojo, M. Ajala and S. Onagbesan. 2012. Application of SSR markers for genetic purity analysis of parental inbred lines and some commercial hybrid maize (*Zea mays L*.). American Journal of Experimental Agriculture 2: 597.

Derera, J. 2005. Genetic effects and associations between grain yield potential, stress tolerance and yield stability in southern African maize (*Zea mays L.)* base germplasm. PhD thesis, University of KwaZulu Natal, Pietermaritzburg, South Africa.

Duvick, D.N. 2001. Biotechnology in the 1930s: the development of hybrid maize. Nature Reviews Genetics 2: 69-74

Duvick, D.N. 2005. The contribution of breeding to yield advances in maize *(Zea mays L.).* Advances in agronomy 86: 83-145.

Duvick, D.N., J. Smith and M. Cooper. 2004. Long-term selection in a commercial hybrid maize breeding program. Plant Breeding Reviews 24: 109-152.

Eberhart, S.A. and Russel, W.A. (1966). Stability parameters for comparing varieties. Crop Science, 6, 36–40.

East, E. 1908. Inbreeding in corn, pp. 419–428 in. Reports of the Connecticut Agricultural Experiment Station for Years 1907–1908.

Edmeades, G.O., Chapman, S.C., Bolaños, J., Bänziger, M. and Lafitte, H.R., 1995. Recent evaluation of progress in selection for drought tolerance in tropical maize. Research for Stress Environments. Proceedings of the Fourth Eastern and Southern Africa Regional Maize Conference.

Edmeades, G., H. Lafitte, J. Bolaños, S. Chapman, M. Banziger and J. Deutsch. 1994. Developing maize that tolerates drought or low nitrogen conditions. Mexico, D.F.: CIMMYT, pp: 21-68.

Elçi, E. and T. Hançer. 2015. Genetic analysis of maize *(Zea mays L.)* hybrids using Microsatellite Markers. Tarım Bilimleri Dergisi 21: 192-198.

Ertiro, B.T., K. Semagn, B. Das, M. Olsen, M. Labuschagne, M. Worku et al. 2017. Genetic variation and population structure of maize inbred lines adapted to the mid-altitude sub-humid maize agro-ecology of Ethiopia using single nucleotide polymorphic (SNP) markers. BMC genomics 18: 777-779

Eyherabide, G.H. and A. Damilano. 2001. Comparison of genetic gain for grain yield of maize between the 1980s and 1990s in Argentina. Maydica 46: 277-282.

Fakorede, M., B. Badu-Apraku, A. Kamara, A. Menkir and S. Ajala. 2003. Maize revolution in West and Central Africa: an overview. Maize revolution in West and Central Africa: 3-15.

Falconer, D.S. and F.C. Mackay. 1995. Introduction to Quantitative Genetics (4th ed.).Longman. ISBN 978-0582243026..

Fan, X.-M., M.S. Kang, H. Chen, Y. Zhang, J. Tan and C. Xu. 2007. Yield stability of maize hybrids evaluated in multi-environment trials in Yunnan, China. Agronomy journal 99: 220-228

Fischer, R., D. Byerlee and G. Edmeades. 2015. Crop yields and global food security. ACIAR: Canberra, ACT: 8-11.

Foster, J.T., G.J. Allan, A.P. Chan, P.D. Rabinowicz, J. Ravel, P.J. Jackson et al. 2010. Single nucleotide polymorphisms for assessing genetic diversity in castor bean (Ricinus communis). BMC Plant Biology 10: 13.

Gauch, H.G. 1993. MATMODEL Version 2.0: AMMI and related analyses for two-way data matricesSoil, Crop, and Atmospheric Sciences, Cornell University.

Gabriel, K.R. 1971. The biplot graphic display of matrices with application to principal component analysis. Biometrika 58: 453-467.

Gallais, A. and M. Coque. 2005. Genetic variation and selection for nitrogen use efficiency in maize: a synthesis. Maydica 50: 531-537

Gauch, H.G. 2006. Statistical analysis of yield trials by AMMI and GGE. Crop science 46: 1488-1500.

Grain SA. 2012. Focus on improved maize for african soils. https://www.grainsa.co.za/focus-on-improved-maize-for-african-soils. (Accessed 3 September 2018)

Gowda, M., M. Worku, S.K. Nair, N. Palacios-Rojas, G. Huestis and B. Prasanna. 2017. Quality Assurance/Quality Control (QA/QC) in Maize Breeding and Seed Production: Theory and Practice. Internationa Maize and Wheat Improvement Center (CIMMYT).22-pages

Hallauer, A. 1988. Hereditary variance: Mating design. p. 45–114. AR Hallauer and JB Miranda (ed.) Quantitative genetics in maize breeding. Iowa State Univ. Press, Ames. Hereditary variance: Mating design. p. 45–114. In AR Hallauer and JB Miranda (ed.) Quantitative genetics in maize breeding. Iowa State University. Press, Ames.: -.

Heisey, P.W. and G.W. Norton. 2007. Fertilizers and other farm chemicals. Handbook of Agricultural Economics 3: 2741-2777

Heisey, P.W. and W. Mwangi. 1996. Fertilizer use and maize production in sub-Saharan AfricaCIMMYT. n Africa's Emerging Maize Revolution, ed. D. Byerlee and C. K. Eicher. Boulder, CO: Lynne Rienne.

Hill, W.G., M.E. Goddard and P.M. Visscher. 2008. Data and theory point to mainly additive genetic variance for complex traits. PLoS genetics 4: e1000008.

Kang, M.S. 1997. Using genotype-by-environment interaction for crop cultivar development. Advances in Agronomy. Elsevier. page 199-252.

Kang, M.S. 2003. Ecovalence and stability variance. Handbook of formulas and software for plant geneticists and dreeders. Haworth Press Inc. New York: 123.

Kamara, A., A. Menkir, M. Fakorede, S. Ajala, B. Badu-Apraku and I. Kureh. 2004. Agronomic performance of maize cultivars representing three decades of breeding in the Guinea savannas of West and Central Africa. The Journal of Agricultural Science 142: 567-575.

Kaya, Y., Ackura, M. and Taner, S. 2006. GGE Bi-plot analysis of multi-environment yield trials in bread wheat. Turkey Journal of Agriculture 62:199-246.

Kashiani, P., G. Saleh, N. Abdullah and S. Abdullah. 2010. Variation and genetic studies on selected sweet corn inbred lines. Asian Journal of Crop Science 2: 78-84.

Kim, S.K., 1997. Achievements, challenges and future direction of hybrid maize research and production in West and Central Africa. Contributing to food self sufficiency in maize research and development in West and Central Africa. Proceedings of a regional maize workshop.

Lafitte, H. R., & Banziger, M. 1997. Maize population improvement for low soil N: Selection gains and the identification of secondary traits. Developing Drought-and Low N-Tolerant Maize. Proceedings of a Symposium; El Batan, Tex.(Mexico); 25-29 Mar 1996. ^ TDeveloping Drought-and Low N-Tolerant Maize. Proceedings of a Symposium; El Batan, Tex.(Mexico); 25-29 Mar 1996^ AEdmeades, GO Banziger, M. Mickelson, HR Peña-Valdivia, CB^ AMexico, DF (Mexico)^ BCIMMYT^ C1997, Centro Internacional de Mejoramiento de Maiz y Trigo (CIMMYT), Mexico DF (Mexico).

Lafitte, H.R. 2000. Abiotic stresses affecting maize. In: Paliwal, R.L. (Ed.), Tropical Maize Improvement and Production. *FAO Plant Production and Protection series No 28*. Rome,Italy, pp. 93–103.

Laghari, K.A., M.A. Sial, M.A. Arain, A.A. Mirbahar, A. Pirzada, M. Dahot et al. 2010. Heritability studies of yield and yield associated traits in bread wheat. Pakstan Journal of Botany 42: 111-115.

Lambers, H., F.S. Chapin and T.L. Pons. 2008. Photosynthesis. Plant physiological ecology. Springer. page 11-99.

Larson, R. 2005. Interventions for improving livelihoods in sub-Saharan Africa..

Lin, C.-S. and M.R. Binns. 1988. A superiority measure of cultivar performance for cultivar× location data. Canadian Journal of Plant Science 68: page 193-198.

Lu, Y., T. Shah, Z. Hao, S. Taba, S. Zhang, S. Gao, et al. 2011. Comparative SNP and haplotype analysis reveals a higher genetic diversity and rapider LD decay in tropical than temperate germplasm in maize. PloS ONE 6:24861

Ma, D., R. Xie, F. Zhang, J. Li, S. Li, H. Long et al. 2015. Genetic contribution to maize yield gain among different locations in China. Maydica. e 2016, 6, 1070.

Machikowa, T. and P. Laosuwan. 2011. Path coefficient analysis for yield of early maturing soybean. Songklanakarin Journal of Science & Technology 33. 24-26

Makumbi, D. 2006. Phenotypic and genotypic characterization of white maize inbreds, hybrids and synthetics under stress and non-stress environments. Texas A&M University

Mashingaidze, K. 1994. Maize Research and Development. In: Rukuni, C. and Eicher, C.K. (Eds.), Zimbabwe's Agricultural Revolution. *University of Zimbabwe Publications Office*, Harare, Zimbabwe, pp. 208–218.

Mashingaidze, K. 2012. Agricultural Research Council-Grain Crop Institute (ARC-GCI).

Melchinger, A.E. and R.K. Gumber. 1998. Overview of heterosis and heterotic groups in agronomic crops. Concepts and breeding of heterosis in crop plants: 29-44.

Menkir, A. and M. Ayodele. 2005. Genetic analysis of resistance to gray leaf spot of midaltitude maize inbred lines. Crop Science 45: 163-170.

Muchow, R.C. and Davis, R. 1988. Effect of nitrogen supply on the comparative productivity of maize and sorghum in a semi-arid tropical environment II. Radiation interception and biomass accumulation. Field Crops Research, 18, 17–30.

Najeeb, S., A. Rather, G. Parray, F. Sheikh and S. Razvi. 2009. Studies on genetic variability, genotypic correlation and path coefficient analysis in maize under the high altitude temperate conditions of Kashmir. Maize Genetics Cooperation Newsletter. 83:1-8.

Ndhlela, T. 2012. Improvement strategies for yield potential, disease resistance and drought tolerance of Zimbabwean maize inbred lines. PhD thesis, University of the Free State.

Nyquist, W.E. and R. Baker. 1991. Estimation of heritability and prediction of selection response in plant populations. Critical Reviews in Plant Sciences 10: 235-322.

Padmaja, D., K. Radhika, L. Subba Rao and V. Padma. 2008. Studies on variability, heritability and genetic advance for quantitative characters in rice *(Oryza sativa L.).* Journal of Plant Genetic Resource 21: 196-198.

Parida, S.K., M. Mukerji, A.K. Singh, N.K. Singh and T. Mohapatra. 2012. SNPs in stress-responsive rice genes: validation, genotyping, functional relevance and population structure. BioMed Central Genomics 13: 426.

Presterl, T., S. Groh, M. Landbeck, G. Seitz, W. Schmidt and H. Geiger. 2002. Nitrogen uptake and utilization efficiency of European maize hybrids developed under conditions of low and high nitrogen input. Plant Breeding 121: 480-486.

Rafiq, C.M., M. Rafique, A. Hussain and M. Altaf. 2010. Studies on heritability, correlation and path analysis in maize *(Zea mays L.).* Journal of Agricultural Research 48: 35-38.

Ramanujam, S. and D. Thirumalachar. 1967. Genetic variability of certain characters in red pepper *(Capsicum annum L.)*. Mysore Journal of Agriculture Science 1: 30-36.

Rafique, M., A. Hussain, T. Mahmood, A.W. Alvi and M.B. Alvi. 2004. Heritability and interrelationships among grain yield and yield components inmaize *(Zea mays L.).* International Journal of Agriculture and Biology (Pakistan).: 475-482.

Sanaullah, M., C. Rumpel, X. Charrier and A. Chabbi. 2012. How does drought stress influence the decomposition of plant litter with contrasting quality in a grassland ecosystem? Plant and Soil 352: 277-288.

Sabaghnia, N., R. Karimizadeh and M. Mohammadi. 2012. Genotype by environment interaction and stability analysis for grain yield of lentil genotypes. Žemdirbyst 99: 305-312.

Sabaghnia, N., M. Mohammadi and R. Karimizadeh. 2013. Yield stability of performance in multi-environment trials of barley *(Hordeum vulgare L.)* genotypes. Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis 61: 787-793.

Sallah, P.Y.K., N.J. Ehike, and J.L. Geadelmann. 1997. Selection for response to low nitrogen in the La Posta maize population. Developing Drought-and Low N-Tolerant Maize. Proceedings of a Symposium; El Batan, Mex.(Mexico); 25-29 Mar 1996. ^ TDeveloping Drought-and Low N-Tolerant Maize. Proceedings of a Symposium; El Batan, Mex.(Mexico); 25-29 Mar 1996^ AEdmeades, GO Banziger, M. Mickelson, HR Pena-

Valdivia, CB^ AMexico, DF (Mexico)^ BCIMMYT^ C1997, Centro Internacional de Mejoramiento de Maiz y Trigo (CIMMYT), Mexico DF

Semagn, K., C. Magorokosho, B.S. Vivek, D. Makumbi, Y. Beyene, S. Mugo et al. 2012. Molecular characterization of diverse CIMMYT maize inbred lines from eastern and southern Africa using single nucleotide polymorphic markers. BioMed Central genomics 13: 113-116

Shukla, G.K. (1972). Some statistical aspects of partitioning genotype environmental components of variability. Heredity 29, 237-245.

Smithson, J.B. and W. Grisley. 1992. First African Bean Yield and Adaptation (AFBYAN I): Part II. Performance across environments. Occasional publications series No. 3B. (CIAT). Dar es Salaam, Tanzania.

Sommer, R., M. Glazirina, T. Yuldashev, A. Otarov, M. Ibraeva, L. Martynova et al. 2013. Impact of climate change on wheat productivity in Central Asia. Agriculture, Ecosystems & Environment 178: 78-99.

Sreckov, Z., A. Nastasic, J. Bocanski, I. Djalovic, M. Vukosavljev and B. Jockovic. 2011. Correlation and path analysis of grain yield and morphological traits in test–cross populations of maize. Pakistan Journal of Botany 43: 1729-1731.

Tollenaar, M. 1989. Genetic improvement in grain yield of commercial maize hybrids grown in Ontario from 1959 to 1988. Crop Science 29: 1365-1371.

Tollenaar, M., A. Ahmadzadeh and E. Lee. 2004. Physiological basis of heterosis for grain yield in maize. Crop Science 44: 2086-2094.

Tully, K., C. Sullivan, R. Weil and P. Sanchez. 2015. The state of soil degradation in Sub-Saharan Africa: Baselines, trajectories, and solutions. Sustainability 7: 6523-6552

Udensi, O. and E. Ikpeme. 2012. Correlation and path coefficient analyses of Seed yield and its contributing traits in Cajanus cajan (L.) Millsp. American Journal of Experimental Agriculture 2: 351-358.

USDA/NIFA 2018. Plant and soil science online library(Accessed 2 October 2018). .http://passel.unl.edu/pages/informationmodule.php?idinformationmodule=1099686649&topicorder=4&maxto=10

Van Eeuwijk, F. 1995. Linear and bilinear models for the analysis of multi-environment trials: I. An inventory of models. Euphytica 84: 1-7.

Vance, C.P. 2001. Symbiotic nitrogen fixation and phosphorus acquisition. Plant nutrition in a world of declining renewable resources. Plant physiology 127: 390-397.

Vanlauwe, B., A. Bationo, J. Chianu, K.E. Giller, R. Merckx, U. Mokwunye et al. 2010. Integrated soil fertility management: operational definition and consequences for implementation and dissemination. Outlook on Agriculture 39: 17-24.

Vargas, M., J. Crossa, F.A. van Eeuwijk, M.E. Ramírez and K. Sayre. 1999. Using partial least squares regression, factorial regression, and AMMI models for interpreting genotype× environment interaction. Crop Science 39: 955-967.

Vasal, S. K., Cordova, H. S., Beck, D. L., & Edmeades, G. O 1997. Choices among breeding procedures and strategies for developing stress tolerant maize germplasm. Developing Drought-and Low N-Tolerant Maize. Proceedings of a Symposium; El Batan, Tex.(Mexico); 25-29 Mar 1996. ^ TDeveloping Drought-and Low N-Tolerant Maize. Proceedings of a Symposium; El Batan, Tex.(Mexico); 25-29 Mar 1996^ AEdmeades, GO Banziger, M. Mickelson, HR Peña-Valdivia, CB^ AMexico, DF (Mexico)^ BCIMMYT^ C1997, Centro Internacional de Mejoramiento de Maiz y Trigo (CIMMYT), Mexico DF (Mexico).

Verheye, W. 2010. Growth and production of maize: traditional low-input cultivation. Land use, land cover and soil sciences. UNESCO-EOLSS Publishers.

Wallace, D., J. Baudoin, J. Beaver, D. Coyne, D. Halseth, P. Masaya et al. 1993. Improving efficiency of breeding for higher crop yield. Theoretical and Applied Genetics 86: 27-40..

Wricke, G. (1962). On a method of understanding the biological diversity in field research. Z. Pfl.- Zücht, 47, 92–146.

Wang, T., X. Ma, Y. Li, D. Bai, C. Liu, Z. Liu et al. 2011. Changes in Yield and Yield Components of Single-Cross Maize Hybrids Released in China between 1964 and 200 1. Crop Science 51: 512-525.

Wanzala, M. 2010. Implementation of the Abuja Declaration on Fertilizer for an African Green Revolution. Seventh Progress Report January-December.

Wray, N. and P. Visscher. 2008. Estimating trait heritability. Nature Education 1: 29.

Xu, Y., Y. Lu, C. Xie, S. Gao, J. Wan and B.M. Prasanna. 2012. Whole-genome strategies for marker-assisted plant breeding. Molecular Breeding 29: 833-854.

Yan, W., Hunt, L. A., Sheng, Q. and Szlavnics, Z. 2000. Cultivar evaluation and mega-environment investigation based on the GGE Bi-plot. Crop Science 40:597-605.

Yan, W., Kang, M. S., Ma, B., Woods, S. and Cornelius, L.P. 2007. GGE Bi-plot vs. AMMI analysis of genotype by environment data. Crop Science 47:643-655.

Yan, W., K.D. Glover, M.S. Kang, R.-C. Yang, J. Crossa and J. Burgueño. 2010. Comment on" Biplot Analysis of Genotype× Environment Interaction: Proceed with Caution,"/Reply. Crop Science 50: 1121-1124

Yan, W. 2001. GGEbiplot—a Windows application for graphical analysis of multienvironment trial data and other types of two-way data. Agronomy Journal 93: 1111-1118.

Yan, W. 2002. Singular-value partitioning in biplot analysis of multienvironment trial data. Agronomy Journal 94: 990-996.

Yang, R.-C., J. Crossa, P.L. Cornelius and J. Burgueño. 2009. Biplot analysis of genotype× environment interaction: Proceed with caution. Crop Science 49: 1564-1576.

Zambezi, B.T. and C. Mwambula. 1997. The impact of drought and low soil nitrogen on maize production in the SADC Region. In G. O. Edmeades, M. Banziger, H. R. Mickelson, & C. B. Peña-Valdivia (Eds.), Developing Drought- and Low N-Tolerant Maize. (pp. 29-34). Proceedings of a Symposium; El Batan, Tex. (Mexico); 25- 29 Mar 1996. Mexico, DF (Mexico): CIMMYT.

CHAPTER 3

GRAIN YIELD PERFORMANCE OF MAIZE HYBRIDS, ESTIMATION OF GENETIC PARAMETERS, CORRELATION AND PATH COEFFICIENT ANALYSES ACROSS LOW NITROGEN AND OPTIMUM ENVIRONMENTS

Abstract

Development of maize hybrid varieties tolerant to low nitrogen (N) offers a sustainable solution for farmers to achieve resilience towards the problem of soil fertility decline in Africa. However, selection for low N tolerance in maize hybrids requires a multifaceted approach due to the complex nature of the grain yield trait. The objective of this study was to identify low N stress tolerant maize hybrids based on yield performance and low N selection index as selection criteria. Heritability estimates were estimated and the nature of relationship that exist between grain yield and secondary traits were studied using correlation and path coefficient analyses. One hundred and seventy maize hybrids were evaluated across low N and optimum environments at three locations in South Africa (SA) during 2017/18 summer season. The results revealed that variance due to environment, genotype and genotype by environment interaction (GEI) were highly significant ($P<0.001$) for almost all the traits under low N and optimum conditions except for GEI where anthesis-silking interval and grain moisture were not significant across the same conditions. The study led to the identification of 13 low N stress tolerant hybrids, based on a low N selection index, that can be recommended for further evaluation and release in South Africa. The study also revealed lower heritability values for grain yield (0.29) than those for secondary traits which included days to anthesis, plant height, ear height and anthesis-silking interval, which had heritability estimates of 0.85, 0.43, 0.38 and 0.52, respectively. Under low N, grain yield positively correlated with field weight, plant height and ear height, but negatively correlated with days to silking, anthesis-silking interval and leaf senescence. Under optimum environment, grain yield was positively correlated with field weight and ears per plant, and negatively correlated with days to anthesis, days to silking, anthesis-silking interval, plant height and ear height. Highest positive direct effect on grain yield was observed for days to silking and field weight under low N, while under optimum, field weight and days to anthesis exhibited the highest direct effects. Higher phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) were observed under low N and optimum environments.

Key words: Heritability, Correlation, Patch analysisis, Optimum and Low Nitrogen

3.1 Introduction

Maize plays an important role in human life and livestock industry being the popularly grown cereal in many African countries including South Africa (SA). The crop has wider adaptability and is capable of growing successfully throughout the world. However, nitrogen stress is among the major abiotic constraints that impede production in Africa (Badu-Apraku *et al.,* 2010; Ismaila *et al.,* 2010). Deficiency in soil nitrogen is due to nutrient loss through soil erosion, volatilization or leaching among other factors (Ogunniyan and Olakojo, 2014). Hence, a possible approach to reduce N deficiency in African soils is to lower nitrogen-use crop requirements through selection for low N tolerance (Smith *et al.,* 1995).

Progress in developing maize varieties that are high yielding under stress conditions is slowed down by the complex nature of the grain yield trait. Grain yield is generally under the influence of environmental factors and various physiological processes. Consequently, grain yield heritability under low N conditions is low and the use of secondary traits to aid the selection process is thus, recommended (Lafitte *et al.,* 2003). The following secondary traits; anthesis-silking interval, leaf senescence, and number of ears per plant have been suggested as ideal for selection of maize genotypes under low N stress (Banziger and Lafitte, 1997; Banziger, 2000). Additionally, the use of a low N selection index formula derived from secondary traits for low N selection has been reported to enhance selection efficiency under low N (Banziger and Lafitte, 1997). In plant breeding, the primary goal is to increase grain yield (Amini *et al.,* 2013), but selection based on grain yield alone has proved not to be effective and efficient since grain yield is a complex trait that depends on a number of variables (Saryam *et al.,* 2015). Therefore, there is a need for breeders to have thorough understanding of the association that exists between grain yield and yield related traits under low N. The extent of this association can be studied through correlation and path coefficient analyses.

Correlation coefficients simply measure the association between two traits (Pavlov *et al.,* 2015). It shows whether selection for one character would result in simultaneous selection of all positively correlated characters. Correlation between characters is important as it depicts the extent to which characters are associated with economic productivity (Muhammad *et al.* 2008). Correlations among the agronomic traits provide a platform for indirect selection in crop improvement programmes (Yousuf and Saleem, 2001). Positive and significant correlations indicate a strong association of the traits involved with grain yield. Additionally, positive and significant correlations coupled with moderate to high heritability are necessary for indirect selection to enhance grain yield. However, correlation coefficients only show the strength of the relationship between two traits and do not consider the traits with direct effects on a basic variable. Thus, path analysis is used to provide reliable information among the traits.

Path analysis partitions the effects on grain yield into direct and indirect effects via other yield components (Del Moral *et al.,* 2003). The primary goal of path analysis is to give a closer insight of the correlation between the traits based on the model of cause and effect relationship. This relationship helps to estimate the importance of an independent variable on a specific dependant variable. Hence, this analysis leads to the partitioning of correlation coefficients into direct and indirect effects (Rafiq *et al.,* 2010). Therefore, correlation and path coefficient analyses aid to identify important secondary traits that are useful in the improvement of complex traits such as grain yield (Kusaksiz, 2010).

Heritability can be defined as the measure of phenotypic variance due to genetic causes and bears a predictive role in plant breeding (Bello *et al.,* 2012). It provides information on the extent to which a particular character can be passed over to the next generation. Knowledge of heritability helps the breeder in decision making on the effective selection method to improve the trait of interest, predicts selection gain and establishes the genetic effects that are relatively important (Laghari *et al.,* 2010). Traits having high heritability values are easy to fix and are selected for using simple phenotypic selection, while for those with low heritability, selection should be delayed and strategies that would create variation such as hybridisation and mutation breeding should be used. According to Najeeb *et al.* (2009), heritability value alone has no meaningful importance without genetic advance. Genetic advance indicates the extent of genetic gain of a trait under a particular selection pressure. Hence, high genetic advance together with high heritability estimates offer the ideal condition for selection.

Additional to heritability and genetic advance estimates, knowledge of genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) of the trait of interest must be considered since most of the economic traits including yield are complex in inheritance and are highly influenced by several genes interacting with various environmental conditions (Bello *et al.,* 2012). Additional to comparing the relative amount of phenotypic and genotypic variations among different traits, PCV and GCV are very useful in estimating the scope of improvement by selection. The reliability of the parameter to be selected for breeding programme among other factors is dependent on the magnitude of its coefficient of variation especially GCV. The difference between GCV and PCV indicate the environmental influence. A high proportion of GCV to PCV is desirable in breeding work. Therefore, the present study was conducted to identify high yielding maize hybrids, estimate variance components that govern grain yield and related traits and conduct correlation and path coefficient analyses for grain yield and related traits across low N and optimum conditions in SA.

3.2 Materials and methods

3.2.1 Germplasm

One hundred and seventy single-cross maize hybrids including three commercial checks were planted in the 2017/18 summer season in SA (Appendix 3.1). The 167 experimental single-cross maize hybrids were developed at the Agricultural Research Council- Grain Crop Institute (ARC-GCI), while the three commercial hybrids used as checks were developed under the Water Efficient Maize for Africa (WEMA) project. The list of hybrids is given in Appendix 3.1.

3.2.2 Experimental sites

Maize hybrids were evaluated at three locations in South Africa namely; Potchefstroom (ARC-GCI) in North West Province, Cedara in KwaZulu-Natal Province and Vaalharts in Northern Cape Province during the 2017/18 summer season. The trial consisted of five environments with Potchefstroom and Cedara having both low N and optimum environments and Vaalharts having optimum environment only. Low N environments in Cedara and Potchefstroom were developed by depleting the soil of nitrogen to a yield potential of less than 60%. This was achieved by continuous growing of maize at a very high population without fertiliser application followed by removal of biomass after each harvest for consecutive years. However, soil samples were not taken for nitrogen analysis due to logistical reasons. Geographical coordinates and meteorological data were collected for all the three locations as presented in Tables 3.1 and 3.2

Table 3.1 Description of trial locations

	Potchefstroom	**Cedara**	**Vaalharts**
Altitude (m)	1349	1068	1180
Latitude(^{0}S)	-26.73607	-29.5419	-27.9576
Longitude(^{0}E)	27.07553	30.26498	24.8399
Soil type	Spodosols, sandy-loam	Vertisols, clay-loam	Spodosols, sandy
Average monthly rainfall (mm)	25.50	98.40	48.51

Table 3.2 Rainfall and temperature data for the three trial locations in the 2017/18 growing season

	Potchefstroom			Cedara			Vaalharts		
Months	Max Temp (^{0}C)	Min Temp (^{0}C)	Total Rainfall (mm)	Max Temp (^{0}C)	Min Temp (^{0}C)	Total Rainfall (mm)	Max Temp (^{0}C)	Min Temp (^{0}C)	Total Rainfall (mm)
Nov-17	29.1	12.7	69.3	24.1	10.5	135.6	31.8	12.1	2.8
Dec-17	29.3	15.7	62.5	23.5	12.5	98.3	33.3	15.5	38.6
Jan-18	31.0	16.1	47.2	27.0	14.1	65.4	34.1	17.2	62.7
Feb-18	27.7	15.6	68.3	26.5	15.2	227.6	31.3	17.0	83.6
Mar-18	27.5	14.6	58.9	25.7	13.6	155.7	29.5	14.4	147.3
Apr-18	25.3	11.1	35.6	24.3	12.4	64.8	26.1	11.7	44.8
Mean	28.3	14.3		25.2	13.5		31.0	14.7	
Total			341.8			747.4			379.8

3.2.3 Field trial design and management

One hundred and seventy single-cross maize hybrids including three checks were laid out in a 34 x 5 (0,1) alpha lattice experimental design replicated twice at each location. Each replication had 34 incomplete blocks, each with five entries. Entries were planted in 4 m two-row plots, spaced 0.25 m apart within the row and 0.75 m between rows. Two border rows on each side of the experimental field were planted to reduce border effects. Under optimum environment, basal dressing fertiliser was applied at planting in the form of compound (N.P.K) 2:3:2 at 250 kg ha^{-1} (56 kg N ha^{-1}, 83 kg P ha^{-1} and 111 kg K ha^{-1}). Top dressing fertiliser was applied in the form of Limestone Ammonium Nitrate (LAN, 28% N) at a rate of 250 kg ha^{-1} four weeks after emergence. Under low N, basal fertiliser was applied as super phosphate (P_2O_5) at 25 kg ha^{-1} and potassium chloride (K_2Cl) at 25 kg ha^{-1}. Standard cultural practices for maize were followed including hand weeding, rouging off-types and chemical control for pests at each environment. Supplemental irrigation was applied as required at Potchefstroom and Vaalharts.

3.2.4 Data collection

All the data were collected according to CIMMYT standard procedures (Magorokosho *et al.,* 2009). The following yield and yield related traits were collected during the study:

- **Days to anthesis (AD):** Number of days after planting when 50% of the plants per plot start to shed pollen.
- **Days to silking (SD):** Number of days after planting when 50% of the plants per plot start showing silks.
- **Anthesis–silking interval (ASI**): Calculated as the difference between days to silking and anthesis.
- **Plant height (PH):** 10 representative plants within a plot measured from the base of the plants to the insertion point of the first tassel branch of the same plant.
- **Ear height (EH):** 10 representative plants within a plot measured from the base of the plant to the insertion of the top most ear of the plant.
- **Leaf senescence (LS):** Recorded on a scale of 1-9 for each plot under the low N environments.
- **Grain moisture (GM):** Measured as percentage water content of the grain after shelling on the same day that grain weight was measured.
- **Grain weight (GW):** Measured as weight of the grain after shelling.
- **Grain yield (GY):** Calculated as the grain weight adjusted to 12.5% moisture content.
- **Ears per plant (EPP):** Calculated as total number of ears in a plot divided by the total number of plants present in the plot at the time of harvesting.

3.2.5 Statistical analyses

3.2.5.1 Single site analysis of variance

Variance estimates for all the collected traits were calculated independently for each environment using GenStat software 18th edition (Payne *et al.,* 2014). The following statistical model was used:

$$Y_{ijk} = \mu + H_i + r_j + B_{K(j)} + \varepsilon_{ijk}$$

Where, Y_{ijk} = the individual observation in each plot; μ = overall mean or grand mean; H_i = the effect of the i^{th} hybrid and i=1,2,3…170; r_j = number of replications and j=1,2; $B_{k(j)}$=

estimate of the incomplete block within replication and k=1,2, 3 ….34; and ε_{ijk} = overall random error.

3.2.5.2 Combined sites analysis of variance

Variance estimates for all the collected traits for combined environments under low N and under optimum, respectively, were performed in GenStat software 18th edition (Payne *et al.,* 2014). A Linear Mixed model was used for the combined analysis. The model includes additive terms for main effects of genotype and environment collectively as well as extra additive terms that account for interaction.

$$Y_{ijkl} = \mu + r_j + B_K + S_l + H_i + SH_{il} + \varepsilon_{ijkl}$$

Where, Y_{ijkl} = the individual observation in each plot; μ = overall mean or grand mean; r_j = effect of the jth replication; $B_{k=}$ effect of the kth block nested in jth replication and k=1,2,3…34, while j=1,2; S_l =the effect the l^{th} environemt and l =1,2,3…5; H_i = the effect of the i^{th} hybrid and i=1,2,3…170; SH_{il} = interaction effect of the ith hybrid and lth environment; and ε_{ijkl} = random error.

The least significant difference (LSD) test at 5% level of significance was used for mean separation using GenStat 18th edition. The hybrid means were ranked according to yield, which was the principal selection criterion at all sites.

3.2.5.3 Low N selection index formula and yield reduction

The selection of tolerant maize hybrids under low N was done using the low N selection index formulae proposed by Banzinger *et al.* (2000).

IN = 5.0 YN + 2.0 EPP - 2.0 LS - 1.0 ASI

Where; YN = yield in low N plots; EPP = number of ears per plant in low N plots, LS = leaf senescence in low N plots and ASI = anthesis-silking interval.

The selection index combined the standardised means of grain yield, number of ears per plant, leaf senescence, and anthesis-silking interval. Each of these traits was standardised using a mean of zero and standard deviation of one to minimise the effects of different scales. A

positive value of the low N tolerant index is an indicator of tolerance while a negative value is an indicator of susceptibility to low N (Meseka *et al.*, 2006; Ifie, 2013; Meseka *et al.*, 2013).

Percentage grain yield reduction under low N and optimum conditions was calculated as the difference between grain yield under low N and optimum, divided by the yield under optimum. Hybrids with lower percentage yield reduction were tolerant and efficient under low N, larger differences meant the hybrids performed very well under optimum but poorly under low N environments. A negative difference meant the hybrids performed better under low N than optimum.

3.2.6 Heritability estimates and genetic advance

3.2.6.1 Variance components

Variance components were estimated using REML method of PROC MIXED in SAS version 9.3 (SAS Institute, 2010). The environment was considered as fixed while the genotypes were regarded as random. Genotypic (σ_g^2), genotype*location ($\sigma_{g*l/l}^2$) and error variance ($\sigma_{e/r*l}^2$) were obtained directly from the PROC MIXED output. These variance components were used to calculate phenotypic variance (σ_p^2) using the formula:

$$\sigma_p^2 = \sigma_g^2 + \frac{\sigma_{gl}^2}{l} + \frac{\sigma_e^2}{rl}$$

Where, l =the number of environments and r =number of replications.

3.2.6.2 Phenotypic and genotypic coefficient of variation

Phenotypic (PCV) and genotypic coefficient of variation (GCV) were calculated for all the quantitative traits according to Singh and Chaudhary (2004), using the formulae:

Phenotypic coefficient of variation (PCV) = $\frac{\sqrt{\sigma_p^2}}{mean} \times 100$ and

Genotype coefficient variation (GCV) = $\frac{\sqrt{\sigma_g^2}}{mean} \times 100$

Where, σ_p^2= phenotypic variance, σ_g^2= genotypic variance and mean = grand mean of the trait.

3.2.6.3 Heritability

Heritability estimates in the broad sense were calculated as the proportion of the genetic variance over the total phenotypic variance. Broad-sense heritability across sites was calculated according to Hallauer and Miranda (1988) as follows:

$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_{gl}^2 / l + \sigma^2 e / rl}$$

Where σ_g^2 = genotypic variance, σ_{gl}^2 = variance due to genotype x environment interaction, l and r are the number of environments and replications per environment, respectively.

3.2.6.4 Genetic advance

Genetic advance using broad-sense heritability was calculated for grain yield and other traits using the following formula from Singh and Chaudhary (2004) for combined data

Genetic advance (GA) = K δp X (H^2)

Where, GA=genetic advance, K= standardized selection differential at 5% selection intensity (2.063), σ_p =phenotypic variance of base population and H^2 is the broad-sense heritability of the character under selection.

Genetic advance was expressed as a percentage of the mean of the unselected parental population

$GAM\% = \frac{GA}{mean} \times 100$, Where, GAM % = genetic advance as percentage of mean,

and *mean* = grand mean of the character.

3.2.7 Correlation analyses

Pearson phenotypic correlation coefficients were calculated using genotype means of traits' data from all sites using the IBM SPSS version 25 software (SPSS, 2014). The phenotypic correlation coefficients were calculated as follows:

$$r_p = \frac{\text{cov}(x, y)}{\sqrt{(\text{var}(x) \times \text{var}(y))}}$$

Where, r_p = phenotypic correlation; $\mathrm{cov}(x, y)$ = phenotypic variance of x and y characters; and $\sqrt{(\mathrm{var}(x) \times \mathrm{var}(y))}$ = square root of the phenotypic variance of x and y characters.

3.2.8 Path coefficient analysis

Path coefficient was calculated using Microsoft Office Excel software by taking the Pearson phenotypic correlation data to determine the contribution (direct and indirect) of each variable to the effect and each variable via other variables to that effect as suggested by Akintunde (2012). The following equation was used to calculate the path coefficients:

$$y = a + b_1X_1 + b_2X_2 + b_3X_3 + U$$

Where, *y* = single response variable (grain yield) and $a+b_1X_1+b_2X_2+b_3X_3+U$= variables from correlation data with the assumptions that values of variables are random, normally distributed and that the causal variables are independently contributing to the dependent variable (grain yield).

3.3 Results

3.3.1 Analysis of variance across low N and optimum environments

The analysis of variance outputs for all the traits in each environment and combined environments under low N and optimum are presented in Tables 3.4 and 3.5, respectively.

In all the environments, Potchefstroom (optimum and low N), Cedara (optimum and low N**),** Vaalharts (optimum), the genotype mean squares were highly significant (P<0.001) for all the traits measured thus: days to anthesis, anthesis-silking interval, ear height, ears per plant, t, grain yield, plant height and days to silking (Tables 3.3,3.4, 3.5, 3.6 and3.7).

Under the combined environments for low N, mean squares for environment were highly significant (P<0.001) for all the traits namely, days to anthesis, anthesis-silking interval, ear height, ears per plant, grain yield, leaf senescence, plant height and days to silking (Table3.8). The genotype mean squares were also significant for all the traits. The genotype by environment interaction was highly significant (P<0.001) for days to anthesis, ear height, ears per plant, grain yield, leaf senescence, plant height, days to silking and but non-significant for anthesis-silking interval.

For the combined environments under optimum conditions, the mean squares for environment and genotypes were highly significant (P<0.001) for all the traits namely, days to anthesis, anthesis-silking interval, ear height, ears per plant, grain yield, plant height and days to silking (Table 3.9). The genotype by environment interaction was significant for days to anthesis, ear height, ears per plant, grain yield, plant height, days to silking and anthesis-silking interval and non-significant for grain moisture.

Table 3.3 Mean squares from analysis of variance of 170 single-cross maize hybrids for grain yield and secondary traits evaluated under Potechfstroom Optimum

Potchefstroom Optimum								
Sources	**DF**	**AD**	**ASI**	**EH**	**EPP**	**GY**	**PH**	**SD**
Rep	1	16.54**	0.14	1185.77***	0.02	0.74	1619.00***	19.78**
Rep*Incomplete block	66	11.13***	0.76***	279.69***	0.09***	2.76***	528.69***	13.33***
Genotype	169	13.41***	0.72***	135.73***	0.06***	3.18***	256.89***	15.83***
Residual	103	2.14	0.23	36.55	0.03	0.63	62.62	2.23
Total	339							

DF=degrees of freedom, AD= days to anthesis, ASI=Anthesis-silking interval, EH=Ear height, EPP=Ears per plant, GY= grain yield, PH=plant height, days to silking. *, **, **** indicate level of significance of the data is at P=0.05, 0.01 and 0.001, respectively. ns indicates non-significant at 5% probability level.

Table 3.4 Mean squares from analysis of variance of 170 single-cross maize hybrids for grain yield and secondary traits evaluated under Cedara Optimum

Cedara Optimum								
Sources	**DF**	**AD**	**ASI**	**EH**	**EPP**	**GY**	**PH**	**SD**
Rep	**1**	**0.03**	**0.05**	**89.8**	**0.02**	**1.61***	**12.94**	**0.00**
Rep*Incomplete block	**66**	**17.73*****	**0.69*****	**174.80*****	**0.05*****	**0.86*****	**236.55*****	**19.65*****
Genotype	**169**	**22.73*****	**0.78*****	**241.25*****	**0.03****	**1.96*****	**275.30*****	**24.29*****
Residual	**103**	**4.31**	**0.27**	**55.77**	**0.02**	**0.4**	**81.01**	**4.6**
Total	**339**							

DF=degrees of freedom, AD= days to anthesis, ASI=Anthesis-silking interval, EH=Ear height, EPP=Ears per plant, GY= grain yield, PH=plant height, days to silking. *, **, **** indicate level of significance of the data is at P=0.05, 0.01 and 0.001, respectively. ns indicates non-significant at 5% probability level.

Table 3.5 Mean squares from analysis of variance of 170 single-cross maize hybrids for grain yield and secondary traits evaluated under Vaarharts Optimum

Vaalharts Optimum								
Sources	**DF**	**AD**	**ASI**	**EH**	**EPP**	**GY**	**PH**	**SD**
Rep	1	74.36***	0	483.23*	0.01	4.24*	1561.50**	73.42**
Incomplete block/Rep	66	25.82***	0.11	247.62***	0.08***	5.82***	457.60***	26.19***
Genotype	169	18.94***	0.22***	274.2***	0.06***	6.24***	411.00***	19.68***
Residual	103	6.919	0.08953	77.25	0.03468	0.7832	198.2	7.136
Total	339							

DF=degrees of freedom, AD= days to anthesis, ASI=Anthesis-silking interval, EH=Ear height, EPP=Ears per plant, GY= grain yield, PH=plant height, days to silking. *, **, **** indicate level of significance of the data is at P=0.05, 0.01 and 0.001, respectively. ns indicates non-significant at 5% probability level.

Table 3.6 Mean squares from analysis of variance of 170 single-cross maize hybrids for grain yield and secondary traits evaluated under Potchefstroom Low Nitrogen

		Potchefstroom Low Nitrogen							
Sources	**DF**	**AD**	**ASI**	**EH**	**EPP**	**GY**	**PH**	**LS**	**SD**
Rep	1	17.89**	4.47***	3969.60***	0.09**	0	3953.55***	2.56***	4.71
Incomplete block/Rep	66	9.20***	1.06***	169.03***	0.06***	0.59***	502.93***	0.66***	12.34***
Genotype	169	11.63***	0.77***	137.68***	0.06***	0.84***	465.18***	0.59***	14.34***
Residual	103	1.76	0.27	36.97	0.01	0.03	91.86	0.1	2.035
Total	339								

DF=degrees of freedom, AD= days to anthesis, ASI=Anthesis-silking interval, EH=Ear height, EPP=Ears per plant, GY= grain yield, PH=plant height, days to silking. *, **, **** indicate level of significance of the data is at P=0.05, 0.01 and 0.001, respectively. ns indicates non-significant at 5% probability level.

Table 3.7 Mean squares from analysis of variance of 170 single-cross maize hybrids for grain yield and secondary traits evaluated under VaarhartsLow Nitrogen

		Vaalharts Low Nitrogen							
Sources	**DF**	**AD**	**ASI**	**EH**	**EPP**	**GY**	**PH**	**LS**	**SD**
Rep	1	1.297	3.01***	3234.42***	0.10*	0.97	7245.09***	0.24	**SD**
Incomplete block/Rep	66	13.47***	1.04***	536.62***	0.02	1.14***	688.82***	1.16***	0.36
Genotype	169	5.10***	1.11***	253.78***	0.02	0.96***	280.39***	0.94***	19.02***
Residual	103	2.088	0.1925	76.97	0.02	0.3975	98.45	0.26	8.20***
Total	339								

DF=degrees of freedom, AD= days to anthesis, ASI=Anthesis-silking interval, EH=Ear height, EPP=Ears per plant,, GY= grain yield, PH=plant height, days to silking. *, **, **** indicate level of significance of the data is at P=0.05, 0.01 and 0.001, respectively. ns indicates non-significant at 5% probability level.

Table 3.8 Mean squares from analysis of variance of 170 hybrids for grain yield and secondary traits under combined Low Nitrogen

		Combined Low Nitrogen							
Sources	**DF**	**AD**	**ASI**	**EH**	**EPP**	**GY**	**LS**	**PH**	**SD**
Block/Replication* Environment	132	1495.77***	1.051***	352.82***	0.04***	0.86***	0.91***	553.29***	15.68***
Replication/Environment	2	19.19**	3.74***	3602.01***	0.10**	0.49	1.40***	5623.95***	2.53
Environment	1	52571.24***	46.07***	353253.36***	0.11*	44.54***	151.09***	682473.79***	49487.65***
Genotype	169	2399.50***	1.25***	251.09***	0.04***	1.16***	0.81***	415.08***	19.37***
Genotype*Environment	169	426.76*	0.6299	140.37***	0.04***	0.64***	0.72***	197.01***	3.17**
Residual	206	396.54	0.23	56.97	0.02	0.21	0.18	58.09	2.18
Total	679								

DF=degrees of freedom, AD= days to anthesis, ASI=Anthesis-silking interval, EH=Ear height, EPP=Ears per plant, GY= grain yield, PH=plant height, days to silking. *, **, **** indicate level of significance of the data is at P=0.05, 0.01 and 0.001, respectively. ns indicates non-significant at 5% probability level.

Table 3.9 Mean squares from analysis of variance of 170 hybrids for grain yield and secondary traits under combined Optimum

	Combined Optimum							
Source	**DF**	**AD**	**ASI**	**EH**	**EPP**	**GY**	**PH**	**SD**
Block/Replication* Environment	198	18.22***	0.52***	234.04***	0.07***	3.15***	407.60***	19.72***
Replication/Environment	3	30.31***	0.06	586.27***	0.02	2.20*	1064.50***	31.07***
Environment	2	61142.89***	39.87***	258715.70***	4.99***	531.55***	272307.90***	63318.87***
Genotype	169	41.30***	0.71***	446.66***	0.08***	5.53***	524.70***	45.73***
Genotype*Environment	338	6.89***	0.51***	102.27***	0.04***	2.93***	209.30***	7.04***
Residual	309	4.46	0.2	56.52	0.03	0.6	113.9	4.66
Total	1019							

DF=degrees of freedom, AD= days to anthesis, ASI=Anthesis-silking interval, EH=Ear height, EPP=Ears per plant, GY= grain yield, PH=plant height, days to silking. *, **, **** indicate level of significance of the data is at P=0.05, 0.01 and 0.001, respectively. ns indicates non-significant at 5% probability level.

3.3.2 Mean performance for grain yield and other agronomic traits of selected hybrids under combined low N and optimum environments

Under the combined environments for low N and optimum, the mean performances for the selected top 20 hybrids for grain yield and secondary traits ranked based on grain yield are presented in the Tables 3.10 and 3.11, respectively**.** Mean performance for all the evaluated 170 hybrids under combined low N and optimum environments is presented in Appendix 3.2 and Appendix 3.3, respectively.

Under the combined low N environments, there were highly significant ($p< 0.001$) differences in the performances of the hybrids for all the traits, namely, days to anthesis, anthesis-silking interval, ear height, plant height, ears per plant, grain yield, leaf senescence and days to silking(Table 3.8). Grain yield for the three commercial hybrid checks ranged from 3.42 t ha^{-1} (G170) to 4.61 t ha^{-1} (G169) and the yield performance of the other check was 4.46 t ha^{-1} (G168). Grain yield for the experimental hybrids ranged from 1.56 t ha^{-1} for G38 (CKDHL0295/CML442) to 4.94 t ha^{-1} for G12 (CB339/CML442) with an overall mean of 3.88 t ha^{-1} (Appendix 3.2 and Table 3.10). Means for anthesis-silking interval ranged from 1.00 to 4.25. The smallest ear height was 58.67 cm for G164 (U2540W/CML448) and the largest ear height was 115.53 cm for G61 (CML216/I-42), while plant height ranged from 148.20 cm for G125 (I-38/CML488) to 210.00 cm for G62 (CML216/RO549W). Means for ears per plant ranged from 0.72 for G72 (CML442/CML488) to 1.45 for G70 (CML442/CML443).

Under the combined optimum environments, entry effects for all the traits measured; days to anthesis, anthesis-silking interval, ear height, plant height, ears per plant, field weight, grain moisture, grain weight, grain yield and days to silking were highly significant ($p< 0.001$) (Table 3.9). Grain yield for the three commercial hybrid checks was 6.75 t ha^{-1} (G170), 7.16 t ha^{-1} (G168) and 7.53 t ha^{-1}, while for the experimental hybrids grain yield ranged from 2.53 t ha^{-1} for G38 (CKDHL0295/CML442) to 8.97 t ha^{-1} for G18 (CK21/CKDHL0089) with an overall mean of 6.66 t ha^{-1} (Appendix 3.3 and Table 3.11). Means for anthesis-silking interval ranged from 0.67 for G108 (CZL068/CK21) to 3.00 for G30 (CK21/CML547). The minimum ear height was 82.7 cm for G16 (CK21/CB323) and the hybrid with largest ear height was G69 (CML442/CML216) with a value of 130.20 cm. Plant height ranged from 174.40 cm for G29 (CK21/CML544) to 230.50 cm for G63 (CML216/U2540W). Means for ears per plant ranged from 0.93 for G153 (RO549W/CML543) to 1.63 for G122 (I-38/CML216).

Table 3.10 Mean perfomance of selected maize hybrids for grain yield and related traits under combined low N environments

Traits	GY (tha^{-1})	ASI (days)	EH (cm)	PH (cm)	EPP
Genotype Code					
G12	4.94	1.25	92.02	178.00	1.08
G18	4.87	2.50	85.31	180.40	0.95
G62	4.87	2.25	109.38	210.00	0.96
G133	4.85	2.00	80.82	181.90	0.90
G55	4.84	1.75	85.49	171.00	0.99
G101	4.79	2.50	92.05	173.00	1.03
G19	4.77	1.75	99.35	189.90	0.99
G44	4.75	2.25	86.18	176.90	1.05
G115	4.72	2.25	89.18	174.30	0.94
G102	4.71	2.50	87.40	170.50	0.97
G45	4.69	1.75	87.92	180.40	0.99
G146	4.69	2.00	104.64	199.10	0.85
G142	4.68	1.75	90.00	179.90	0.97
G99	4.66	1.75	79.82	165.60	1.05
G68	4.64	2.25	87.63	182.20	0.88
G26	4.61	1.00	85.68	169.00	1.03
G54	4.58	2.00	101.45	185.40	0.83
G117	4.57	3.25	97.47	184.90	0.90
G148	4.52	1.75	110.97	191.60	0.94
G52	4.50	1.75	92.09	185.60	1.06
Checks					
G169	4.61	1.75	78.34	175.40	0.95
G168	4.48	2.50	104.04	189.70	0.98
G170	3.42	1.50	89.51	163.80	0.89
Min	1.56	1.00	58.67	148.20	0.72
Max	4.94	4.25	115.53	210.00	1.45
Mean	3.88	2.31	90.09	179.95	0.95
CV (%)	12.25	20.77	8.38	6.21	13.98
$LSD_{(0.05)}$	0.94	0.67	10.52	22.00	0.18
S.E.	0.48	0.48	7.55	11.17	0.13

GY=Grain yield, ASI=Anthesis-silking interval, EH=Ear height, PH=Plant height, EPP=Ears per plant,

Table 3.11 Mean perfomance of selected maize hybrids for grain yield and secondary traits under combined optimum environments

Traits	GY (tha^{-1})	ASI (days)	EH (cm)	PH (cm)	EPP
Genotype Code					
G18	8.97	1.83	111.80	196.10	1.40
G24	8.89	2.17	111.50	210.60	1.16
G46	8.62	1.67	110.80	201.60	1.18
G33	8.39	1.67	101.70	198.80	0.99
G100	8.27	1.50	95.00	183.70	1.06
G66	8.25	1.00	102.10	204.90	1.12
G12	8.20	1.17	100.30	202.00	1.05
G133	8.18	1.00	93.20	201.20	1.12
G137	8.04	1.33	116.50	212.90	1.23
G134	8.03	2.00	108.60	207.60	1.03
G48	8.02	2.00	108.20	209.20	1.19
G96	7.99	2.17	117.20	216.00	1.17
G65	7.93	1.67	95.60	198.50	0.97
G1	7.89	1.00	91.30	194.40	1.08
G6	7.88	1.50	101.70	202.60	1.14
G32	7.86	2.00	110.10	198.00	1.27
G101	7.85	1.67	104.70	201.00	1.36
G13	7.84	1.50	93.30	201.00	1.03
G34	7.80	1.50	117.20	213.10	1.11
G28	7.78	1.00	95.10	209.10	1.20
Checks					
G169	7.53	2.17	93.70	205.00	1.03
G168	7.16	1.50	113.30	198.30	1.21
G170	6.75	1.00	94.00	179.30	1.29
Min	2.53	0.67	82.70	174.40	0.93
Max	8.97	3.00	130.20	230.50	1.63
Mean	6.66	1.59	103.92	200.52	1.15
CV (%)	11.67	27.86	7.23	5.32	14.25
LSD(0.05)	1.50	0.50	8.54	12.13	0.19
S.E.	0.78	0.44	0.58	10.67	0.16

GY=Grain yield, ASI=Anthesis-silking interval, EH=Ear height, PH=Plant height, EPP=Ears per plant,

3.3.3 Grain yield reduction and low N selection index for the selected hybrids under combined low N and optimum environments

The yield reduction percentage and selection index values for selected top 20 hybrids based on grain yield under combined low N environments are presented in Table 3.12 and for all the experimental hybrids are presented in Appendix 3.4. For comparison purposes and yield reduction calculations, mean grain yield for two optimum and low N environments from the same locations (Cedara and Potchefstroom) were used. Negative values for low N selection index indicate susceptibility to low nitrogen while positive values indicate tolerance; negative values for yield reduction means the hybrid performed better under low N than optimum (Meseka *et al.*, 2006; Ifie, 2013; Meseka *et al.*, 2013). Low N selection index values ranged from -17.19 for G63 to 15.35 for G12. Yield reduction ranged from 9.39% for G105 to 65% for G34. Out of the 170 hybrids evaluated, 79 had negative indices while 91 had positive indices.

Table 3.12 Grain yield reduction and selection index among the selected hybrids for combined environments under low N

Hybrids	GY (tha^{-1}) Opt	GY (tha^{-1}) Low N	Low N Selection Index	Index Ranking	Yield Reduction (%)
G12	8.24	4.94	15.35	1	40.08
G18	6.61	4.87	8.58	19	26.32
G62	7.52	4.87	8.47	18	35.25
G133	8.06	4.85	9.66	11	39.81
G55	7.62	4.84	9.69	10	36.52
G101	7.43	4.79	8.71	15	35.51
G19	6.20	4.77	9.94	9	23.08
G44	5.95	4.75	10.64	7	20.11
G115	5.57	4.72	6.36	28	15.29
G102	7.11	4.71	6.45	27	33.82
G45	6.24	4.69	4.29	12	24.83
G146	5.86	4.69	9.38	45	20.01
G142	6.70	4.68	11.37	6	30.19
G99	6.32	4.66	11.73	5	26.27
G68	5.40	4.64	5.71	32	13.99
G26	7.78	4.61	12.61	3	40.69
G54	7.01	4.58	5.03	38	34.68
G117	6.39	4.57	3.80	50	28.50
G148	5.46	4.52	3.86	49	17.29
G52	6.42	4.50	12.39	4.	29.85
Checks					
G169	6.84	4.61	7.85	23	32.63
G168	6.712	4.48	3.72	52	33.24
G170	6.174	3.42	-4.56	132	44.57
Min	**1.42**	**1.56**	**-17.19**		**9.39**

Hybrids	GY (tha^{-1}) Opt	GY (tha^{-1}) Low N	Low N Selection Index	Index Ranking	Yield Reduction (%)
Max	**9.27**	**4.94**	**15.35**		**65.87**
Mean	**6.38**	**3.88**	**0.00**		**37.49**

GY=Grain yield, low N= Low nitrogen, Opt= Optimum

3.3.4 Mean grain yield performance for the selected maize hybrids in each environment

Grain yield performance of the selected 20 maize hybrids and checks in each environment is presented in Table 3.13. The list for all the 170 hybrids is in Appendix 3.5. The highest performing hybrids were as follows: under Potchefstroom low N was G142 (I-42/CML544) with yield of 5.03 t ha^{-1}, at Potchefstroom optimum was G96 (CML543/I-38) with yield of 13.38 t ha^{-1}, Cedara optimum was G77 (CML443/CK21) with yield of 7.23 t ha^{-1}, Cedara low N was G12 (CB339/CML442) with yield of 5.33 t ha^{-1} and Vaalharts optimum was G18 (CK21/CKDHL0089) with yield of 11.86 t ha^{-1}. All the three checks had low grain yield compared to the top 20 selected experimental hybrids. Among the three optimum environments, Potchefstroom registered the highest mean grain yield of 13.38 t ha^{-1} for G96 (CML543/I-38) followed by Vaalharts with grain yield of 11.86 t ha^{-1} for G18 (CK21/CKDHL0089) and the last was Cedara with yield of 7.23 t ha^{-1} for G77 (CML443/CK21). Under low N environments, Cedara registered the highest grain yield of 5.03 t ha^{-1} for G142 (I-42/CML544) followed by Potchefstroom with grain yield of 5.33 t ha^{-1} for G12 (CB339/CML442).

Table 3.13 Selcted top 20 perfoming hybrids for each environment ranked based on grain yield (t ha^{-1})

Hybrids	Potch Low N	Hybrids	Potch Opt	Hybrids	Ced Low N	Hybrids	Ced Opt	Hybrids	Val Opt
G142	5.03	G96	13.38	G12	5.33	G77	7.23	G18	11.86
G62	4.91	G100	12.04	G88	5.31	G40	7.18	G9	11.43
G11	4.92	G18	10.67	G133	5.31	G54	7.03	G6	11.36
G44	4.76	G134	10.5	G55	5.28	G12	6.93	G20	11.32
G130	4.72	G34	10.49	G15	5.26	G162	6.93	G13	11.08
G102	4.71	G59	10.36	G5	5.26	G25	6.91	G48	10.43
G45	4.65	G24	10.29	G26	5.18	G4	6.90	G66	10.38
G105	4.65	G26	10.04	G109	5.18	G55	6.88	G116	10.28
G18	4.64	G92	9.86	G68	5.18	G133	6.83	G33	10.22
G54	4.60	G46	9.58	G86	5.17	G111	6.57	G1	10.08
G21	4.59	G85	9.56	G19	5.16	G113	6.57	G158	10.03
G101	4.58	G12	9.54	G9	5.16	G24	6.56	G137	9.95
G74	4.54	G25	9.53	G117	5.16	G32	6.54	G23	9.87
G12	4.53	G101	9.40	G3	5.15	G100	6.50	G46	9.82
G58	4.53	G133	9.28	G99	5.14	G46	6.47	G24	9.81
G79	4.52	G103	9.12	G135	5.13	G98	6.46	G65	9.77
G69	4.51	G121	9.09	G146	5.11	G91	6.43	G114	9.70
G48	4.49	G33	9.07	G77	5.10	G124	6.30	G2	9.69
G152	4.47	G91	8.93	G83	5.10	G41	6.27	G142	9.59
G33	4.46	G50	8.84	G18	5.09	G1	6.19	G45	9.57
Checks									
G169	4.37	G169	8.67	G169	4.84	G168	5.79	G169	8.91
G168	4.21	G170	8.24	G168	4.75	G169	5.01	G168	8.05
G170	3.14	G168	7.63	G170	3.71	G170	4.11	G170	7.89
MIN	**1.67**		**1.45**		**0.26**		**0.47**		**0.58**
MAX	**5.03**		**13.38**		**5.33**		**7.23**		**11.86**
MEAN	**3.62**		**7.53**		**4.13**		**5.23**		**7.22**
CV (%)	**4.86**		**10.53**		**15.25**		**12.12**		**12.26**
SE	**0.18**		**0.79**		**0.63**		**0.63**		**0.89**

Potch= Potchefstroom, Ced= Cedara, Val= Vaalharts, Opt= Optimum

3.3.5 Heritability estimates and genetic parameters under low N and optimum environments

Broad-sense heritability values for different traits under low N and optimum environments are presented in Tables 3.14 and 3.15, respectively. Heritability estimates were interpreted according to the classification of Bhateria *et al.* (2006), where values >0.5 are high, 0.3 – 0.5 medium and < 0.3 are low. Genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) were interpreted according to Deshmukh *et al*. (1986) where PCV and GCV values greater than 20% are considered as high, values between 10 and 20% are medium and values lower than 10% are considered low. Genetic advance as percentage of mean (GAM) was interpreted according to Johnson *et al.* (1955). Values for GAM from 0 to 10% are low, 10 to 20% are moderate and 20% and above are classified as high.

Under low N, there was high broad-sense heritability for days to anthesis, days to silking and antheis-silking interval with values of 0.85, 0.52 and 0.86, respectively. Medium broad-sense heritability estimates were observed for ear height, and plant height at 0.38, 0.46, respectively. Low heritability values were observed for ears per plant, grain yield, field weight and leaf senescence at 0.00, 0.29, 0.20 and 0.23, respectively. Genetic advance ranged from 0.00 (ears per plant) to 10.38 (plant height) while genetic advance as a percentage of the mean ranged from 0.00% (ears per plant) to 29.54% (anthesis-silking interval). Genotypic coefficient of variation (GCV) ranged from 0.00% (ears per plant) to 19.80% (anthesis-silking interval) and phenotypic coefficient (PCV) of variation ranged from 2.58% (days to anthesis) to 23.36 % (anthesis-silking interval). Grain yield specifically exhibited moderate GAM value of 10.10%, lower GCV value of 8.5% and moderate PCV value of 14.86 %. The study revealed the general trend of slightly higher PCV values than those for GCV.

Under optimum environments, heritability values for the traits varied across all the three defined heritability ranges. High broad-sense heritability estimates were exhibited for days to anthesis, ear height, plant height and days to silking at 0.85, 0.78, 0.58 and 0.86, respectively. Medium heritability values were observed for grain yield, field weight and ears per plant at 0.41, 0.46 and 0.50, respectively. Low broad-sense heritability values were observed only for anthesis-silking interval at 0.29. Genetic advance ranged from 0.23 (anthesis-silking interval) to 15.07 (ear height), while genetic advance as percentage of mean ranged from 6.02% (plant height) to 14.50% (ear height) with grain yield trait having moderate GAM value of 13.15%. Genotypic coefficient of variation (GCV) ranged from 3.49% (days to anthesis) to 12.83% (anthesis-silking interval) with grain yield having lower GCV value of 9.95 % while phenotypic

coefficient of variation (PCV) ranged from 3.79% (days to anthesis) to 23.93% (anthesis-silking interval) with grain yield having the moderate PCV value of 15.53%. The study also revealed slightly higher values for PCV than those for GCV.

Table 3.14 Heritability estimates and genetic parameters for grain yield and related secondary traits for maize hybrids under combined low N environments

Traits	σ^2_g	$\sigma^2_{g*l/l}$	$\sigma^2_{e/r*l}$	σ^2_p	H^2	MEANS	GCV (%)	PCV (%)	GA	GAM (%)
AD	3.91	0.25	0.46	4.62	0.85	83.40	2.37	2.58	3.75	4.50
ASI	0.21	0.13	0.06	0.40	0.52	2.31	19.80	27.36	0.68	29.54
EH	28.15	31.58	13.62	73.35	0.38	90.09	5.89	9.51	6.78	7.53
EPP	0.00	0.01	0.00	0.01	0.00	0.95	0.00	11.55	0.00	0.00
FW	0.04	0.10	0.04	0.17	0.20	3.26	5.75	12.84	0.17	5.31
GY	0.11	0.17	0.05	0.33	0.29	3.88	8.53	14.86	0.39	10.10
PH	55.21	51.01	14.08	120.31	0.46	179.95	4.13	6.10	10.38	5.77
SD	5.48	0.41	0.52	6.41	0.86	85.71	2.73	2.95	4.47	5.21
LS	0.00	0.19	0.04	0.23	0.01	3.29	1.56	14.67	0.01	0.34

AD=Days to anthesis, ASI=Anthesis-silking interval, EH=Ear height, EPP=Ears per plant, FW=Field weight, GY=Grain yield, PH=Plant height, SD=Days to silking and LS= Leaf senescence. σ^2_g = genotypic variance, $\sigma^2_{g*l/l}$ = genotypic*location/location variance, $\sigma^2_{e/r*l}$ = standard error/replication* location variance, GCV=genotypic coefficient of variation, PCV= phenotypic coefficient of variation, H^2=broad-sense heritability, GA=genetic advance, GAM%= genetic advance as percent of means.

Table 3.15 Heritability estimates and genetic parameters for grain yield and related secondary traits for maize hybrids under combined optimum environments

Traits	σ^2_g	$\sigma^2_{g*l/l}$	$\sigma^2_{e/r*l}$	σ^2_p	H2	Mean	GCV (%)	PCV (%)	GA	GAM (%)
AD	7.08	0.50	0.75	8.33	0.85	0.81	3.49	3.79	5.06	6.64
ASI	0.04	0.07	0.03	0.14	0.29	2.13	12.83	23.93	0.23	14.18
EH	68.56	9.96	9.54	88.07	0.78	3.10	7.97	9.03	15.07	14.50
EPP	0.01	0.00	0.00	0.01	0.50	0.49	7.41	10.53	0.12	10.77
FW	0.25	0.25	0.05	0.55	0.46	2.19	9.67	14.30	0.70	13.49
GY	0.44	0.54	0.10	1.07	0.41	2.83	9.95	15.53	0.88	13.15
PH	58.65	23.48	18.33	100.47	0.58	3.42	3.82	5.00	12.07	6.02
SD	7.94	0.52	0.78	9.23	0.86	0.82	3.62	3.90	5.39	6.92

AD=Days to anthesis, ASI=Anthesis-silking interval, EH=Ear height, EPP=Ears per plant, FW=Field weight, GY=Grain yield, PH=Plant height, SD=Days to silking . σ^2_g = genotypic variance, $\sigma^2_{g*l/l}$ = genotypic*location/location variance, $\sigma^2_{e/r*l}$ = standard error/replication* location variance, GCV=genotypic coefficient of variation, PCV= phenotypic coefficient of variation, H^2=broad-sense heritability, GA=genetic advance, GAM%= genetic advance as percent of means.

3.3.6 Correlation analysis for grain yield and secondary traits under low N and optimum environments

Phenotypic correlations (r) for grain yield with secondary traits under low N and optimum environments are shown in Tables 3.16 and 3.17.

Under low N conditions, grain yield was highly significant (P<0.01) positively correlated) to days to anthesis (r= 0.229**), plant height (0.349**), ear height (r=0.344**), ears per plant (r=0.141**) and field weight (r=0.853**) and negatively correlated to days to silking (r=0-.201**), anthesis-silking interval (r=-0.310**) and leaf senescence (r=-0.069**). Ear height was highly significant (P<0.01) and positively correlated to days to anthesis, days to silking, field weight and plant height (r=0.823**, r=0.813**, r=0.557** and r=0.948**, respectively) but negatively correlated to anthesis-silking interval (-0.209**). Plant height was highly significant (P<0.01) and positively correlated to days to anthesis, days to silking, leaf senescence and field weight (r=0.863**, 0.851**, 0.519** and 0.572**, respectively) and highly significant but negatively correlated to anthesis-silking interval and ears per plant (r=-0.233** and r=-0.102**, respectively). Leaf senescence was highly significant (P<0.01) and positively correlated to days to anthesis, days to silking, and field weight (r=0.494**, r= 0.503** and r= 0.187**, respectively) and highly significant but negatively correlated to ears per plant (0.242**). Anthesis-silking interval was highly significant (P<0.01) and negatively correlated to days to

anthesis, plant, height, ear height, and field weight (r=-0.0180**, r=-0.233**, r=-0.209**and r=-0.239**). Ears per plant was highly significant (P<0.01) and positively correlated to field weight (r=0.153**).

Grain yield under optimum conditions was highly significant (P<0.01) and positively correlated to field weight and ears per plant, (r= 0.887** and r=0.307**, respectively) and highly significant but negatively correlated to days to anthesis, days to silking, anthesis-silking interval, plant height and ear height (r=-0.628**, r=-0.615**, r= -0.124**, r= -0.469** and -0.473**, respectively). Plant height was highly significant (P<0.01) and positively correlated to days to anthesis, days to silking and ear height (r=0.848**, r=0.847** and r=0.917**, respectively) and negatively correlated to ears per plant and field weight (r=-0.383** and r=-0.177**, respectively). Ear height was highly significant and positively correlated to days to anthesis and days to silking (r=0.866** and r=0.865**, respectively) and negatively correlated to ears per plant and field weight (r=-0.400** and r=-0.182**, respectively). Anthesis-silking interval was positively correlated to days to silking (r=0.095*) and negatively correlated to field weight (r=-0.133**).

Table 3.16 Pearson correlation coefficients for grain yield and secondary traits across low N environments

	AD	SD	ASI	PH	EH	LS	EPP	FW	GY
AD	1	0.995**	-.0180**	0.863**	0.823**	0.494**	-0.083*	0.488**	0.229**
SD		1	-0.082*	0.851**	0.813**	0.503**	-0.090*	0.461**	-0.201**
ASI			1	-0.233**	-0.209**	0.018	-0.060	-0.329**	-0.310**
PH				1	0.948**	0.519**	-0.102**	0.572**	0.349**
EH					1	0.557**	-0.095*	0.557**	0.344**
LS						1	-0.242**	0.187**	-0.063**
EPP							1	0.153**	0.141**
FW								1	0.853**
GY									1

*,**. Significant at P= 0.05 and P=0.01, respectively. AD=Days to anthesis, SD=days to silking, LS= Leaf senescence, ASI=Anthesis-silking interval, EH=Ear height, EPP=Ears per plant, FW=Field weight, GY=Grain yield, PH=Plant height.

Table 3.17 Pearson correlation coefficients for grain yield and secondary traits across optimum environments

	AD	SD	ASI	PH	EH	EPP	FW	GY
AD	1	0.997**	0.015	0.848**	0.866**	-0.439**	-0.353**	-0.628**
SD		1	0.095*	0.847**	0.865**	-0.441**	-0.341**	-0.615**
ASI			1	0.041	0.029	-0.050	-0.133**	-0.124**
PH				1	0.917**	-0.383**	-0.177**	-0.469**
EH					1	-0.400**	-0.182**	-0.473**
EPP						1	0.153**	0.307**
FW							1	0.887**
GY								1

*,** Significant at P= 0.05 and P=0.01, respectively. AD=Days to anthesis days, SD=days to silking, ASI=Anthesis-silking interval, EH=Ear height, EPP=Ears per plant, FW=Field weight, GY=Grain yield, PH=Plant height.

3.3.7 Path coefficient analysis for grain yield and secondary traits under optimum and low N environments

Under low N conditions, path coefficient analysis revealed that days to silking (2.0290) exhibited the highest direct effect on grain yield followed by field weight (0.9683), plant height (0.0004) and ear height (0.0055). On the other hand, days to anthesis (-2.3119), anthesis-silking interval (-0.2418), ears per plant (-0.0310) and leaf senescence (-0.0026) had negative direct effects on grain yield. Among the studied traits, days to silking via field weight (0.4464) and anthesis-silking (0.0198); and ears per plant via days to anthesis (0.1919) and anthesis-silking interval (0.0144) revealed positive indirect effects on grain yield. Similarly, days to anthesis, days to silking, plant height, ear height, ears per plant and field weight exhibited positive indirect association with each other towards grain yield with an overall effect of 0.229**, 0.201**, 0.347**, 0.344**, 0.141** and 0.853**, respectively.

Under optimum conditions, path coefficient analysis revealed that field weight (0.77) exhibited the highest direct effect on grain yield followed by days to anthesis (0.769), anthesis-silking interval (0.112) and ears per plant (0.041). On the other hand, days to silking (-1.007), plant height (-0.099), and ear height (-0.024) had negative direct effect on grain yield. Among the studied traits, field weight via days to silking (0.343) and plant height (0.018); and ear height via days to anthesis (0.666) and anthesis-silking interval (0.003) revealed positive indirect effects on grain yield. Similarly, anthesis-silking interval, ears per plant and field weight exhibited positive indirect association with each other towards grain yield with a total effect of 0.124**, 0.307** and 0.887**, respectively.

Table 3.18 The direct (diagonal) and indirect (out diagonal) contribution of maize traits on grain yield across low N environments

	AD	SD	ASI	PH	EH	LS	EPP	FW	Correlation to GY
AD	**-2.3119**	2.0187	0.0435	0.0004	0.0045	-0.0013	0.0026	0.4725	0.229**
SD	-2.3003	**2.0290**	0.0198	0.0004	0.0045	-0.0013	0.0028	0.4464	0.201**
ASI	0.4161	-0.1663	**-0.2418**	-0.0001	-0.0012	-4.8E-05	0.0018	-0.3186	-0.310**
PH	-1.9951	1.7265	0.0563	**0.0004**	0.0052	-0.0014	0.0032	0.5538	0.349**
EH	-1.9027	1.6494	0.0505	0.0004	**0.0055**	-0.0015	0.0029	0.5393	0.344**
LS	-1.1421	1.0204	-0.0044	0.0002	0.0031	**-0.0026**	0.0075	0.1811	-0.063**
EPP	0.1919	-0.1826	0.0144	-4.5E-05	-0.0005	0.0006	**-0.0310**	0.1481	0.141**
FW	-1.1282	0.9353	0.0795	0.0002	0.0031	-0.0005	-0.0047	**0.9683**	0.853**

AD=Days to anthesis days, SD=days to silking, ASI=Anthesis-silking interval, EH=Ear height, EPP=Ears per plant, FW=Field weight, GY=Grain yield, PH=Plant height

Table 3.19 The direct (diagonal) and indirect (out diagonal) contribution of maize traits on grain yield across optimum environments

	AD	SD	ASI	PH	EH	EPP	FW	Correlation to GY
AD	**0.769**	-1.004	0.002	-0.084	-0.021	-0.018	-0.273	-0.628**
SD	0.767	**-1.007**	0.011	-0.084	-0.021	-0.018	-0.263	-0.615**
ASI	0.011	-0.096	**0.112**	-0.004	-0.001	-0.002	0.103	0.124**
PH	0.652	-0.853	0.005	**-0.099**	-0.022	-0.016	-0.137	-0.469**
EH	0.666	-0.871	0.003	-0.091	**-0.024**	-0.016	-0.141	-0.473**
EPP	-0.338	0.444	-0.006	0.038	0.010	**0.041**	0.118	0.307**
FW	-0.272	0.343	0.015	0.018	0.004	0.006	**0.772**	0.887**

AD=Days to anthesis days, ASI=Anthesis-silking interval, EH=Ear height, EPP=Ears per plant, FW=Field weight, GY=Grain yield, PH=Plant height, LS=Leaf senescence, SD= Days to silking

3.4 Discussion

3.4.1 Genotype performance for grain yield and secondary traits for individual and combined environments

Significant differences observed among the hybrids for grain yield and related traits under low N and optimum environments suggest that hybrids' levels of tolerance for low N stress were different. Makumbi *et al*. (2011) also observed significant variances for grain yield amongst hybrids under low N stress. Significant differences for anthesis-silking interval and plant height observed were also important for grain yield under stress. Increased plant height in plants under stress is desirable as it indicates higher amounts of carbohydrate reserves for enhancement of stress tolerance (Blum, 1997). Extended stress periods during the vegetative phase could influence the cell size development, reduce length of internodes and overall height of plant (Denmead and Shaw, 1960). As such, plant height can demonstrate stress tolerance to nitrogen deficiency through rapid growth and early attainment of reproductive stage. The results also showed that days to silking and anthesis-silking interval were less under optimum and more under low N environments. This is consistent with findings of Banziger *et al.* (2000), Betran *et al.* (2003a), Worku (2005) and Ifie (2013) who reported that these traits are delayed by low N stress.

Significant differences among the environments suggest that the environments were different and it was justifiable to evaluate the hybrids under low N and optimum environments separately. Significant genotype by environment interaction (GEI), suggests that hybrid performance for all the traits was not consistent across the environments. The results are in line with Ifie's (2013) findings who reported siginificant GEI for grain yield and related secondary traits under low N. Bello and Olaoye (2009), Aly *et al.* (2011) and Abdel-Moneam *et al.* (2014) reported significant mean squares for all traits under contrasting environments affirming that the experimental growing conditions were different. This validates the importance of evaluating the hybrids across different locations. Significant differences for all traits among the experimental hybrids under low N suggest that there was adequate variability for these traits and selection is possible to identify the best hybrids under low N.

3.4.2 Grain yield reduction and low nitrogen selection index

Information on grain yield reduction due to low N stress is important to determine if the level of stress is sufficient to discriminate among genotypes and allow for identification of tolerant genotypes (Ifie, 2013). The overall grain yield mean under combined optimum conditions for Potchefstroom and Cedara (Table 3.12) was 6.38 t ha^{-1}, compared to 3.88 t ha^{-1} under low N,

representing a yield reduction of 39% due to low N stress. The yield reduction obtained in this study is similar to that of Blum (1997) and Presterl *et al.,* (2003), who reported yield reductions under low N of 35% and 37%, respectively. There was substantial grain yield reduction under low N among the hybrids ranging from 9.39% for G105 (CML547/I-42) to 65% for G34 (CKDHL0089/RO549W) confirming that nitrogen stress is the major limiting factor for increased maize productivity. This further confirms that grain yield increase in maize is associated with availability of nitrogen. This observation is in line with the findings from other studies under low N where yield increase was directly proportional to nitrogen levels (Akintoye *et al.,* 1999; Bänziger *et al.,* 1999; Badu-Apraku *et al.,* 2011; Ifie, 2013; Noelle, 2014). The results also revealed that in each environment, the selected top 20 hybrids out yielded the three commercial checks. Therefore, it is ideal that these top 20 hybrids be tested for stability across environments.

The experimental hybrids also exhibited a wide range of low N index values ranging from -17.19 for hybrid G63 (CML216/U2540W) to 15.35 for G12 (CB339/CML442) confirming that there were different levels of low N stress tolerance among the hybrids. Hybrids bearing negative indices indicate that they were susceptible to low N and those with positive values were tolerant to low N. All the selected top 20 hybrids based on grain yield exhibited positive low N indices and 13 of them were among the top 20 low N tolerant hybrids based on index ranking. Out of the 170 hybrids evaluated, 79 had negative indices while 91 had positive indices meaning that 53% of the hybrids were tolerant to low N stress. Among the three checks used, one check G170 exhibited negative index for low N tolerance of -4.56 with higher yield reduction of 44.57% indicating that it is not an ideal candidate for low nitrogen conditions. The yield of the best hybrids under low N yielded 4.94 t ha^{-1} for G4 (CB322/I-42) slightly higher than what was observed by The *et al.,* (2013) who reported a yield of 3.0 t ha^{-1} for the best hybrids under low N. Such differences in yield may be due to different levels of nitrogen stress under which the genotypes were evaluated as well as different genetic potential of the material used. Overall, the results suggest that complementing selection for low N based on grain yield with low N index and yield reduction percentage is effective and reliable than selecting based on grain yield alone.

3.4.3 Heritability estimates, variance components and coefficients of variation under combined low N and optimum environments

Heritability values are important in predicting the expected progress to be achieved through selection. Heritability estimates along with GCV provide a reliable prediction on the extent of genetic advance to be realised through phenotypic selection (Gidey *et al.,* 2013). Higher

heritability values for days to anthesis, anthesis-silking interval, and days to silking and moderate heritability values for plant height and ear height indicate that early generation selection for these traits to improve low N tolerance would be effective. For traits with low heritability values such as grain yield, ears per plant, field weight and leaf senescence; selection needs to be delayed if progress is to be realised from selection.

Heritability for grain yield was low (0.29) under low N and this is in contrast to moderate heritability values of 41% reported by Sibale and Smith (1997). Moderate broad-sense heritability for grain yield suggests that the narrow-sense heritability value for this trait might be even lower than the calculated (Falconer and Mackay 1996), which may lead to low genetic gain from selection. Heritability estimates for grain yield were lower than for days to anthesis, days to silking and anthesis-silking interval under low N. Comparison of heritability values for grain yield and most of the traits between low N and optimum environments revealed lower values under low N than optimum environment while heritability of some other traits remained high. This trend suggests that differences in grain yield under low N were not due to genotypic effects alone, hence selection of low N tolerant maize hybrids based on grain yield alone may not be reliable and effective.

The study further revealed that PCV values under low N were higher that GCV values suggesting that the environment played an important role on the expression of the traits under study. Generally, quantitative traits are highly influenced by the environments hence the observation was expected. This is in line with Teklu *et al.* (2014) who also observed higher PCV values than GCV values in sesame genotypes. All the measured traits under low N had lower GCV values except anthesis-silking interval which had a medium GCV value of 19.8% while for PCV, athesis-silking interval registered a higher value of 27.36% and ears per plant, field weight, grain yield and leaf senescence recorded medium PCV values of 11.5%, 12.84%, 14.86% and 14.67%, respectively. Days to anthesis days, ear height, plant height, and days to silking registered lower values of PCV. The breeding implication of the observed values for GCV and PCV is that effective response from selection may be achieved based on these characters with high and medium GCV and PCV and their phenotypic expression would be a good indication of their genetic potential. Low values indicate the need for creating variability through hybridisation or mutation followed by selection (Tiwari *et al.,* 2011).

Under optimum environments, results showed that PCV values were higher that GCV values indicating that environment played a critical role on the expression of the characters (Bello *et al.,* 2012). All the measured traits under optimum had lower GCV values except anthesis-silking interval which had a medium GCV value of 12.83% while for PCV, only athesis-silking

interval had a higher value of 23.93% and ears per plant, field weight, grain yield had medium PCV values of 10.53%, 14.30%, and 15.53%, respectively. Days to anthesis days, ear height, plant height, and days to silking registered lower values of PCV. These GCV and PCV values suggest that postive response from selection can be achieved based on the traits having high and medium values and their phenotypic expression would be a good indication of their genetic potential.

Generally grain yield in maize is the most important trait, topmost priority by the plant breeders. The presence of low to moderate values for grain yield heritability, GAM, GCV and PCV under both optimum and low N conditions, respectively have displayed comparatively high environmental influence on grain yield trait expression within the breeding materials used in this sudy. Hence selection and genetic improvement based on this trait will be less effective.

3.4.4 Correlation between grain yield and secondary traits under low N and optimum environments

Secondary traits highly correlated with grain yield coupled with high heritability have potential to speed up the selection process for genotypes that are tolerant to low N stress (Banzinger *et al.,* 2000). Under low N, days to anthesis, ears per plant, field weight, plant height and ear height showed positive significant correlation with grain yield except for anthesis-silking interval, leaf senescence and days to silking, which showed significant negative correlation. The results are in line with Parajuli *et al.* (2018) who reported a significant negative correlation for leaf senescence and anthesis-silking interval with grain yield under low N. High positive correlations between grain yield and related traits indicate the possibility of simultaneous selection since higher values for those traits would result to increase in grain yield. Grain yield and anthesis-silking interval (ASI) had significant negative correlation confirming the importance of shorter ASI for increased grain yield under low N. Maize hybrids with reduced number of days to anthesis, silking and short anthesis-silking interval indicate tolerance to low N. When the two traits positively correlate, simultaneous selection is possible. The results are in agreement with Noelle (2014) who also reported significant correlations for grain yield with days to silking and anthesis-silking interval among the list of significant traits revealed under low N study. The results are in agreement with Adofo-Boateng (2015) who reported positive correlations for plant height, ear height, ears per plant with grain yield under low N and negative correlations for anthesis-silking interval and days to silking. Badu-Apraku *et al.* (2011) reported that under low N, the most reliable traits were days to anthesis, days to silking, stay green characteristics, anthesis-silking interval, plant height, number of ears per plant, ear aspects and plant aspects. Hence, the significant correlations of some of the traits listed by

Badu-Apraku *et al*. (2011), in this study, further justify the importance of using these secondary traits under low N selection. Significant, negative correlations observed between grain yield and leaf senescence as well as with days to silking demonstrate that reduced leaf senescence and ability of the plant to produce silks a few days after anthesis denotes genotype tolerance to low N stress. Other researchers including Bolaños and Edmeades, (1993b), Lafitte and Edmeades (1995), Bänziger and Lafitte (1997), Bänziger *et al*. (2002), and Betrán *et al*. (2003 reported similar results.

Under optimum conditions, grain yield had significant positive correlation with field weight, ears per plant and negative correlations were observed with days to anthesis, days to silking, plant height and ear height. The results are in agreement with Noelle (2014) who reported similar correlations for plant height and days to silking. Mhoswa *et al.* (2016) and Sreckov *et al.* (2011) also reported significant negative correlations for grain yield with plant height and ear height in maize. The characters with positive correlations can be used as selection criteria for the improvement of grain yield in maize (Kumar *et al.* 2015). This means that ears per plant and field weight could be used in predicting the grain yield under optimum conditions. The negative correlation between days to silking and days to anthesis is important in identifying early and late maturing cultivars and shows that yield is compromised when silk emergence is delayed. Furthermore, negative correlation of grain yield with days to anthesis and days to silking shows that breeding for earliness would be possible among the tested hybrids.

3.4.5 Path coefficient analysis for grain yield and secondary traits under optimum and low N environments

Grain yield being a complex quantitative trait is under the influence of environmental factors. Therefore, indirect selection through yield related secondary traits is the best approach to enhance selection efficiency. The yield components are sequentially inter-related during growth cycle and correlation can hardly segregate the traits to show their level of importance in determining grain yield. Therefore, selection based on correlation coefficients alone can be misleading since the correlation between two characters may be due to a third factor. There is therefore need to complement correlation with path coefficient analysis to provide more insights on which variables have direct or indirect influence on grain yield (Aycicek and Yildirim, 2006).

Under low N, path coefficient analysis revealed that days to silking, field weight, plant height and ear height had direct positive influence on grain yield. Mushongi (2010) reported similar results, particularly for plant height. Kumar *et al.* (2015) and Hepziba *et al.* (2013) also

reported similar postive direct effects for plant height and ear height on maize grain yield. Direct postive effects of these traits towards grain yield indicate that these traits can play an important role in develoment of superior low N stress tolerant maize hybrids. Days to silking and field weight had the highest significant direct effects on grain yield. Hence selection for grain yield can be done through these traits. Negative direct effects were observed for leaf senescence, anthesis-silking interval and days to anthesis. The results suggest that maize grain yield under low N could be improved by selection for lower leaf senescence, early silking and short anthesis-silking interval. Hybrids with low leaf senescence afford a plant wider leaf surface area for photosynthesis that will translate to higher yield than hybrids that lose more leaves. The results agree with the findings of Banziger *et al*. (2000) where anthesis-silking interval and leaf senescence were among the important secondary traits that could guide selection for both drought and low N tolerance in addition to grain yield. Badu-Apraku *et al.* (2011) also reported similar results where days to anthesis, leaf senescence anthesis-silking interval and days to silking were among the most reliable recommended secondary traits for selection under low N. The results also revealed that days to anthesis, anthesis-silking interval and days to silking were associated with high heritability values of 0.85, 0.52 and 0.86, respectively, which agreed with Banziger *et al.* (2000) that good secondary traits must have high heritability and, be genetically correlated with grain yield under stress, among other factors.

Under optimum conditions, path coefficient analysis revealed that days to anthesis, field weight, ears per plant and anthesis-silking interval had higher direct positive impact on grain yield. The results complement the findings of Kumar *et al*. (2015) and Pavan *et al.* (2011) under low N. This means that improvement of grain yield is possible based on the traits that have higher positive direct effects. On the other hand, days to silking, ear height and plant height had direct negative effects on grain yield under optimum environments. This is due to the fact that 2017/18 summer season was shorter and the crop under optimum environment were also partially affected by the water stress. This means that selection of yield based on these traits may lead to loss of maize grain yield. These findings corroborate those of Geeth and Jayaraman (2000), Vijayabharathi *et al.* (2009), Bello et al. (2010), Dipika *et al.* (2014) and Sridhar *et al*. (2016). Jakhar *et al.* (2017) and Matin *et al.* (2017) also observed negative direct effect for days to silking towards grain yield among the characters studied.

3.5 Conclusions

The main objective of the study was to identify maize hybrids that are tolerant to low N stress based on grain yield, low N selection index as well as yield reduction percentage. Based on

grain yield and low N selection index, 13 maize hybrids were identified having satisfied the selection criteria used in this study viz. G12 (CB339/CML442), G18 (CK21/CKDHL0089), G62 (CML216/RO549W), G133 (I-42/CB339), G55 (CML202/CK21), G101 (CML544/I-38), G19 (CK21/CKDHL0295), G44 (CKDHL0378/I-42), G45 (CKDHL0378/U2540W), G142 (I-42/CML544), G99 (CML544/CK21), G26 (CK21/CML444) and G52 (CKL05022/I-42). These hybrids are potential candidates for further evaluation and release as low N tolerant maize hybrids. Low heritability estimates for grain yield under low N as compared to heritability values of secondary traits and higher values for PCV than GCV confirmed that selection for improved yield under low N based on grain yield would be not effective since the environment played a large part. Hence, secondary traits with higher heritability under low N should be used to identify superior genotypes under low N environments.

Under low N, there were positive significant correlations for ear height, plant height, ears per plant and field weight with grain yield, while grain yield negatively correlated with anthesis-silking interval, days to silking and leaf senescence. Under optimum, there were significant positive correlations for field weight and ears per plant with grain yield while negative correlations were obtained with days to anthesis, days to silking, anthesis-silking interval, plant height and ear height. High positive significant correlation for secondary traits with grain yield indicate that the breeder could use those traits in predicting grain yield increase. Under low N, path analysis revealed positive direct effects for field weight, days to silking, plant height and ear height toward grain yield while days to anthesis, anthesis-silking interval and leaf senescence had negative direct effects. Under optimum, field weight, days to anthesis, anthesis-silking interval and ears per plant had positive direct effects on grain yield while days to silking, plant height and ear height had negative direct effects. Direct postive effects indicate that these traits have an important role in development of superior low N stress tolerant maize hybrids and that improvements in those traits would result in improvement of grain yield.

3.6 References

Abdel-Moneam, M. A., Sultan, M. S. Sadek, S. E. and Shalof, M. S. 2014. Estimation of heterosis and genetic parameters for yield and yield components in maize using the diallel cross method. Asian Journal of Crop Science 6:101-111.

Aycicek, M. and Yildirim, T. 2006. Path coefficient analysis of yield and yield components in bread wheat *(Triticum aestivum L.)* genotypes. Pakistan Journal of Botany 38(2): 417-424.

Adofo-Boateng, P. 2015. Development of high-yielding and stable maize *(Zea mays L.)* hybrids tolerant to low soil nitrogen (Doctoral dissertation). Retrieved from University of Ghana http://ugspace. ug. edu. Ghana

Akintoye, H., Lucas, E. and Kling, J. 1999. Grain yield and yield components of single, double, and synthetic maize lines grown at four N levels in three ecological zones of West African Tropical Agriculture 76: 51-56.

Amini, Z., M. Khodambashi and Houshmand, S. 2013. Correlation and path coefficient analysis of seed yield related traits in maize. International Journal of Agriculture and Crop Sciences 5: 2217.

Aly, R. S. H., Metwali, E. M. R. and Mousa, S. T. M. 2011. Combining ability of maize (*Zea mays* L.) inbred lines for grain yield and some agronomic traits using topcross mating design. Global Journal of Molecular Science 6:1-8.

Badu-Apraku, B., A. Menkir, S. Ajala, R. Akinwale, M. Oyekunle and K. Obeng-Antwi. 2010. Performance of tropical early-maturing maize cultivars in multiple stress environments. Canadian Journal of Plant Science 90: 831-852.

Badu-Apraku, B., M. Oyekunle, R. Akinwale and A.F. Lum. 2011. Combining ability of early-maturing white maize inbreds under stress and nonstress environments. Agronomy Journal 103: 544-557.

Bänziger, M. 2000. Breeding for drought and nitrogen stress tolerance in maize: from theory to practice, International Maize and Wheat Improvement Centre (CIMMYT).

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

Bänziger, M., G. Edmeades and H. Lafitte. 1999. Selection for drought tolerance increases maize yields across a range of nitrogen levels. Crop Science 39: 1035-1040.

Banziger, M., G.O. Edmeades, D. Beck and M. Bellon. 2000. Breeding for Drought Tolerance in Maize. CIMMYT Special Publication. Mexico. D.F: CIMMYT. p 68.

Bänziger, M. and J. de Meyer. 2002. Collaborative maize variety development for stress-prone environments in southern Africa. Farmers, scientists, and plant breeding: Integrating knowledge and practice. CABI, Oxon, UK: 269-296

Bello, O. and Olaoye, G. 2009. Combining ability for maize grain yield and other agronomic characters in a typical southern guinea savanna ecology of Nigeria. African Journal of Biotechnology 8: 2518-2522.

Bello, O. B., Ige, S. A., Azeez, M. A., Afolabi, M. S., Abdulmaliq, S. Y. and Mahamood, J. 2012. Heritability and genetic advance for grain yield and its component characters in maize *(Zea mays* L.). International Journal of Plant Research 2:138-145.

Betran, F., D. Beck, M. Bänziger and G. Edmeades. 2003. Genetic analysis of inbred and hybrid grain yield under stress and nonstress environments in tropical maize. Crop Science 43, 807-817

Bhateria, S., S.P. Sood and A. Pathania. 2006. Genetic analysis of quantitative traits across environments in linseed *(Linum usitatissimum* L*.).* Euphytica 150: 185-194.

Blum 1997. Constitutive traits affecting plant performance under stress. Proceedings of a Symposium, El Batan, Mex.(Mexico), 25-29 Mar 1996, CIMMYT.

Bolaños, J. and G. Edmeades. 1993. Eight cycles of selection for drought tolerance in lowland tropical maize. I. Responses in grain yield, biomass, and radiation utilization. Field Crops Research 31: 233-252.

Del Moral, L., Y. Rharrabti, D. Villegas and C. Royo. 2003. Evaluation of Grain Yield and Its Components in Durum Wheat under Mediterranean Conditions Funding for this study was provided by the Spanish government throughout INIA Project SC97-039-C2 and CICYT Project AGF99-0611-CO3. Agronomy Journal 95: 266-274.

Denmead, O. and R.H. Shaw. 1960. The Effects of Soil Moisture Stress at Different Stages of Growth on the Development and Yield of Corn 1. Agronomy Journal 52: 272-274.

Deshmukh, S., M. Basu and P. Reddy. 1986. Genetic variability, character association and path coefficients of quantitative traits in Virginia bunch varieties of groundnut. Indian Journal of Agricultural Sciences 56:816- 821

Dipika, S. M., Srikant, V. and Gedion, J. S. 2014. Inter-relationship and coefficeint analysis for different physiological and yiled contributing traits in maize. Annals of Plant and Soil Research 16:35-40

Falconer, D. and Mackay, T. 1996. Introduction to Quantitative Genetics. Longman, London.

Geeth, K. and Jayaraman, N. 2000. Path analysis in maize (*Zea mays* L.). Agriculture Science Digest 20:60-61. Muhammad, B. A., Muhammad, R., Muhammad, S. T, Amerr, H., Tariq, M. and Muhammad, S. 2003. Character association and path coefficient analysis of grain yield and yield components in maize (*Zea mays L*.). Pakistan Journal of Biological Science 6:136-138.

Gidey, Y.T., S.A. Kebede and G.T. Gashawbeza. 2013. Assessment of genetic variability, genetic advance, correlation and path analysis for morphological traits in sesame genotypes. International Journal of Plant Breeding and Genetics 7: 21-24

Hallauer, A. and J. Miranda. 1988. Quantitative Genetics in Maize Breeding, 2nd edn, Iowa State University Press, Ames, IA.

Hepziba, S.J., K. Geetha and S. Ibrahim. 2013. Research Note Evaluation of genetic diversity, variability, character association and path analysis in diverse inbreds of maize (*Zea mays* L.). Electronic Journal of Plant Breeding 4: 1067-1072

Ifie, B.E. 2013. Genetic analysis of Striga resistance and low soil nitrogen tolerance in early maturing maize *(Zea mays L.)* inbred lines.PhD thesis, University of Ghana.

Ismaila, U., A. Gana, N. Tswanya and D. Dogara. 2010. Cereals production in Nigeria: Problems, constraints and opportunities for betterment. African Journal of Agricultural Research 5: 1341-1350.

Jakhar, D.S., R. Singh and A. Kumar. 2017. studies on path coefficient analysis in maize *(Zea mays L.)* for grain yield and its attributes. International Journal of Current Microbiology and Applied Science 6: 2851-2856.

Johnson, H.W., H. Robinson and R. Comstock. 1955. Estimates of Genetic and Environmental Variability in Soybeans 1. Agronomy Journal 47: 314-318.

Kumar, V., S. Singh, P. Bhati, A. Sharma, S. Sharma and V. Mahajan. 2015. Correlation, path and genetic diversity analysis in maize (*Zea mays L*.). Environment and Ecology 33: 971-975.

Kusaksiz, T. 2010. Adaptability of some new maize *(Zea mays L.)* cultivars for silage production as main crop in Mediterranean environment. Turkish Journal of Field Crops 15: 193-197.

Lafitte, R., A. Blum and G. Atlin. 2003. Using secondary traits to help identify drought-tolerant genotypes. Breeding rice for drought-prone environments: 37-48.

Lafitte, H. and G. Edmeades. 1995. Association between traits in tropical maize inbred lines and their hybrids under high and low soil nitrogen. Maydica (Italy). 40: 259-267.

Laghari, K.A., M. A. Sial, M. A. Arain, A. A. Mirbahar, A. Pirzada, M. Dahot et al. 2010. Heritability studies of yield and yield associated traits in bread wheat. Pakstan Journal of Botany 42: 111-115

Magorokosho, C., Vivek, B. and MacRobert, J. 2009. Characterization of maize germplasm grown in Eastern and Southern Africa: Results of the 2008 regional trials coordinated by CIMMYT. CIMMYT, Harare, Zimbabwe.

Makumbi, D., J.F. Betrán, M. Bänziger and J.-M. Ribaut. 2011. Combining ability, heterosis and genetic diversity in tropical maize *(Zea mays L.)* under stress and non-stress conditions. Euphytica 180: 143-162.

Makumbi, D., J.F. Betrán, M. Bänziger and J.-M. Ribaut. 2011. Combining ability, heterosis and genetic diversity in tropical maize *(Zea mays L.)* under stress and non-stress conditions. Euphytica 180: 143-162.

Matin, M.Q.I., M.S. Uddin, M.M. Rohman, M. Amiruzzaman, A.K. Azad and B.R. Banik. 2017. Genetic Variability and Path Analysis Studies in Hybrid Maize (*Zea mays L.).* American Journal of Plant Sciences 8: 3101-3109

Meseka, S., A. Menkir, A. Ibrahim and S. Ajala. 2006. Genetic analysis of performance of maize inbred lines selected for tolerance to drought under low nitrogen. Maydica 51: 487- 495.

Meseka, S.K., A. Menkir, A.E.S. Ibrahim and S.O. Ajala. 2013. Genetic analysis of maize inbred lines for tolerance to drought and low nitrogen. Jonares 1: 29-36.

Mhoswa, L., J. Derera, F.N. Qwabe and T.R. Musimwa. 2016. Diversity and path coefficient analysis of Southern African maize hybrids. Chilean journal of agricultural research 76: 143-151

Muhammad, A., M. Hader, S. Muhammad and A. Muhammad. 2008. Contribution of various leaf morpho-physiological parameters towards grain yield in maize. International journal of agriculture and biology 10: 546-550.

Mushongi, A. 2010. Genetic Studies of Secondary Traits and Yield Heterosis in Maize Under High and Low Nitrogen Conditions, Incorporating Farmer Perceptions and Preferences for Varieties. PhD thesis,University of KwaZulu-Natal, Pietermaritzburg.

Najeeb, S., A. Rather, G. Parray, F. Sheikh and S. Razvi. 2009. Studies on genetic variability, genotypic correlation and path coefficient analysis in maize under the high altitude temperate conditions of Kashmir. Maize Genetics Cooperation Newsletter. 83:1-8.

Noelle, H. 2014. Genetic Analysis of tolerance to Low Soil Nitrogen in Intermediate Maturing Maize *(Zea Mays L.)* Inbred Lines. PhD thesis, University of Ghana,

Ogunniyan, D and Olakojo S. 2014. Genetic variation, heritability, genetic advance and agronomic character association of yellow elite inbred lines of maize (*Zea mays* L.). Nigerian Journal of Genetics 28: 24-28.

Parajuli, S., B. Ojha and G. Ferrara. 2018. Quantification of Secondary Traits for Drought and Low Nitrogen Stress Tolerance in Inbreds and Hybrids of Maize *(Zea mays L.).* Journal of Plant Genetics and Breeding 2: 2.Pavan, R., H. Lohithaswa, M. Wali, P. Gangashetty and B. Shekara. 2011. Research Note Correlation and path coefficient analysis of grain yield and yield contributing traits in single cross hybrids of maize *(Zea mays L.).* Electronic Journal of Plant Breeding 2: 253-257.

Pavlov, J., N. Delić, K. Marković, M. Crevar, Z. Čamdžija and M. Stevanović. 2015. Path analysis for morphological traits in maize *(Zea mays L.).* Genetika 47: 295-301Payne, R. W., Murray, D. A., Harding, S.A., Baird, D. B. and Soutar, D. M. 2014. GenStat for Windows (18th ed.) Introduction. VSN International, Hemel Hempstead.

Payne R, Murray D, Harding S, Baird D, Soutar D. 2014. GenStat for Windows. 18th ed., Hemel Hempstead: VSN International

Presterl, T., G. Seitz, M. Landbeck, E. Thiemt, W. Schmidt and H. Geiger. 2003. Improving nitrogen-use efficiency in european maize. Crop Science 43: 1259-1265.

Rafiq, C.M., M. Rafique, A. Hussain and M. Altaf. 2010. Studies on heritability, correlation and path analysis in maize *(Zea mays L.).* Journal of Agricultural Research 48: 35-38.

Saryam, D.K., S. Mittra, A. Mehta, S. Prajapati and S. Kadwey. 2015. Correlation and path co-efficient analysis of quantitative traits in *okra [Abelmoschus esculentus (L.)* moench]. The Bioscan 10: 735-739

SAS Institute Inc. 2010. SAS/STAT® 9.3 User's Guide . Cary, NC: SAS Institute Inc.

Sibale, E.M., & Smith, M.E. 1997. Relationship between secondary traits and grain yield of maize grown in low nitrogen soil in Malawi. p. 245-248. *In* G.O. Edmeades et al. (ed.) Developing drought- and low N-tolerant maize. Proceedings of a symposium, March 25-29, 1996. CIMMYT, El Batan, Mexico. CIMMYT,Mexico City.

Smith, M.E., Miles, C.A. and Van Beem, J. 1995. Genetic improvement of maize for nitrogen use efficiency, pp 306. In: Jewell DC, Waddington SR, Ransom JK, Pixley KV, editors. Maize research for stress environments. Proceedings of the Fourth Eastern and Southern Africa Regional Maize Conference, held at Harare, Zimbabwe, 28 March-1 April 1994. Mexico D.F. CIMMYT

SPSS. 2014. Statistics for Windows, version 25 (SPSS Inc., Chicago, llinois., USA). http://www-01.ibm.com/support/docview.wss?uid=swg21476197

Sreckov, Z., A. Nastasic, J. Bocanski, I. Djalovic, M. Vukosavljev and B. Jockovic. 2011. Correlation and path analysis of grain yield and morphological traits in test–cross populations of maize. Pakistan Journal of Botany 43: 1729-1731.

Singh, R. K. and Chaudhary, B. D. 2004. Biometrical Methods in Quantitative Genetic Analysis. Kalyani Publishers, New Delhi, India.

Sridhar, M., Muralikrishna, K. Sultana, R. and Bhave, M. H. V. 2016. Character association and path analysis for grain yield and yield component traits in popcorn (*Zea mays* var. *everta* Sturt.). International Journal of Agricultaral Science and Research 6:145-150.

Teklu, D.H., S.A. Kebede and D.E. Gebremichael. 2014. Assessment of genetic variability, genetic advance, correlation and path analysis for morphological traits in sesame genotypes. Asian Journal of Agricultural Research 8: 181-194.

The, C., Ngonkeu, M. L., Zonkeng, C. and Apala, H. M. 2013.Evaluation and selection of maize *(Zea mays L.)* genotypes tolerant to low N soil In:. Optimizing Productivity of Food Crop Genotypes in Low Nutrient Soils. IAEA-TECDOC-1721. 251-264

Tiwari, D.K., P. Pandey, S. Tripathi, S.P. Giri and J.L. Dwivedi. 2011. Studies on genetic variability for yield components in rice *(Oryza sativa L.).* Advances in Agriculture & Botanics 3 (1): 76-81.

Yousuf, M. and M. Saleem. 2001. Correlation analysis of S1 families of maize for grain yield and its components. International Journal of Agriculture and Biology 4: 387-388

Vijayabharathi, A., Anandakumar, C. R. and Gnanamalar, R. P. 2009. Analysis of correlations and path effects for popping expansion in popcorn (*Zea mays* var. *everta* Sturt*).* Journal of Plant Breeding 1:60-64

3.7 Appendices

APPENDIX 3.1

List of single-cross maize hybrids used in this study

GENOTYPE CODE	PEDIGREE	SOURCE
G1	CB322/CK21	ARC-GCI
G2	CB322/I-42	ARC-GCI
G3	CB322/RO549W	ARC-GCI
G4	CB322/U2540W	ARC-GCI
G5	CB323/CK21	ARC-GCI
G6	CB323/CML442	ARC-GCI
G7	CB323/I-38	ARC-GCI
G8	CB323/I-42	ARC-GCI
G9	CB323/RO549W	ARC-GCI
G10	CB323/U2540W	ARC-GCI
G11	CB339/CK21	ARC-GCI
G12	CB339/CML442	ARC-GCI
G13	CB339/I-38	ARC-GCI
G14	CB339/U2540W	ARC-GCI
G15	CK21/CB322	ARC-GCI
G16	CK21/CB323	ARC-GCI
G17	CK21/CB339	ARC-GCI
G18	CK21/CKDHL0089	ARC-GCI
G19	CK21/CKDHL0295	ARC-GCI
G20	CK21/CKDHL0378	ARC-GCI
G21	CK21/CKDHL0470	ARC-GCI
G22	CK21/CKL05022	ARC-GCI
G23	CK21/CML202	ARC-GCI
G24	CK21/CML216	ARC-GCI
G25	CK21/CML443	ARC-GCI
G26	CK21/CML444	ARC-GCI
G27	CK21/CML488	ARC-GCI
G28	CK21/CML511	ARC-GCI
G29	CK21/CML544	ARC-GCI
G30	CK21/CML547	ARC-GCI
G31	CK21/CZL068	ARC-GCI
G32	CKDHL0089/CK21	ARC-GCI
G33	CKDHL0089/CML442	ARC-GCI
G34	CKDHL0089/RO549W	ARC-GCI
G35	CKDHL0089/U2540W	ARC-GCI
G36	CKDHL0089/I-38	ARC-GCI
G37	CKDHL0295/CK21	ARC-GCI
G38	CKDHL0295/CML442	ARC-GCI
G39	CKDHL0295/U2540W	ARC-GCI
G40	CKDHL0295/I-38	ARC-GCI
G41	CKDHL0295/I-42	ARC-GCI
G42	CKDHL0378/CML442	ARC-GCI
G43	CKDHL0378/I-38	ARC-GCI
G44	CKDHL0378/I-42	ARC-GCI
G45	CKDHL0378/U2540W	ARC-GCI

GENOTYPE CODE	PEDIGREE	SOURCE
G46	CKDHL0470/CK21	ARC-GCI
G47	CKDHL0470/RO549W	ARC-GCI
G48	CKDHL0470/I-38	ARC-GCI
G49	CKDHL0470/I-42	ARC-GCI
G50	CKDHL0470/U2540W	ARC-GCI
G51	CKL05022/I-38	ARC-GCI
G52	CKL05022/I-42	ARC-GCI
G53	CKL05022/RO549W	ARC-GCI
G54	CKL05022/U2540W	ARC-GCI
G55	CML202/CK21	ARC-GCI
G56	CML202/CML442	ARC-GCI
G57	CML202/I-38	ARC-GCI
G58	CML202/I-42	ARC-GCI
G59	CML216/CK21	ARC-GCI
G60	CML216/CML442	ARC-GCI
G61	CML216/I-42	ARC-GCI
G62	CML216/RO549W	ARC-GCI
G63	CML216/U2540W	ARC-GCI
G64	CML442/CB322	ARC-GCI
G65	CML442/CB323	ARC-GCI
G66	CML442/CB339	ARC-GCI
G67	CML442/CKDHL0089	ARC-GCI
G68	CML442/CKDHL0378	ARC-GCI
G69	CML442/CML216	ARC-GCI
G70	CML442/CML443	ARC-GCI
G71	CML442/CML444	ARC-GCI
G72	CML442/CML488	ARC-GCI
G73	CML442/CML511	ARC-GCI
G74	CML442/CML544	ARC-GCI
G75	CML442/CML547	ARC-GCI
G76	CML442/CZL068	ARC-GCI
G77	CML443/CK21	ARC-GCI
G78	CML443/CML442	ARC-GCI
G79	CML443/I-38	ARC-GCI
G80	CML443/I-42	ARC-GCI
G81	CML444/CK21	ARC-GCI
G82	CML444/CML442	ARC-GCI
G83	CML444/I-38	ARC-GCI
G84	CML444/I-42	ARC-GCI
G85	CML488/CK21	ARC-GCI
G86	CML488/CML442	ARC-GCI
G87	CML488/I-38	ARC-GCI
G88	CML488/RO549W	ARC-GCI
G89	CML488/U2540W	ARC-GCI
G90	CML511/CK21	ARC-GCI
G91	CML511/CML442	ARC-GCI
G92	CML511/I-38	ARC-GCI
G93	CML511/I-42	ARC-GCI
G94	CML511/RO549W	ARC-GCI
G95	CML511/U2540W	ARC-GCI
G96	CML543/I-38	ARC-GCI
G97	CML543/RO549W	ARC-GCI
G98	CML543/U2540W	ARC-GCI

GENOTYPE CODE	PEDIGREE	SOURCE
G99	CML544/CK21	ARC-GCI
G100	CML544/CML442	ARC-GCI
G101	CML544/I-38	ARC-GCI
G102	CML544/I-42	ARC-GCI
G103	CML544/RO549W	ARC-GCI
G104	CML547/CML442	ARC-GCI
G105	CML547/I-42	ARC-GCI
G106	CML547/RO549W	ARC-GCI
G107	CML547/U2540W	ARC-GCI
G108	CZL068/CK21	ARC-GCI
G109	CZL068/I-38	ARC-GCI
G110	CZL068/I-42	ARC-GCI
G111	CZL068/RO549W	ARC-GCI
G112	CZL068/U2540W	ARC-GCI
G113	I-38/CB322	ARC-GCI
G114	I-38/CB323	ARC-GCI
G115	I-38/CB339	ARC-GCI
G116	I-38/CKDHL0089	ARC-GCI
G117	I-38/CKDHL0295	ARC-GCI
G118	I-38/CKDHL0378	ARC-GCI
G119	I-38/CKDHL0470	ARC-GCI
G120	I-38/CKL05022	ARC-GCI
G121	I-38/CML202	ARC-GCI
G122	I-38/CML216	ARC-GCI
G123	I-38/CML443	ARC-GCI
G124	I-38/CML444	ARC-GCI
G125	I-38/CML488	ARC-GCI
G126	I-38/CML511	ARC-GCI
G127	I-38/CML543	ARC-GCI
G128	I-38/CML544	ARC-GCI
G129	I-38/CML547	ARC-GCI
G130	I-38/CZL068	ARC-GCI
G131	I-42/CB322	ARC-GCI
G132	I-42/CB323	ARC-GCI
G133	I-42/CB339	ARC-GCI
G134	I-42/CKDHL0295	ARC-GCI
G135	I-42/CKL05022	ARC-GCI
G136	I-42/CML202	ARC-GCI
G137	I-42/CML216	ARC-GCI
G138	I-42/CML443	ARC-GCI
G139	I-42/CML444	ARC-GCI
G140	I-42/CML488	ARC-GCI
G141	I-42/CML511	ARC-GCI
G142	I-42/CML544	ARC-GCI
G143	I-42/CML547	ARC-GCI
G144	I-42/CZL068	ARC-GCI
G145	RO549W/CB322	ARC-GCI
G146	RO549W/CKDHL0295	ARC-GCI
G147	RO549W/CKDHL0470	ARC-GCI
G148	RO549W/CKL05022	ARC-GCI
G149	RO549W/CML216	ARC-GCI
G150	RO549W/CML444	ARC-GCI
G151	RO549W/CML488	ARC-GCI

GENOTYPE CODE	PEDIGREE	SOURCE
G152	RO549W/CML511	ARC-GCI
G153	RO549W/CML543	ARC-GCI
G154	RO549W/CML544	ARC-GCI
G155	RO549W/CML547	ARC-GCI
G156	RO549W/CZL068	ARC-GCI
G157	U2540W/CB322	ARC-GCI
G158	U2540W/CB323	ARC-GCI
G159	U2540W/CKDHL0295	ARC-GCI
G160	U2540W/CKDHL0378	ARC-GCI
G161	U2540W/CKDHL0470	ARC-GCI
G162	U2540W/CKL05022	ARC-GCI
G163	U2540W/CML216	ARC-GCI
G164	U2540W/CML488	ARC-GCI
G165	U2540W/CML511	ARC-GCI
G166	U2540W/CML543	ARC-GCI
G167	U2540W/CML547	ARC-GCI
G168	WE3128	ARC-GCI
G169	WE4145	ARC-GCI
G170	WE6208B	ARC-GCI

APPENDIX 3.2

Performance of maize hybrids for grain yield and agronomic traits under combined low N environments during the 2017/18 summer season

Traits	GY (tha^{-1})	ASI (days)	EH (cm)	PH (CM)	EPP
Hybrid Code					
G1	4.0	1.3	77.1	170.0	1.0
G2	3.9	1.5	92.4	196.5	0.9
G3	4.2	1.8	88.7	179.7	0.9
G4	4.0	2.5	89.2	184.1	0.9
G5	4.2	2.0	85.8	173.3	0.9
G6	3.8	2.0	73.7	156.0	1.0
G7	3.7	3.3	85.8	164.7	0.9
G8	3.4	2.3	71.2	166.3	0.9
G9	4.5	1.8	95.0	188.0	0.8
G10	3.4	2.0	82.7	172.8	0.9
G11	4.3	1.5	80.0	167.1	1.0
G12	4.9	1.3	92.0	178.0	1.1
G13	3.9	1.5	87.5	178.2	0.9
G14	3.5	2.0	98.6	191.4	1.0
G15	4.4	1.8	91.8	188.3	0.9
G16	4.2	1.3	80.0	173.7	0.9
G17	4.2	1.8	86.8	163.6	1.0
G18	4.9	2.5	85.3	180.4	1.0
G19	4.8	1.8	99.4	189.9	1.0
G20	4.2	1.8	80.6	167.0	1.0
G21	4.4	2.0	96.5	184.6	0.9
G22	3.8	2.0	92.7	187.1	1.1
G23	4.1	1.8	88.4	175.6	1.0
G24	4.2	1.8	102.3	203.0	0.9
G25	4.0	2.0	86.7	173.3	1.0
G26	4.6	1.0	85.7	169.0	1.0
G27	4.3	1.8	84.6	162.9	1.0
G28	4.2	1.3	78.3	177.9	1.1
G29	4.2	1.8	85.5	168.1	1.0
G30	3.6	2.0	86.2	171.9	0.9
G31	3.3	1.3	85.9	180.4	1.2
G32	3.4	1.5	91.4	181.3	0.8
G33	4.3	2.0	86.1	174.7	1.0
G34	2.6	3.5	101.9	192.1	0.9
G35	3.8	2.3	81.4	179.3	0.9

Traits	GY (tha^{-1})	ASI (days)	EH (cm)	PH (CM)	EPP
G36	2.8	3.0	82.5	172.9	0.9
G37	3.9	2.3	93.7	185.6	0.9
G38	1.6	2.3	82.0	173.4	1.1
G39	4.4	3.0	112.3	193.5	1.0
G40	4.4	2.5	94.8	178.2	1.0
G41	3.9	2.5	94.2	184.3	0.9
G42	4.5	2.3	87.3	182.6	0.9
G43	4.4	2.8	92.7	180.6	1.0
G44	4.8	2.3	86.2	176.9	1.1
G45	4.7	1.8	87.9	180.4	1.0
G46	4.0	2.3	88.0	175.9	0.9
G47	3.2	3.3	100.0	185.5	0.9
G48	3.9	3.3	85.7	188.2	0.9
G49	4.1	2.5	86.2	188.7	0.9
G50	3.3	2.5	89.9	180.9	1.0
G51	3.5	3.0	90.1	173.0	0.9
G52	4.5	1.8	92.1	185.6	1.1
G53	3.9	3.0	104.8	191.5	0.8
G54	4.6	2.0	101.5	185.4	0.8
G55	4.8	1.8	85.5	171.0	1.0
G56	4.1	1.8	88.7	176.9	0.9
G57	3.4	2.3	76.8	162.7	0.9
G58	3.9	1.8	80.0	165.9	0.9
G59	3.8	1.8	103.7	202.6	0.9
G60	3.4	3.5	96.3	193.5	0.9
G61	3.8	2.8	115.5	201.1	0.9
G62	4.9	2.3	109.4	210.0	1.0
G63	2.8	3.8	110.1	204.8	0.9
G64	3.7	1.3	95.8	179.6	0.9
G65	3.7	2.5	81.2	171.4	0.9
G66	4.0	1.8	89.2	181.1	0.9
G67	4.2	3.3	90.0	180.5	0.9
G68	4.6	2.3	87.6	182.2	0.9
G69	4.2	1.5	107.3	202.6	1.0
G70	4.1	2.5	91.6	173.5	1.5
G71	3.7	2.3	92.8	184.2	0.9
G72	3.2	2.3	81.8	165.2	0.7
G73	3.1	1.5	92.3	191.1	0.8
G74	4.4	1.8	90.0	172.4	1.0
G75	3.9	2.0	99.1	196.4	1.0
G76	3.3	2.3	87.7	185.4	1.1
G77	4.2	2.3	91.2	183.5	0.9

Traits	GY (tha^{-1})	ASI (days)	EH (cm)	PH (CM)	EPP
G78	2.5	3.5	69.3	151.9	0.9
G79	3.9	2.8	76.8	167.4	1.0
G80	4.4	2.8	83.2	175.7	1.1
G81	4.0	3.5	95.7	202.1	0.9
G82	4.4	2.5	102.8	186.0	1.0
G83	4.4	2.3	94.2	185.2	0.9
G84	3.6	2.8	90.8	176.8	0.8
G85	3.8	1.8	77.1	162.5	1.0
G86	4.2	2.0	80.1	159.6	0.9
G87	3.7	1.5	84.5	162.3	1.2
G88	4.1	1.8	89.3	178.6	1.4
G89	3.2	1.8	85.3	166.3	1.1
G90	4.4	1.5	82.1	184.2	1.0
G91	3.6	2.3	87.5	182.8	1.0
G92	4.2	3.0	87.3	178.4	0.9
G93	4.0	2.0	77.3	173.0	1.0
G94	3.2	3.0	87.8	179.4	1.0
G95	3.6	2.8	94.0	195.7	0.9
G96	3.7	3.5	97.5	191.5	1.0
G97	3.8	3.5	102.5	197.4	0.9
G98	3.7	3.8	104.6	196.7	0.9
G99	4.7	1.8	79.8	165.6	1.1
G100	4.2	1.8	91.2	175.5	1.0
G101	4.8	2.5	92.1	173.0	1.0
G102	4.7	2.5	87.4	170.5	1.0
G103	2.6	3.8	76.3	162.9	1.0
G104	4.1	3.3	88.7	176.3	1.0
G105	4.5	2.5	99.2	201.9	0.9
G106	3.3	2.8	100.0	191.1	1.0
G107	3.3	2.5	98.1	188.0	0.9
G108	3.3	2.0	85.1	179.3	1.3
G109	4.4	1.8	89.1	187.1	0.9
G110	4.3	2.3	84.4	183.7	1.0
G111	4.0	2.5	87.7	181.1	0.8
G112	3.8	2.8	89.1	184.5	0.9
G113	4.0	2.3	91.6	181.6	0.9
G114	3.7	1.8	90.4	180.5	0.9
G115	4.7	2.3	89.2	174.3	0.9
G116	3.6	3.0	95.2	183.9	0.8
G117	4.6	3.3	97.5	184.9	0.9
G118	4.1	2.5	86.6	175.1	0.9
G119	3.4	2.8	86.5	189.3	1.0

Traits	GY (tha^{-1})	ASI (days)	EH (cm)	PH (CM)	EPP
G120	3.3	2.8	91.6	177.6	0.8
G121	4.2	2.5	81.1	158.9	1.0
G122	2.2	3.0	105.7	193.6	1.4
G123	3.8	2.5	80.3	169.2	1.0
G124	3.7	3.5	90.1	165.6	1.4
G125	3.9	2.0	69.1	148.2	0.9
G126	4.0	2.3	88.0	187.4	0.9
G127	4.1	3.3	99.6	187.1	1.0
G128	4.0	2.5	91.7	182.0	1.0
G129	4.1	2.5	95.6	187.7	0.9
G130	4.4	2.0	85.5	182.5	1.0
G131	3.4	1.8	76.0	165.1	0.9
G132	2.9	2.3	81.1	175.7	0.8
G133	4.9	2.0	80.8	181.9	0.9
G134	3.8	4.3	83.1	170.6	0.9
G135	4.3	1.8	94.6	176.2	0.9
G136	4.0	1.8	82.6	165.7	1.0
G137	3.7	2.5	99.4	198.0	1.0
G138	3.6	2.0	78.6	166.4	1.0
G139	3.7	2.3	86.3	173.0	0.9
G140	4.0	2.0	81.1	167.0	1.0
G141	3.8	1.8	78.5	176.5	1.0
G142	4.7	1.8	90.0	179.9	1.0
G143	2.6	2.0	87.7	191.1	0.8
G144	3.4	2.5	84.5	184.7	0.9
G145	3.6	2.5	85.9	170.6	0.9
G146	4.7	2.0	104.6	199.1	0.9
G147	3.4	2.3	98.9	190.4	1.0
G148	4.5	1.8	111.0	191.6	0.9
G149	3.4	3.3	106.0	188.6	1.1
G150	4.4	1.8	87.4	169.5	1.0
G151	3.9	2.5	92.7	168.4	0.9
G152	3.8	2.8	80.5	172.7	0.9
G153	4.1	3.3	106.4	199.9	0.9
G154	4.0	1.5	96.3	172.6	0.9
G155	3.2	3.8	102.7	197.9	0.7
G156	3.6	2.0	91.8	184.8	1.0
G157	3.8	1.8	97.1	185.5	0.9
G158	3.7	2.3	103.0	163.8	0.8
G159	3.7	3.3	100.1	193.4	1.0
G160	3.8	1.5	84.2	172.9	0.9
G161	3.5	3.0	99.9	187.0	0.9

Traits	GY (tha^{-1})	ASI (days)	EH (cm)	PH (CM)	EPP
G162	3.5	1.8	95.7	178.2	0.8
G163	3.4	2.8	98.4	190.7	0.9
G164	1.8	2.0	58.7	148.8	0.9
G165	3.5	2.8	84.6	178.1	0.9
G166	3.3	3.5	106.7	198.4	0.8
G167	3.1	3.5	101.0	194.4	0.7
G168	4.5	2.5	104.0	189.7	1.0
G169	4.6	1.8	78.3	175.4	1.0
G170	3.4	1.5	89.5	163.8	0.9
Min	1.6	1.0	58.7	148.2	0.7
Max	4.9	4.3	115.5	210.0	1.5
Mean	3.9	2.3	90.1	180.0	1.0
CV (%)	11.9	20.8	8.4	4.2	14.0
S.E.	0.0	0.0	0.6	0.6	0.0
P-value	<.0001	<.0001	<.0001	<.0001	<.0001

APPENDIX 3.3

Performance of maize hybrids for grain yield and related traits under combined optimum environments during the 2017/18 summer season

Traits	GY (tha^{-1})	ASI (days)	EH (cm)	PH (CM)	EPP
Hybrid Code					
G1	7.9	1.0	91.3	194.4	1.1
G2	6.7	1.0	92.3	207.0	1.2
G3	7.1	1.2	102.0	203.5	1.0
G4	7.2	1.5	104.3	204.9	1.0
G5	7.5	1.3	93.2	199.3	1.1
G6	7.9	1.5	101.7	202.6	1.1
G7	6.0	1.2	94.2	200.6	1.0
G8	7.0	1.7	87.3	196.3	1.2
G9	7.5	1.5	97.5	187.1	1.0
G10	7.4	1.8	91.3	192.8	1.1
G11	6.6	1.3	85.0	191.7	1.1
G12	8.2	1.2	100.3	202.0	1.1
G13	7.8	1.5	93.3	201.0	1.0
G14	7.0	1.5	98.2	199.2	1.0
G15	7.1	1.8	92.2	190.2	1.1
G16	6.2	1.3	82.7	186.9	1.0
G17	7.0	1.2	87.6	181.2	1.1
G18	9.0	1.8	111.8	196.1	1.4
G19	6.9	1.5	101.3	195.0	1.1
G20	7.5	1.8	90.9	184.9	1.3
G21	6.8	1.5	111.7	203.1	1.1
G22	6.5	1.3	101.5	195.2	1.2
G23	7.7	2.0	100.4	194.7	1.2
G24	8.9	2.2	111.5	210.6	1.2
G25	7.7	1.8	101.2	197.2	1.3
G26	7.7	1.2	98.3	191.4	1.3
G27	5.8	1.2	97.7	185.5	1.3
G28	7.8	1.0	95.1	209.1	1.2
G29	5.5	1.7	94.3	174.4	1.1
G30	5.5	3.0	89.7	186.0	1.1
G31	5.2	1.2	95.3	193.4	1.2
G32	7.9	2.0	110.1	198.0	1.3
G33	8.4	1.7	101.7	198.8	1.0
G34	7.8	1.5	117.2	213.1	1.1
G35	6.4	1.5	110.2	208.3	1.3
G36	5.6	1.8	110.7	205.7	1.4
G37	7.1	1.5	106.1	205.1	1.2
G38	2.5	1.7	92.2	196.6	1.3

G39	6.4	1.7	113.1	202.7	1.0
G40	6.5	2.3	114.5	206.8	1.0
G41	7.0	1.7	97.3	198.9	1.1
G42	7.6	1.5	104.5	205.2	1.1
G43	6.4	1.7	106.7	201.9	1.3
G44	6.0	2.2	95.1	194.1	1.3
G45	7.1	1.2	97.7	200.2	1.3
G46	8.6	1.7	110.8	201.6	1.2
G47	6.4	2.2	110.4	183.8	1.0
G48	8.0	2.0	108.2	209.2	1.2
G49	6.3	2.2	96.3	201.3	1.1
G50	6.4	1.8	108.2	199.0	1.2
G51	6.5	1.5	107.9	200.2	1.2
G52	5.9	1.7	104.5	195.0	1.1
G53	7.0	2.0	129.6	221.1	1.0
G54	6.5	1.7	109.2	195.6	1.4
G55	6.9	1.0	103.1	198.7	1.3
G56	6.2	1.8	102.2	197.8	1.0
G57	6.7	2.0	107.4	205.1	1.1
G58	6.2	1.3	95.4	193.1	1.1
G59	6.9	1.8	114.6	206.1	1.2
G60	7.6	2.0	122.2	220.7	0.9
G61	7.2	1.3	107.7	214.4	1.5
G62	6.7	2.0	120.9	218.3	1.1
G63	7.0	1.2	126.9	230.5	1.2
G64	6.9	1.2	104.0	200.8	1.0
G65	7.9	1.7	95.6	198.5	1.0
G66	8.3	1.0	102.1	204.9	1.1
G67	6.7	1.7	109.0	204.7	1.0
G68	6.4	1.0	103.3	200.4	1.3
G69	7.0	1.7	130.2	224.5	1.1
G70	7.0	1.5	102.6	195.5	1.2
G71	6.8	1.5	101.2	195.0	1.0
G72	6.0	1.5	93.6	185.2	1.3
G73	6.5	2.0	94.1	200.4	1.1
G74	6.0	2.0	102.1	187.7	1.0
G75	6.2	1.5	106.2	212.1	1.3
G76	5.1	1.0	91.2	192.9	1.1
G77	6.4	2.2	103.9	201.3	1.2
G78	6.5	1.7	100.9	212.3	1.1
G79	6.2	2.0	99.5	182.3	1.2
G80	7.2	1.8	100.5	198.5	1.3
G81	6.7	1.5	108.7	201.9	1.2
G82	7.1	1.2	113.9	198.9	1.0

G83	6.5	2.0	107.7	191.4	1.1
G84	7.6	1.8	114.4	204.1	1.4
G85	7.0	1.5	96.7	187.8	1.2
G86	6.8	1.0	96.0	185.4	1.3
G87	6.7	1.3	108.1	198.4	1.4
G88	2.7	1.2	97.9	185.0	1.1
G89	5.6	1.3	98.9	182.0	1.3
G90	7.0	1.3	88.6	193.5	1.2
G91	7.1	1.5	107.3	208.7	1.2
G92	7.2	2.0	102.9	210.4	1.1
G93	7.1	2.0	97.6	200.5	1.3
G94	6.3	1.7	107.1	212.7	1.1
G95	5.7	1.8	100.1	209.7	1.1
G96	8.0	2.2	117.2	216.0	1.2
G97	5.6	1.5	122.5	213.9	1.0
G98	6.2	1.7	120.4	209.8	1.0
G99	7.1	1.2	99.9	190.2	1.1
G100	8.3	1.5	95.0	183.7	1.1
G101	7.9	1.7	104.7	201.0	1.4
G102	7.2	1.3	97.4	191.7	1.2
G103	7.1	1.2	116.3	201.5	1.1
G104	5.9	1.7	107.4	209.1	1.2
G105	5.7	2.0	100.6	208.3	1.3
G106	6.4	2.2	118.2	210.7	1.1
G107	5.1	2.0	115.5	217.9	1.0
G108	4.0	0.7	98.7	193.8	1.4
G109	4.9	1.7	96.8	192.6	1.4
G110	6.7	1.7	105.7	204.8	1.3
G111	6.4	1.7	104.5	199.9	1.2
G112	6.2	1.7	103.5	198.6	1.1
G113	7.7	1.5	103.7	209.5	1.0
G114	7.3	1.3	95.4	202.8	1.1
G115	6.5	1.0	94.4	186.5	1.1
G116	7.4	1.3	108.8	206.3	1.0
G117	7.1	2.5	119.6	210.3	1.1
G118	6.0	2.0	104.4	201.7	1.3
G119	6.3	2.0	105.4	206.6	1.2
G120	5.6	1.5	113.2	214.4	1.1
G121	7.8	1.2	107.3	202.7	1.1
G122	3.3	1.5	115.4	217.2	1.6
G123	7.1	1.8	107.4	204.0	1.2
G124	6.2	2.0	110.9	204.2	1.2
G125	6.4	1.3	101.3	183.1	1.5
G126	5.8	2.0	99.5	206.8	1.0

G127	7.0	1.7	109.9	202.2	1.2
G128	7.0	1.8	105.2	196.4	1.2
G129	5.8	2.0	109.6	228.5	1.2
G130	6.3	1.5	102.7	202.0	1.1
G131	5.1	1.0	95.3	202.0	1.3
G132	6.8	1.0	86.1	199.5	1.2
G133	8.2	1.0	93.2	201.2	1.1
G134	8.0	2.0	108.6	207.6	1.0
G135	7.2	1.2	102.0	202.3	1.2
G136	6.4	1.2	99.1	198.8	1.1
G137	8.0	1.3	116.5	212.9	1.2
G138	6.8	1.5	91.2	194.5	1.2
G139	6.5	1.2	92.2	184.5	1.6
G140	6.8	1.2	94.4	190.4	1.3
G141	7.1	2.0	90.1	197.6	1.1
G142	7.7	1.2	105.3	201.0	1.3
G143	5.8	1.7	105.9	211.7	1.2
G144	5.3	1.0	100.7	200.3	1.0
G145	6.1	1.2	96.1	180.1	1.1
G146	6.0	1.8	113.2	203.0	1.0
G147	7.2	2.0	116.1	207.3	1.1
G148	6.8	1.5	111.8	198.5	1.1
G149	4.3	2.5	112.4	201.7	1.0
G150	7.0	1.8	108.2	190.5	1.2
G151	5.9	1.2	108.4	191.2	1.2
G152	4.8	2.2	93.4	185.6	1.0
G153	5.6	1.5	124.1	208.5	0.9
G154	7.1	1.5	110.5	198.8	1.0
G155	7.3	1.8	119.6	219.5	1.1
G156	6.0	2.0	109.3	196.9	1.1
G157	6.5	1.5	108.8	211.9	1.0
G158	6.6	1.2	94.3	194.9	1.2
G159	7.1	1.8	117.5	215.3	1.1
G160	7.6	1.5	99.5	191.5	1.3
G161	5.9	1.8	106.5	194.2	1.0
G162	6.5	1.3	114.7	198.5	1.2
G163	7.7	2.2	116.6	218.9	1.1
G164	2.7	1.5	83.8	182.9	1.2
G165	5.5	1.2	96.1	193.8	1.1
G166	7.3	1.5	121.2	217.9	1.1
G167	6.2	2.3	121.2	219.9	1.0
G168	7.2	1.5	113.3	198.3	1.2
G169	7.5	2.2	93.7	205.0	1.0
G170	6.8	1.0	94.0	179.3	1.3

Min	2.5	0.7	82.7	174.4	0.9
Max	9.0	3.0	130.2	230.5	1.6
Mean	6.7	1.6	103.9	200.5	1.2
CV (%)	11.7	27.9	7.2	5.3	14.3
S.E.	0.1	0.0	0.6	0.8	0.0
P-value	<.0001	<.0001	<.0001	<.0001	<.0001

APPENDIX 3.4

Low N selection index, index ranking and yield reduction percentage for 170 maize hybrids grown under combined low N Environments in 2017/18 summer season

Traits Hybrid Codes	GY (tha^{-1}) Opt	GY L (tha^{-1}) Low N	Low N Selection Index	Index Ranking	Yield Reduction (%)
G12	8.24	4.94	15.34	2	40.08
G62	6.61	4.87	8.58	18	26.32
G18	7.52	4.87	8.47	19	35.25
G133	8.06	4.85	9.66	11	39.81
G55	7.62	4.84	9.69	10	36.52
G101	7.43	4.79	8.71	15	35.51
G19	6.20	4.77	9.94	9	23.08
G44	5.95	4.75	10.64	7	20.11
G115	5.57	4.72	6.36	28	15.29
G102	7.11	4.71	6.45	27	33.82
G146	6.24	4.69	4.29	45	24.83
G45	5.86	4.69	9.38	12	20.01
G142	6.70	4.68	11.37	6	30.19
G99	6.32	4.66	11.73	5	26.27
G68	5.40	4.64	5.71	32	13.99
G26	7.78	4.61	12.61	3	40.69
G169	6.84	4.61	7.85	23	32.63
G54	7.01	4.58	5.03	38	34.68
G117	6.39	4.57	3.80	50	28.50
G148	5.46	4.52	3.86	49	17.29
G52	6.42	4.50	12.39	4	29.85
G168	6.71	4.48	3.72	52	33.24
G9	5.58	4.48	3.27	60	19.72
G42	6.64	4.48	6.19	30	32.51
G105	4.94	4.48	2.30	68	9.39
G130	6.19	4.43	8.96	14	28.50
G39	6.48	4.42	3.87	48	31.79
G90	6.57	4.42	5.16	36	32.73
G15	6.08	4.42	5.17	34	27.36
G83	6.58	4.41	4.38	44	32.88
G74	6.15	4.41	5.56	33	28.36
G82	6.73	4.40	4.88	39	34.65
G21	6.91	4.39	2.37	67	36.40
G40	7.12	4.39	5.90	31	38.34
G109	6.14	4.39	2.99	63	28.44
G80	7.04	4.36	8.30	20	38.04

Traits Hybrid Codes	GY (tha^{-1}) Opt	GY L (tha^{-1}) Low N	Low N Selection Index	Index Ranking	Yield Reduction (%)
G150	6.26	4.36	4.54	43	30.34
G43	6.22	4.35	4.04	47	30.02
G33	7.47	4.34	3.65	56	41.96
G135	7.15	4.33	3.70	55	39.49
G110	7.29	4.30	3.71	54	41.02
G11	6.04	4.28	8.61	17	29.10
G27	6.06	4.27	4.86	40	29.52
G20	5.66	4.24	7.95	22	25.08
G67	6.12	4.24	3.37	59	30.82
G5	6.74	4.23	3.06	62	37.23
G28	7.07	4.21	8.66	16	40.38
G69	6.80	4.21	4.76	41	38.17
G92	7.01	4.20	3.71	53	40.04
G24	8.43	4.20	2.15	70	50.12
G3	6.68	4.20	-1.20	101	37.21
G16	6.06	4.19	6.29	29	30.84
G17	6.84	4.19	3.59	57	38.77
G86	6.38	4.17	1.51	74	34.63
G29	6.07	4.16	5.17	35	31.48
G100	9.27	4.16	5.07	37	55.18
G77	7.04	4.15	2.05	72	40.98
G121	7.24	4.15	0.44	86	42.70
G56	6.53	4.13	2.29	69	36.67
G118	5.85	4.13	2.79	64	29.40
G127	6.89	4.12	1.42	75	40.13
G88	6.78	4.12	15.35	1	39.22
G70	6.41	4.12	10.18	8	35.75
G49	6.17	4.11	1.54	73	33.44
G104	6.17	4.10	0.93	79	33.45
G23	6.55	4.10	4.07	46	37.42
G153	6.56	4.07	1.27	78	38.05
G129	5.07	4.06	0.65	84	19.93
G111	6.25	4.04	-1.25	102	35.29
G4	6.55	4.03	2.61	65	38.51
G128	7.22	4.02	3.09	61	44.30
G93	6.98	4.02	3.51	58	42.41
G140	6.95	4.02	0.19	88	42.19
G136	6.79	4.02	9.31	13	40.84
G66	7.19	4.01	0.01	91	44.18
G1	6.79	4.00	8.17	21	41.17
G25	8.22	4.00	1.31	77	51.39

Traits Hybrid Codes	GY (tha^{-1}) Opt	GY L (tha^{-1}) Low N	Low N Selection Index	Index Ranking	Yield Reduction (%)
G81	6.07	3.99	-3.71	123	34.18
G126	6.58	3.99	-0.61	96	39.35
G46	8.02	3.98	1.37	76	50.40
G113	7.60	3.97	-0.79	98	47.80
G154	6.65	3.97	0.49	85	40.33
G53	6.02	3.94	-3.69	121	34.50
G79	6.86	3.94	0.74	82	42.55
G58	6.03	3.93	7.82	24	34.84
G151	6.35	3.93	-2.17	107	38.15
G13	6.22	3.93	-0.07	92	36.83
G2	5.25	3.92	0.74	83	25.37
G41	7.35	3.91	-4.13	128	46.88
G48	6.82	3.90	-2.90	111	42.75
G125	6.41	3.90	-3.42	119	39.15
G37	7.33	3.86	-3.07	115	47.39
G75	5.85	3.85	0.88	80	34.07
G85	7.56	3.84	3.77	51	49.27
G112	5.18	3.82	-4.14	130	26.17
G123	6.90	3.82	-1.27	103	44.73
G141	6.42	3.79	2.06	71	40.90
G152	5.39	3.79	0.80	81	29.76
G59	8.05	3.77	-0.53	95	53.09
G97	5.75	3.77	-4.98	134	34.43
G160	6.71	3.77	-1.17	100	43.86
G134	8.22	3.77	-4.03	127	54.19
G6	6.13	3.76	0.11	89	38.64
G22	5.68	3.76	4.55	42	33.74
G61	6.05	3.76	-5.09	135	37.87
G157	6.24	3.75	-2.22	108	39.87
G35	5.51	3.75	-0.41	94	31.97
G65	7.01	3.74	-3.84	125	46.58
G7	4.64	3.74	-4.60	133	19.50
G124	6.54	3.73	6.82	26	42.99
G139	5.84	3.71	-2.92	112	36.46
G137	7.08	3.70	-3.05	114	47.74
G159	6.65	3.69	-1.84	106	44.58
G71	6.30	3.69	-6.66	142	41.52
G158	4.89	3.68	-2.56	110	24.67
G64	6.73	3.68	-5.43	137	45.36
G96	8.96	3.67	-3.26	118	59.04
G114	6.09	3.65	-2.41	109	39.97

Traits Hybrid Codes	GY (tha^{-1}) Opt	GY L (tha^{-1}) Low N	Low N Selection Index	Index Ranking	Yield Reduction (%)
G87	6.00	3.65	0.11	90	39.17
G95	5.89	3.64	-3.04	113	38.19
G30	6.50	3.63	-4.38	131	44.18
G138	6.67	3.62	0.20	87	45.66
G116	5.93	3.61	-6.13	140	39.08
G145	5.30	3.61	-3.19	117	31.89
G84	6.89	3.60	-5.86	139	47.73
G91	7.68	3.60	-0.27	93	53.18
G156	5.46	3.57	-3.77	124	34.66
G51	5.65	3.53	-8.08	150	37.51
G14	6.32	3.51	-5.18	136	44.48
G161	6.19	3.49	-8.05	149	43.59
G162	7.49	3.49	-8.77	152	53.41
G165	5.23	3.46	-1.37	104	33.89
G119	5.34	3.44	-7.08	145	35.54
G147	6.44	3.43	-3.09	116	46.67
G10	6.57	3.43	-4.13	129	47.74
G60	6.60	3.43	-9.90	157	48.05
G170	6.17	3.42	-4.56	132	44.57
G144	6.34	3.42	-9.19	154	46.04
G57	6.48	3.41	-0.78	97	47.38
G8	6.44	3.40	-6.81	144	47.15
G32	7.32	3.39	-6.41	141	53.73
G149	4.53	3.38	-3.88	126	25.34
G131	4.36	3.38	-3.70	122	22.46
G163	6.85	3.36	-7.93	148	51.03
G31	5.66	3.32	2.44	66	41.38
G106	6.31	3.32	-6.75	143	47.47
G50	7.04	3.30	-3.61	120	53.10
G120	5.70	3.30	-7.38	146	42.04
G76	5.55	3.29	-0.93	99	40.67
G107	5.41	3.29	-9.07	153	39.24
G166	6.96	3.29	-9.43	155	52.76
G108	4.39	3.28	7.59	25	25.26
G89	5.16	3.24	-1.71	105	37.30
G47	6.20	3.20	-12.30	161	48.38
G72	5.88	3.16	-10.10	158	46.31
G94	5.63	3.15	-8.09	151	44.03
G155	6.49	3.15	-12.92	162	51.51
G73	6.02	3.07	-9.49	156	49.11
G167	6.16	3.05	-13.50	163	50.48

Traits Hybrid Codes	GY (tha^{-1}) Opt	GY L (tha^{-1}) Low N	Low N Selection Index	Index Ranking	Yield Reduction (%)
G132	5.93	2.91	-11.41	159	50.89
G63	6.27	2.78	-17.19	169	55.66
G36	5.02	2.76	-7.53	147	45.05
G103	7.39	2.63	-12.06	160	64.38
G34	7.66	2.61	-17.08	168	65.87
G143	6.57	2.56	-13.58	164	60.98
G78	6.04	2.48	-15.50	167	58.93
G122	3.71	2.19	-5.69	138	41.07
G164	3.75	1.79	-15.47	166	52.16
G38	3.29	1.56	-14.67	165	52.72
Min	**3.29**	**1.56**	**-17.19**		**9.39**
Max	**9.27**	**4.94**	**15.35**		**65.87**
Mean	**6.41**	**3.88**	**0.04**		**38.80**

APPENDIX 3.5

Mean grain yields (tha^{-1}) of 170 maize hybrids grown under five environments during the 2017/18 summer season

Hybrid Code	Potch Low N	Potch Opt	Ced Low N	Ced Opt	Val Opt
G1	3.24	7.39	4.75	6.20	10.08
G2	3.30	6.16	4.54	4.34	9.69
G3	3.24	8.58	5.15	4.79	7.80
G4	3.58	6.20	4.48	6.90	8.44
G5	3.21	7.69	5.26	5.79	9.11
G6	3.02	7.69	4.51	4.58	11.36
G7	2.95	4.71	4.53	4.58	8.61
G8	3.23	8.49	3.58	4.38	8.25
G9	3.80	7.27	5.16	3.89	11.43
G10	3.11	7.37	3.76	5.77	9.00
G11	4.92	8.31	3.64	3.76	7.71
G12	4.54	9.55	5.33	6.93	8.12
G13	3.43	6.95	4.43	5.48	11.08
G14	2.92	7.53	4.09	5.11	8.33
G15	3.57	6.10	5.26	6.06	9.04
G16	3.97	7.50	4.41	4.62	6.45
G17	3.78	7.92	4.59	5.76	7.41
G18	4.64	10.67	5.10	4.36	11.86
G19	4.37	6.69	5.16	5.71	8.24
G20	4.27	5.42	4.20	5.90	11.32
G21	4.60	8.55	4.19	5.27	6.61
G22	2.60	6.03	4.92	5.32	8.06
G23	4.23	7.53	3.97	5.57	9.87
G24	3.95	10.29	4.46	6.56	9.81
G25	3.81	9.53	4.18	6.91	6.51
G26	4.04	10.04	5.18	5.51	7.66
G27	4.20	6.65	4.35	5.48	5.32
G28	3.65	8.15	4.77	5.99	9.21
G29	3.98	6.39	4.34	5.75	4.41
G30	3.22	8.08	4.04	4.93	3.46
G31	2.86	5.73	3.78	5.58	4.15
G32	4.04	8.09	2.73	6.54	8.93
G33	4.46	9.07	4.21	5.88	10.22
G34	2.40	10.50	2.82	4.82	8.09
G35	2.49	6.89	5.00	4.12	8.17
G36	3.11	7.94	2.40	2.10	6.81
G37	3.22	8.56	4.50	6.11	6.67
G38	1.79	5.25	1.32	1.33	1.00

Hybrid Code	Potch Low N	Potch Opt	Ced Low N	Ced Opt	Val Opt
G39	4.16	7.10	4.68	5.87	6.36
G40	4.18	7.07	4.60	7.18	5.37
G41	4.37	8.43	3.44	6.27	6.20
G42	4.16	8.12	4.79	5.16	9.39
G43	4.28	7.21	4.42	5.22	6.82
G44	4.76	6.60	4.74	5.30	6.03
G45	4.66	6.49	4.72	5.23	9.57
G46	4.26	9.58	3.70	6.47	9.82
G47	2.84	7.65	3.56	4.74	6.93
G48	4.49	7.68	3.31	5.96	10.43
G49	3.23	7.15	4.98	5.18	6.67
G50	2.53	8.84	4.08	5.24	5.16
G51	3.31	6.78	3.75	4.52	8.17
G52	4.40	7.15	4.61	5.68	4.97
G53	3.06	7.06	4.83	4.98	8.90
G54	4.60	6.98	4.55	7.03	5.45
G55	4.40	8.36	5.28	6.88	5.51
G56	3.70	8.10	4.57	4.95	5.58
G57	2.76	8.55	4.05	4.41	7.18
G58	4.53	7.31	3.33	4.76	6.58
G59	3.98	10.37	3.57	5.72	4.61
G60	3.83	7.73	3.03	5.47	9.49
G61	4.22	7.05	3.30	5.06	9.37
G62	4.92	7.62	4.82	5.59	6.80
G63	2.60	6.74	2.97	5.80	8.36
G64	3.02	8.01	4.34	5.46	7.34
G65	3.83	8.41	3.66	5.61	9.77
G66	3.74	8.59	4.28	5.78	10.38
G67	4.06	6.84	4.41	5.40	7.71
G68	4.11	5.08	5.18	5.71	8.53
G69	4.51	7.78	3.90	5.83	7.28
G70	3.35	7.09	4.89	5.73	8.06
G71	3.35	7.39	4.02	5.22	7.72
G72	3.10	7.11	3.22	4.65	6.35
G73	2.24	7.46	3.89	4.59	7.48
G74	4.54	6.46	4.27	5.84	5.69
G75	4.33	7.81	3.38	3.88	6.93
G76	2.59	7.42	3.99	3.68	4.20
G77	3.21	6.84	5.10	7.23	5.16
G78	1.77	7.27	3.19	4.80	7.42
G79	4.52	8.15	3.37	5.57	4.77
G80	4.36	8.27	4.37	5.81	7.61
G81	3.54	6.91	4.45	5.23	8.01

Hybrid Code	Potch Low N	Potch Opt	Ced Low N	Ced Opt	Val Opt
G82	4.18	8.13	4.62	5.33	7.94
G83	3.73	8.59	5.10	4.56	6.19
G84	3.32	7.93	3.88	5.85	8.98
G85	3.60	9.56	4.07	5.57	5.80
G86	3.17	8.27	5.17	4.49	7.53
G87	3.91	7.56	3.39	4.44	8.18
G88	2.93	1.45	5.31	0.48	6.25
G89	2.49	5.85	3.98	4.47	6.46
G90	4.18	7.66	4.66	5.48	7.75
G91	3.37	8.93	3.82	6.44	5.90
G92	3.84	9.86	4.57	4.16	7.46
G93	4.02	7.96	4.02	6.01	7.40
G94	2.83	7.07	3.48	4.19	7.59
G95	2.95	7.09	4.33	4.68	5.44
G96	3.55	13.38	3.79	4.54	6.05
G97	2.82	7.11	4.73	4.39	5.22
G98	3.30	7.46	4.14	6.46	4.73
G99	4.18	7.28	5.14	5.37	8.75
G100	4.34	12.04	3.97	6.50	6.25
G101	4.58	9.40	5.01	5.47	8.69
G102	4.72	8.81	4.70	5.41	7.44
G103	2.02	9.12	3.25	5.67	6.64
G104	3.73	6.94	4.48	5.39	5.44
G105	4.66	6.42	4.30	3.45	7.11
G106	2.65	7.33	3.99	5.29	6.51
G107	3.36	7.55	3.22	3.27	4.59
G108	3.45	4.16	3.12	4.62	3.15
G109	3.60	6.78	5.18	5.49	2.28
G110	4.33	8.44	4.27	6.13	5.57
G111	3.57	5.92	4.51	6.57	6.71
G112	3.44	5.24	4.20	5.11	8.10
G113	3.27	8.63	4.67	6.57	7.88
G114	2.86	7.02	4.45	5.16	9.70
G115	4.41	6.33	5.04	4.82	8.47
G116	3.85	6.73	3.37	5.13	10.28
G117	3.98	7.40	5.16	5.38	8.57
G118	3.35	5.51	4.91	6.19	6.42
G119	4.14	6.59	2.75	4.10	8.27
G120	2.68	6.31	3.93	5.09	5.41
G121	4.22	9.09	4.09	5.40	8.84
G122	2.23	5.16	2.14	2.27	2.35
G123	4.14	8.17	3.49	5.64	7.36
G124	3.60	6.79	3.86	6.30	5.43

Hybrid Code	Potch Low N	Potch Opt	Ced Low N	Ced Opt	Val Opt
G125	4.38	7.02	3.43	5.80	6.27
G126	4.22	8.66	3.77	4.51	4.24
G127	4.39	8.61	3.86	5.16	7.11
G128	3.29	8.72	4.76	5.73	6.57
G129	3.69	5.61	4.43	4.53	7.12
G130	4.72	7.16	4.13	5.23	6.37
G131	3.34	5.29	3.41	3.42	6.66
G132	2.08	6.97	3.75	4.89	8.66
G133	4.39	9.28	5.31	6.83	8.42
G134	3.60	10.50	3.93	5.94	7.66
G135	3.53	8.64	5.13	5.67	7.27
G136	3.70	8.75	4.33	4.83	5.51
G137	3.78	8.31	3.62	5.85	9.95
G138	3.23	7.34	4.02	6.00	7.09
G139	3.10	6.86	4.32	4.83	7.88
G140	3.53	8.56	4.51	5.35	6.54
G141	3.71	7.54	3.88	5.30	8.53
G142	5.03	8.54	4.32	4.86	9.59
G143	2.14	8.07	2.99	5.08	4.36
G144	2.95	7.05	3.89	5.63	3.14
G145	3.43	5.32	3.79	5.28	7.54
G146	4.26	6.92	5.12	5.55	5.50
G147	3.83	8.11	3.04	4.77	8.67
G148	4.01	5.20	5.03	5.72	9.50
G149	1.85	5.03	4.92	4.03	3.85
G150	4.44	7.93	4.27	4.58	8.39
G151	4.16	6.61	3.70	6.10	5.00
G152	4.47	6.61	3.11	4.18	3.57
G153	3.13	7.49	5.00	5.63	3.53
G154	4.46	7.29	3.47	6.01	7.89
G155	3.01	7.49	3.28	5.48	8.84
G156	2.85	6.30	4.28	4.62	7.13
G157	3.23	7.38	4.28	5.11	7.08
G158	3.01	5.01	4.36	4.77	10.03
G159	4.07	8.17	3.30	5.13	8.12
G160	2.47	7.90	5.06	5.51	9.45
G161	3.76	7.77	3.22	4.60	5.35
G162	3.87	8.05	3.12	6.93	4.48
G163	3.22	7.52	3.49	6.18	9.31
G164	3.33	7.03	0.26	0.47	0.58
G165	4.33	5.44	2.58	5.02	6.05
G166	2.85	7.74	3.73	6.17	7.92
G167	1.67	6.75	4.43	5.57	6.13

Hybrid Code	Potch Low N	Potch Opt	Ced Low N	Ced Opt	Val Opt
G168	4.21	7.63	4.75	5.79	8.05
G169	4.37	8.67	4.84	5.01	8.91
G170	3.14	8.24	3.71	4.11	7.90
MIN	1.67	1.45	0.26	0.47	0.58
MAX	5.03	13.38	5.33	7.23	11.86
MEAN	3.62	7.53	4.13	5.23	7.22
CV (%)	4.86	10.53	15.25	12.12	12.26
SE	0.1761	0.7926	0.6304	0.6333	0.885
LSD $_{(0.05)}$	0.3492	1.572	1.25	1.256	1.755
P-VALUE	<.001	<.001	<.001	<.001	<.001

CHAPTER 4

GENOTYPE BY ENVIRONMENT INTERACTION AND STABILITY ANALYSIS OF MAIZE HYBRIDS ACROSS LOW-NITROGEN AND OPTIMUM ENVIRONMENTS

Abstract

Genotype by environment interactions (GEI) complicate interpretation of multi-locational trial results and prediction of genotype performance across environments. Therefore, this study sought to determine the extent of GEI on the grain yield performance of 170 single-cross maize hybrids evaluated across five environments that comprised of low N and optimum conditions, at three locations in South Africa (SA) using the additive main effects and multiplicative interaction (AMMI) and genotype plus genotype by environment (GGE) biplot analyses. The locations were; Potchefstroom and Cedara both having low N and optimum environments and Vaalharts with only optimum environment. The results revealed that variances due to environment, genotype and GEI were all highly significant ($P<0.001$). Based on the calculated AMMI stability values and GGE biplot analyses, hybrids G142 (I-42/CML544), G26 (CK21/CML444), G160 (U2540W/CKDHL0378), G12 (CB399/CML442) and G102 (CML544/I-42) were the most stable. Hybrids G18 (CK21/CKDHL0089), G12 (CB399/CML442), G134 (I-42/CKDHL0295), G24 (CK21/CML216), G102 (CML544/1-42), G33 (CKDHL0089/CML442), G101 (CML544/I-38), G46 (CKDHL0470/CK21) and G26 (CK21/CML444) emerged as the high yielding hybrids in each specific environment. The following hybrids were considered high yielding and stable across the environments; G134 (I-42/CKDHL0295), G12 (CB399/CML442), G24 (CK21/CML216), G33 (CKDHL0089/CML442) and G102 (CML544/I-42) with higher mean grain yield of 6.32 t ha^{-1}, 6.89 t ha^{-1}, 7.01 t ha^{-1}, 6.77 t ha^{-1} and 6.22 t ha^{-1}, respectively, all above the grand mean of 5.55 t ha^{-1}. These five hybrids have broad adaptation since they are stable and high yielding across environments, and are thus potential candidates for further evaluation and possible release in South Africa.

Key Words: AMMI, GEI, GGE biplot, grain yield, maize, stability,

4.1 Introduction

Maize is the main staple food crop for the majority of countries within the sub-Saharan Africa (SSA) region. The crop serves as a valuable source of several by-products across the continent (Khodarahmpour and Shoushtar, 2011). However, its production is hampered by biotic, abiotic and socio-economic factors leading to variation in grain yield. Among the biotic factors there are drought and soil fertility decline, especially low nitrogen (N) (Edmeades *et al*., 2006). Additional to these factors, the occurrence of genotype x environment interaction (GEI) and low stability of genotypes across locations pose an important constraint that limits farmers from achieving maximum performance of the released cultivars. A general breeding scheme for maize involves evaluation of a large number of genotypes in the early stages followed by multi-location trials for a few selected genotypes. The multi-locational testing reveals GEI that complicates the interpretation of yield performance and reduces selection efficiency for the best genotypes (Annicchiarico and Perenzin, 1994). The analysis of GEI, therefore, becomes an important tool to evaluate genotype performance across environments and selecting parents for breeding programmes.

The performance of the genotype is due to the interaction between the genotype and the environment. The existence of GEI confirms that these two effects are not always additive (Purchase, 1997). Yan and Hunt (2001) termed GEI as the nature of variation that cannot be explained by the genotype main effect or the environment main effect only. Genotype by environment interaction results in inconsistent genotypic expression across environments and diminishes the association between phenotypic and genotypic values causing better selections in one environment that have poor performance in another environment. According to Blanche (2005), the term environment encompasses all the biotic and abiotic factors that have influence on growth and development of a particular plant at that location. These factors include weather related factors such as, planting date, plant stand, disease pressure, soil type and management factors such as irrigation, fertility, use of plant growth regulators, weed control pressure and practices, insect pressure and control (Kerby *et al.,* 2000).

Assessment of GEI is critical in determining the best breeding approach for developing genotypes adapted to the target environments. There are different methods used to evaluate GEI selected according to the plant breeders' view, though adequate knowledge in statistics and biometry is required to select the best stability model for a particular study. Among the multivariate stability analysis models, AMMI and GGE biplot have proved to be more valid and are widely used in assessing GEI due to their complimentary role to each other. AMMI is capable of capturing large portion of the interaction sum of squares while separating the main

as well as interaction effects and shows which genotype will be suitable for which environment (Jeberson *et al.,* 2017). Additionally, GGE biplot is effective in identifying the best performing cultivars across environments, identifying the best cultivars for mega-environments differentiation and assessing the yield stability of the cultivars (Yan *et al.,* 2000; Yan and Kang, 2003). Therefore, these models were used to investigate GEI on grain yield and analyse yield stability among the 170 experimental single-cross maize hybrids across stress and non-stress environments.

4.2 Materials and methods

4.2.1 Germplasm

The list of germplasm used in this study is presented in Appendix 3.1 of Chapter 3. The planting materials comprised of 167 experimental maize single-cross hybrids developed by Agricultural Research Council-Grain Crops Institute (ARC-GCI) and three local commercial check varieties released under the Water Efficient Maize for Africa (WEMA) project. All the maize hybrids used in this study were bred for drought and low N stress tolerance.

4.2.2 Experimental sites, field trial design and management

The same experimental sites and characterization of environmental conditions, field trial design and management presented in Chapter 3 subsection 3.1.2 were used for this study.

4.2.3 Data collection

Grain yield (GY) data were collected for each plot individually in all the five environments. The weight of grain per plot after shelling was determined and used to calculate GY per hectare adjusted to 12.5% moisture content, as follows

$$GY = GW \times \frac{100 - MC}{100 - 12.5} \times \frac{1000}{Plotarea}$$

Where, GW = grain weight (kg plot^{-1}) after shelling, MC = percentage grain moisture content of the shelled grain.

4.2.4 Statistical analyses

4.2.4.1 Analysis of variance

To determine the effects of GEI on grain yield, data were first subjected to analysis of variance using GenStat software 18thth edition. Analyses were done for across the five environments

and in each of the environments to test the levels of significance. The following ANOVA model was used for the combined analysis across environments:

$$Y_{ijkl} = \mu + r_j + B_K + S_l + H_i + SH_{il} + \varepsilon_{ijkl}$$

Where, Y_{ijkl} = the individual observation in each plot; μ = overall mean or grand mean; r_j = effect of the j[th] replication; B_k = effect of the k[th] block nested in j[th] replication and k = 1,2,3…34, while j = 1,2; s_l = the effect the l [th] environment and l =1,2,3,4,5; H_i = the effect of the i [th] hybrid and i=1,2,3…170; SH_{il} = interaction effect of the i [th] hybrid and l [th] environment, and ε_{ijkl} = random error. The least significant difference at 5% level of significance was used for mean separation. The hybrid means were ranked according to grain yield as the principal selection criterion at all sites.

For the single site ANOVA, the model was as follows:

$$Y_{ijk} = \mu + H_i + r_j + B_{K(j)} + \varepsilon_{ijk}$$

Where, Y_{ijk} = the individual observation in each plot; μ = grand mean for each variable; H_i = the effect of the i [th] hybrid and i =1,2,3…170; r_j = effect of the j[th] replication and j = 1,2; $B_{k(j)}$ = effect of the k[th] incomplete block within a replication and k = 1,2 … 34; and ε_{ijk} = overall random error effect.

4.2.4.2 AMMI analysis of variance

The AMMI statistical model adopted from Gauch and Zobel (1989) was used to analyse the grain yield data across the five environments in GenStat 18th Edition, as follows:

$$Y_{ger} = \mu + \alpha_g + \beta_e + \sum \lambda_n Y_{gn} \delta_{en} + \rho_{ge} + \varepsilon_{ger}$$

Where; Y_{ger} = Yield of genotype *g* in environment *e* for replicate *r,* μ = Grand mean, α_g = Genotype mean deviations (genotype means minus grand mean), β_e = Environment mean deviation, n= Number of PCA axes retained in the model, λ_n =Singular value for PCA axis n,

Y_{gn} = Genotype eigenvector values for PCA axis n, δ_{en} = Environment eigenvector values for PCA axis n, ρ_{ge} = Residuals and ε_{er} = Error term

4.2.4.3 AMMI stability values (ASV)

The ASV parameter measures the relative stability of each genotype in each environment and across environments (Dagnachew *et al*., 2014). This parameter was calculated according to the formula suggested by Purchase (1997). The ASV is the distance of interaction principal component axis (IPCA) from coordinate point to the origin in a two dimensional plot of IPCA1 against IPCA2 scores in the AMMI model. Because the IPCA1 contributes more to the GXE interaction sum of squares, then a weighted value has to be estimated for each genotype and environment according to the relative contributions of the first two IPCAs. The following formula was used in the calculation of AMMI stability value (ASV):

$$ASV = \sqrt{\left[\frac{SSPCA1}{SSPCA2}(IPCA1scores)\right]^2 + (IPCA2scores)^2}$$

Where $\frac{SSPCA1}{SSPCA2}$ = weight assigned to the first interaction principal component score due to its high contribution in the GxE model.

The larger the ASV value in either direction (positive or negative), the more specifically adapted the genotype to a certain environment. Smaller ASV indicates a more stable genotype across environments (Purchase, 1997; Dagnachew *et al*., 2014).

4.2.4.4 GGE biplot

The GGE biplot analysis was performed using R statistical package GEAR R version 4.0 (Pacheco *et al*., 2015). It was used to generate graphs showing (i) “which-won-where”, (ii) discriminativeness versus representativeness of the environment, (iii) relationships among the test environments and (iv) means versus stability of hybrids (Yan and Kang, 2003). The GGE biplot represents the first two principal components (PC1 and PC2, referred as primary and secondary effects, respectively) derived from subjecting environment centered yield data (yield variation due to GGE), to singular value decomposition (Yan *et al.,* 2000).

The following model adopted from Yan (2000) for GGE biplot analysis was used:

$$\frac{Y_{ij-}Y_j}{S_j} = \lambda_1 \xi_{i1} \eta_{j1} + \lambda_2 \xi_{i2} \eta_{j2} + \varepsilon_{ij}$$

Where, Y_{ij} = the average yield of genotype i in the j[th] environment; Y_j = the average yield of all genotypes in environment j; S_j = the standard deviation in environment j; λ_1 and λ_2 = the singular values for PC1 and PC2; ξ_{i1} and ξ_{i2} = PC1 and PC2 scores, for genotype i; η_{j1} and η_{j2} = PC1 and PC2 scores, for environment j; and ε_{ij} = the residual of model associated with the genotype i in the environment j. The data were not transformed but standardised and environment centered.

4.3 Results

4.3.1 Combined analysis of variance for grain yield

Analysis of variance for grain yield detected highly significant (P<0.001) differences for all the sources of variation including environment, genotype and GEI (Table 5.1). For individual environments, ANOVA detected highly significant (P<0.001) effects among the experimental hybrids across all the tested environments (Table 5.2). Under combined analysis, the contributions of environment, genotype and GEI to the total grain yield variation (G+ GE + GEI) were 57%, 11%, 18%, respectively. The grand mean yield was 5.55 t ha^{-1} and coefficient of variation (CV) for all five environments across three locations was 12.7 %.

Table 4.1 Mean squares from combined ANOVA for grain yied across five environments.

Source of Variation	**DF**	**SS**	**MS**
Environment	4	4260.65	1065.16***
Environment. Replication	5	7.57	1.51**
Environment. Replication. IB	330	737.04	2.23***
Genotype	169	822.28	4.87***
Environment. Genotype	676	1405.60	2.08***
Residual	515	230.82	0.45
Total	1699	7463.95	4.39
Yield Mean (t ha $^{-1}$)	5.55		
CV (%)	12.07		

DF=degrees of freedom, SS= sum of squares, **, *** indicate level of significance of the data at P=0.01 and P=0.001, respectively

4.3.2 Analysis of variance for grain yield in individual environments

Table 4.2 Mean squares from ANOVA for grain yield in individual environments

Sources	DF	Potch (Low N)	Potch (Opt)	Cedara (Low N)	Cedara (Opt)	Vaalharts (Opt)
Replication	1	0.00	0.74	0.97	1.61*	4.24*
Replication. IB	66	0.59***	2.76***	1.14***	0.86***	5.82***
Genotype	169	0.84***	3.18***	0.96***	1.96***	6.24***
Residual	103	0.03	0.63	0.40	0.40	0.78
GY Mean (t ha^{-1})		3.62	7.53	4.13	5.23	7.22
CV (%)		4.86	10.53	15.25	12.12	12.26

DF=degrees of freedom, *, *** indicate level of significance of the data at P=0.05 and P=0.001, respectively, Potch= Potchefstroom

4.3.3 Mean grain yield of hybrids in individual environments

The selected top 40 hybrids across all the five environments in terms of grain yield are presented in Table 5.3 and the list for all the 170 hybrids is on Appendix 4.2. The hybrids performed differently in each environment. The best performing hybrids in each environment were CML543/I-38-G96 (13.38 t ha^{-1}) for E1, CB339/CML442-G12 (6.93 t ha^{-1}) for E2, CK21/CKDHL0089-G18 (11.86 t ha^{-1}) for E3, I-42/CML544-G142 (5.03 t ha^{-1}) for E4 and CB339/CML442-G12 (5.33 t ha^{-1}) for E5. Bolded and underlined grain yield denotes highest yielder in that particular environment.

Table 4.3 Mean grain yield (t ha^{-1}) among the selected top 40 hybrids in each environnment

Location Hybrid Code	E4 (Low N)	E1 (Optimum)	E5 (Low N)	E2 (Optimum)	E3 (Optimum)	Across all Environments
G18	4.63	10.67	5.10	4.36	**11.86**	7.33
G24	3.95	10.29	4.46	6.56	9.81	7.01
G12	4.54	9.55	**5.33**	**6.93**	8.12	6.89
G133	4.39	9.28	5.31	6.83	8.42	6.85
G33	4.46	9.07	4.21	5.88	10.22	6.77
G46	4.26	9.58	3.70	6.47	9.82	6.76
G101	4.58	9.4	5.01	5.47	8.69	6.63

Location Hybrid Code	E4 (Low N)	E1 (Optimum)	E5 (Low N)	E2 (Optimum)	E3 (Optimum)	Across all Environments
G100	4.34	12.04	3.97	6.50	6.25	6.62
G66	3.74	8.59	4.28	5.78	10.38	6.55
G26	4.04	10.04	5.18	5.51	7.66	6.49
G142	**5.03**	8.54	4.32	4.86	9.59	6.47
G48	4.49	7.68	3.31	5.96	10.43	6.37
G169	4.37	8.67	4.84	5.01	8.91	6.36
G28	3.65	8.15	4.77	5.99	9.21	6.35
G1	3.24	7.39	4.75	6.20	10.08	6.33
G121	4.22	9.09	4.09	5.40	8.84	6.33
G134	3.60	10.5	3.93	5.93	7.66	6.32
G42	4.16	8.115	4.79	5.16	9.39	6.32
G9	3.80	7.27	5.16	3.90	11.43	6.31
G137	3.78	8.31	3.62	5.85	9.95	6.30
G13	3.43	6.95	4.43	5.48	11.08	6.27
G96	3.55	**13.38**	3.79	4.54	6.05	6.26
G65	3.83	8.41	3.66	5.61	9.77	6.25
G23	4.23	7.53	3.97	5.57	9.87	6.23
G6	3.02	7.69	4.51	4.58	11.36	6.23
G20	4.27	5.42	4.20	5.90	11.32	6.22
G102	4.72	8.81	4.70	5.41	7.44	6.22
G5	3.21	7.69	5.26	5.79	9.11	6.21
G113	3.27	8.63	4.67	6.57	7.88	6.20
G25	3.81	9.53	4.18	6.91	6.51	6.19
G99	4.18	7.28	5.14	5.37	8.75	6.14
G45	4.66	6.49	4.72	5.23	9.57	6.13
G117	3.98	7.40	5.16	5.38	8.57	6.10
G168	4.21	7.63	4.75	5.79	8.05	6.09
G55	4.40	8.36	5.28	6.88	5.51	6.08
G80	4.36	8.27	4.37	5.81	7.61	6.08
G160	2.47	7.90	5.06	5.51	9.45	6.08
G32	4.04	8.09	2.73	6.54	8.93	6.07
G135	3.53	8.64	5.13	5.67	7.27	6.05
G82	4.18	8.13	4.62	5.33	7.94	6.04
Mean Yield (t ha^{-1})	**3.62**	**7.53**	**4.13**	**5.23**	**7.22**	**5.55**

NB: Bolded and underlined denote highest yield in that environment, E= Environment, E1= Potchefstroom Optimum, E2= Cedara optimum, E3= Vaalharts optimum, E4= Potchefstroom low N, and E5= Cedara low N. Bolded genotypes are the commercial checks

4.3.4 AMMI analysis

The additive main effect and multiplicative interaction (AMMI) analysis of variance revealed significant effects for genotypes, environment and the GEI (Table 5.4). Partitioning of the variance components showed that 13.45% of the total variation was contributed by the genotype, 60.35% was due to environment, 0.11% was due to block and GEI contributed 26.20% of the total variation.

The four interaction principal component axis (IPCA1, IPCA2, IPCA3 and IPCA4) were highly significant ($p<0.001$). These IPCA's contributed 54.86%, 26.27%, 11.03% and 7.84% respectively, to the total interaction sum of squares and cumulatively they contributed 100% of the total genotype by environment interaction sum of squares.

Table 4.4 AMMI analysis for grain yield aross fiveenvironments

Source of Variation	**DF**	**SS**	**MS**	**Total Variation (%)**	**GE Explained (%)**	**GE Cumulative (%)**
Total	1699	7464	4.39			
Treatments	849	7061	8.32***			
Genotypes	169	950	5.62***	13.45		
Environments	4	4261	1065.16***	60.35		
Replications within Environments	5	8	1.51**	0.11		
GE	676	1850	2.74***	26.20		
IPCA 1	172	1015	5.9***		54.86	54.86
IPCA 2	170	486	2.86***		26.27	81.14
IPCA 3	168	204	1.21***		11.03	92.16
IPCA 4	166	145	0.87***		7.84	100.00
Error	845	395	0.47			

, * Significant at P=0.01 and P=0.001, respectively, DF = Degrees of freedom, SS = Sum of squares, MS = Mean sum of squares, GE = Genotype x Environment interaction, IPCA 1 = Interaction principal component axis 1, IPCA 2 = Interaction principal component axis 2, IPCA 3 = Interaction principal component 3, IPCA 4 = Interaction principal component 4

4.3.4.1 Mean grain yield and AMMI stability values for selected hybrids

Mean grain yield of all the tested genotypes ranged from 2.14 t ha^{-1} for G38 to 7.33 t ha^{-1} for G18 with a grand mean yield of 5.55 t ha^{-1} (Table 4.6 and Appendix 4.1). Fifty-five percent of the hybrids evaluated performed above the grand mean of 5.55 t ha^{-1} and the selected (top) 40 genotypes had a grain mean value above average. Additive main effect and multiplicative interaction stability value (ASV) for the hybrids ranged from 0.02 for G158 to 1.92 for G110 (Table 4.6 and Appendix 4.1).

Table 4.5 Mean grain yield (t ha^{-1}), first, second, third and fourth IPCA scores and ASV of top 40 hybrids

Hybrid	Mean GY	IPCAg1	IPCAg2	IPCAg3	IPCAg4	ASV
G1	6.33	-0.50	-0.02	0.29	0.12	1.04
G100	6.62	-0.25	-0.14	-0.12	0.09	0.54
G101	6.62	0.61	0.76	0.16	-0.05	1.49
G102	6.22	-0.06	0.27	-0.18	0.07	0.29
G113	6.20	-0.35	-0.42	0.02	-0.06	0.84
G117	6.10	-0.64	0.04	0.02	-0.31	1.34
G12	6.89	0.13	0.13	0.17	0.07	0.30
G121	6.33	0.19	-0.23	0.14	0.12	0.46
G13	6.27	-0.75	0.01	0.11	0.02	1.56
G133	6.85	-0.40	0.11	0.23	0.21	0.84
G134	6.32	0.04	0.11	0.18	0.08	0.14
G135	6.05	0.18	0.59	0.18	0.04	0.70
G137	6.30	0.40	0.18	-0.15	0.15	0.86
G142	6.47	-0.24	0.0741	0.03	-0.10	0.51
G160	6.08	-0.12	0.24	-0.04	-0.28	0.35
G168	**6.09**	**0.08**	**-0.14**	**0.41**	**0.43**	**0.22**
G169	**6.36**	**-0.08**	**-0.09**	**-0.01**	**-0.02**	**0.20**
G18	7.32	-0.59	0.72	-0.45	0.20	1.42
G20	6.22	-0.87	-0.35	0.09	-0.33	1.84
G23	6.23	-0.46	0.07	0.01	-0.23	0.95
G24	7.01	-0.19	0.52	0.25	0.02	0.65
G25	6.19	0.37	0.24	0.36	-0.05	0.81
G26	6.49	0.17	0.36	-0.09	0.27	0.51
G28	6.35	-0.27	0.08	0.15	0.10	0.57
G32	6.07	-0.23	0.22	0.36	-0.54	0.53
G33	6.76	-0.37	0.33	0.02	-0.17	0.84
G42	6.32	-0.32	0.11	-0.15	0.06	0.68
G45	6.13	-0.46	-0.24	-0.20	-0.16	0.99
G46	6.76	-0.25	0.44	0.23	-0.27	0.69
G48	6.37	-0.53	0.15	0.11	-0.48	1.13

Hybrid	Mean GY	IPCAg1	IPCAg2	IPCAg3	IPCAg4	ASV
G5	6.21	-0.30	-0.03	0.15	0.311	0.63
G55	6.09	0.51	-0.20	0.17	0.06	1.08
G6	6.23	-0.80	0.25	-0.05	0.22	1.68
G65	6.25	-0.39	0.30	0.105	-0.18	0.86
G66	6.56	-0.47	0.28	0.12	-0.02	1.019
G80	6.08	0.54	0.03	-0.04	-0.35	1.12
G82	6.04	-0.19	-0.12	0.01	0.05	0.42
G9	6.31	-0.83	0.09	-0.40	0.26	1.73
G96	6.26	0.25	-0.10	-0.04	0.23	0.53
G99	6.14	0.50	-0.19	0.35	0.00	1.05
Grand Mean Yield	5.5 t ha^{-1}					

IPCAg [1] = Interaction principal component axis for genotype scores 1, IPCAg [2] = Interaction principal component axis for genotype scores 2, IPCAg [3] = Interaction principal component for genotype scores 3, ASV = AMMI stability value, Mean GY = Mean grain yield. Bolded genotypes are the commercial checks

4.3.4.2 Mean yield and AMMI stability values for the test environments

Mean yield for individual environments ranged from 3.62 t ha^{-1} for E4 to 7.53 t ha^{-1} for E1 (Table 4.6). Two environments (E1 and E3) recorded mean yield above the grand mean of 5.55 t ha^{-1}. Environment E4 had lowest mean yield and E1 was recorded as the highest yielding environment.

Table 4.6 Mean yield, first, second, third and fourth IPCA scores of environments

Environment	Mean GY ($th^{-1)}$	IPCAe1	IPCAe2	IPCAe3	IPCAe4
E1	7.53	1.76	3.16	-0.07	0.43
E2	5.23	0.98	-1.11	2.52	-0.66
E3	7.22	-4.17	0.65	0.07	-0.16
E4	3.62	0.92	-0.95	-1.80	-1.81
E5	4.13	0.51	-1.75	-0.72	2.15

E1= Potchefstroom optimum, E2= Cedara optimum, E3= Vaalharts optimum, E4= Potchefstroom low N and E5= Cedara low N

4.3.4.3 Best four hybrid selections from AMMI per environment

The best four hybrids per environment (Table 4.7) were identified using the AMMI analysis Hybrid 18 (CK21/CKDHLOO89) was the best in E4 and E3, and ranked third in E1. Hybrids G97 (CML543/RO549W), G78 (CML443/CML442) and G69 (CML442/CML216) performed best in environments E1, E2 and E5, respectively.

Table 4.7 Top four hybrids for each environment

			Genotype Ranking per environment			
Environment	Mean GY (t ha^{-1})	Score	1	2	3	4
E1	7.53	1.76	G97	G101	G18	G135
E2	5.22	0.98	G78	G40	G12	G55
E4	3.62	0.92	G18	G102	G62	G143
E5	4.13	0.51	G69	G12	G55	G19
E3	7.22	-4.17	G18	G9	G6	G20

GY= Grain yield. Environments are described in Table 4.6

4.3.5 GGE biplot analysis

For better visualisation and decision-making, GGE biplot analyses were done for the best 40 hybrids presented in Table 4.5 for AMMI stability values. The results revealed that the first two principal components explained a total of 59.46% (PC1= 34.27% and PC2= 25.19%) of the total variation based on the standardised model.

4.3.5.1 'Which-won-where' biplot polygon view

The polygon view of the GGE biplot (Figure 4.1) was formed by connecting the hybrid markers for G18 (CK21/CKDHL0089), G12 (CB339/CML442), G101 (CML544/I-38), G99 (CML544/CK21), G108 (CZL068/CK21), G133 (I-42/CB339) and G9 (CB323/RO549W) which appeared furthest away from the biplot origin to the rest of the genotypes contained within the polygon. There were seven rays, which divided the biplot into seven sectors. According to Kaya *et al.* (2006) rays are the perpendicular lines to the side of the polygon formed in the plot. The five environments fell into two of these seven sectors with environments E1 and E2 within the same sector while environments E3, E5 and E4 fell within the other sector. The vertex genotype for each quadrant denotes the genotype that gave the highest grain yield for the environments that fell within that quadrant. The highest yielding hybrids in environments E1 and E2 are G101 (CML544/I-38) and G12 (CB339/CML442), and for environments E3, E5 and E4 the best hybrids was G18 (CK21/CKDHL0089). Hybrids G99 (CML544/CK21), G121

(I-38/CML202) G168 (local check), G133 (I-42/CB339) and G9 (CB323/RO549W) performed poorly in all the five test environments.

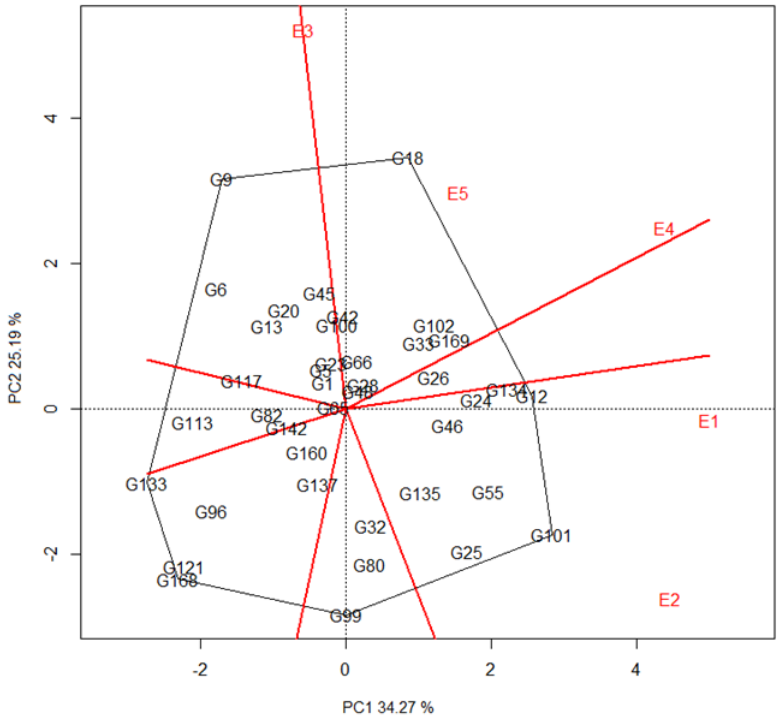

Figure 4.1 Polygon view of “which-won-where” for standardised data of 40 single cross maize hybrids. Environments are described in Table 4.6

4.3.5.2 Discriminating ability vs representativeness of environments

Environment vectors were drawn from biplot origin to connect the environment markers (Figure 4.2). In terms of discriminating ability, the longer the environment vector from the biplot origin to the environment marker, the more discriminating and informative the environment for the hybrids tested. Environment E3 had the longest vector, thus highly discriminating of the hybrids. Environments E4, E1 and E2 were almost the same in terms of discriminating ability, having moderately long vectors from the biplot origin and environment. Environment E5 exhibited the shortest vector. The distance between two environments measures their similarity or dissimilarity in discriminating the genotypes (Yan and Tinker, 2006). Environments E5 and E4 had the least distance between them, while the greatest distance was observed between E3 and E2. In terms of the test environment representativeness, an Average Environment Axis (AEC) was used. The average environment is denoted by small circle at the

end of the arrow and has the average coordinates of all test environments. On the other hand, AEC is the line that passes through the average environment and the biplot origin. The test environment with small angle with the AEC is more representative of other test environments. Therefore, E4 was the most representative followed by E5 and E1 while E3 and E2 were the least representative.

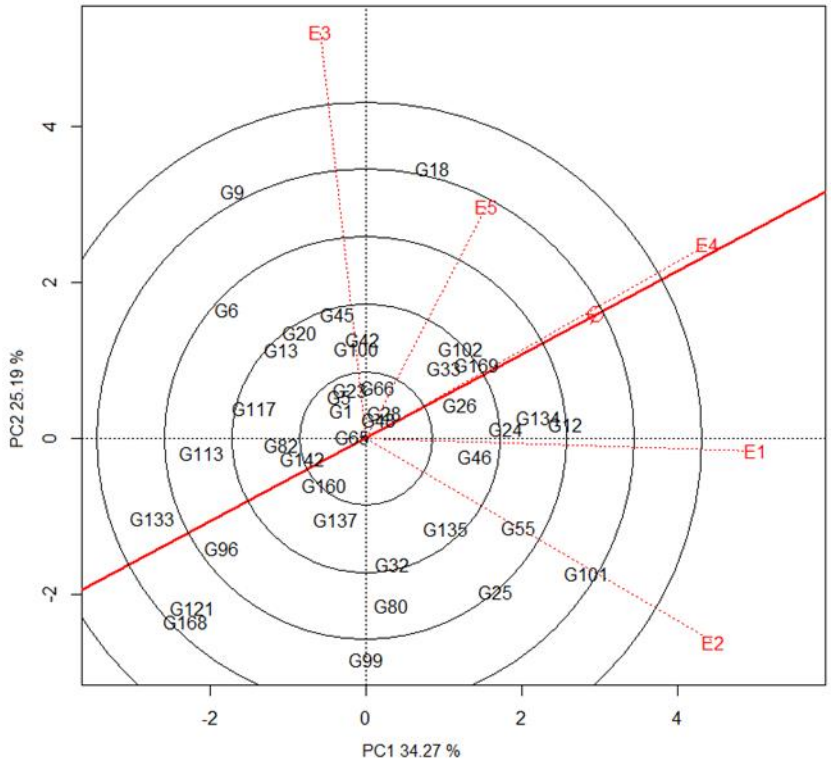

Figure 4.2 Environmental ranking based on discriminating ability and representativeness. Environments are described in Table 4.6

4.3.5.3 Mean yield performance and stability

Mean versus stability biplot (Figure 4.3) were used to investigate the performance and stability of the tested hybrids across the five environments. The single arrowed line is the average environment coordinate (AEC) and it points towards higher mean yield across the environments. The AEC ordinate divided the hybrids into two groups, the one above it and the other group below the AEC. The hybrids on the above side and close to the circle are the best performing hybrids with respect to mean grain yield and these are; G18 (CK21/CKDHL0089),

followed by G12 (CB339/CML442) and G134 (I-42/CKDHL0295). Hybrids G20 (CK21/CKDHL0378), G90 (CML511/CK21) had a mean yield similar to the grand mean and G121 (I-38/CML202), G168 (local check) and G133 (I-42/CB339) had the lowest mean yield. Hybrids G33 (CKDHL0089/CML442) G169 (local check), G26 (CK21/CML444), and G102 (CML544/I-42) had short vectors running from the AEC, while genotypes G18 (CK21/CKDHL0089), G101 (CML544/I-38), G25 (CK21/CML443), G9 (CB323/RO549W) and G99 (CML544/CK21) had the longest vectors. The most three stable hybrids were G169 (local checks), G33 (CKDHL0089/CML442) and G26 (CK21/CML444). Top 10 hybrids ranking based on mean grain yield were; G18 (CK21/CKDHL0089) G12 (CB339/CML442), G134 (I-42/CKDHL0295), G24 (CK21/CML216), G169 (local check), G102 (CML544/I-42), G33 (CKDHL0089/CML442), G101 (CML544/I-38), G26 (CK21/CML444) and G46 (CKDHL0470/CK21). The top 10 stable hybrids were G169 (Local check), G33 (CKDHL0089/CML442), G26 (CK21/CML444) G102 (CML544/I-42), G134 (I-42/CKDHL0295), G24 (CK21/CML216) G60 (CML216/CML442), G142 (I-42/CML544), G160 (U2540W/CKDHL0378) and G12 (CB339/CML442).

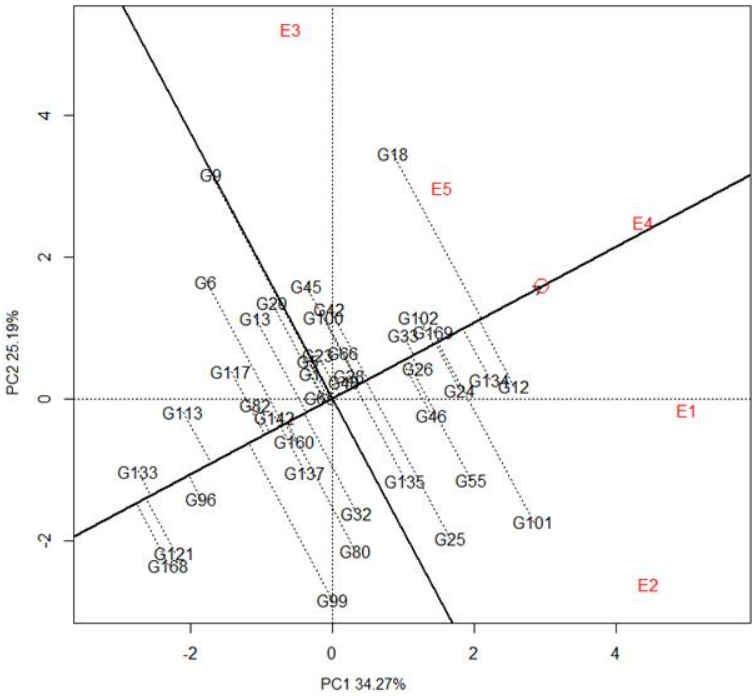

Figure 4.3 Ranking of genotypes based on mean performance and stability

4.3.5.4 Interrelationship among the test environments

The interrelationship among the environments is presented in Figure 4.4. The lines connecting the biplot origin and the marker for each environment are termed as environment vectors and the angle between them is related to the nature of correlation that exists between environments under investigation. The cosine of the angle between the vectors of two environments approximate correlation coefficient between them (Kroonenberg, 1995; Yan, 2002). Yan and Holland (2010) described the angle of < 90° as positive correlation, angle of equal to 90° denotes no correlation and the angle of > 90° as negative correlations. Based on this, environments E1 (Potch optimum), E4 (Potch Low N) and E5 (Cedara Low N) were positively correlated because all the angles among their vectors were less than 90°. However, the angle between vectors of E3 (Vaalharts optimum) and E2 (Cedara optimum) exhibited negative correlation since they were more than and 90°.

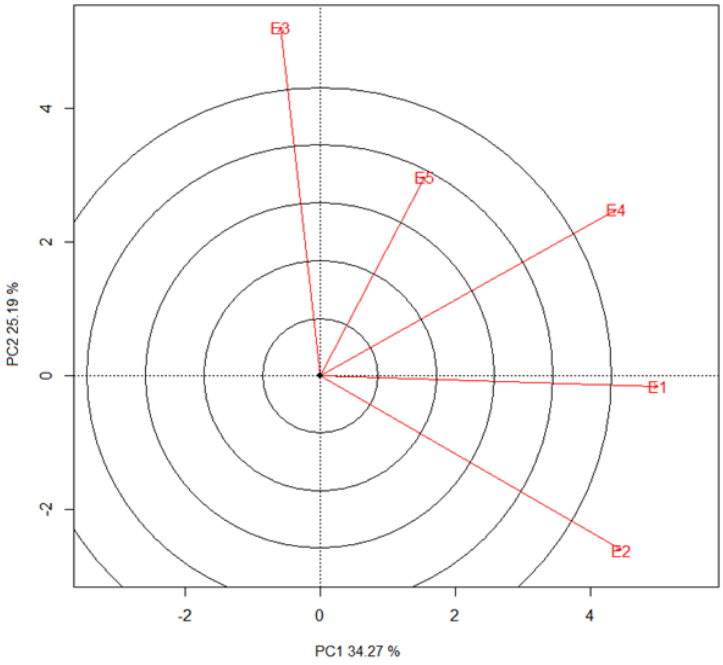

Figure 4.4 Interelationships among the environment

4.4 Discussion

4.4.1 Combined analysis of variance and grain yield performance in each environment

Analysis of variance revealed that the environment contributed the highest proportion of variation in grain yield performance across the environments. Large sum of squares (= variances) for environments indicated that the environments were diverse, with large differences among environmental means causing variation in grain yields. This confirms that low nitrogen stress were responsible for grain yield production across the environments. Similar results were observed in previous GEI studies by the following scientists on various crops (Farshadfar, 2008; Worku and Zelleke, 2008; Silveira *et al.,* 2013). GEI variation contribution toward grain yield performance was slightly larger compared to the contribution made by the genotypes alone. This confirms that genotype response across testing environment was significant, hence the inconsistency in ranking of the genotypes across the environment. This is in agreement with Rad *et al.* (2013) who reported larger contribution of GEI towards yield variation than the contribution made by the genotype in wheat. In another earlier study, the contribution of GEI was also 4.7 times larger than that of the genotypes (Mehari *et al.,* 2014). The existence of GEI confounds the selection process for superior genotypes, recommendation of a genotype for a target environment and reduces the selection efficiency in breeding programmes (Gauch, 2006).

4.4.2 AMMI analysis

The AMMI model demonstrated the presence of GEI, which was partitioned among the four IPCAs (Interaction Principal Components Axes). The AMMI ANOVA also showed that GEI was significant and its 26.20% contribution which was less than 50% of the total sum of squares justify the ability of the AMMI model to determine genotype stability. When GEI accounts for more than 50% of the total variation, it is advisable that regression analysis be done on stability studies (Romagosa and Fox, 1993). The first interaction principal component axis (IPCA1) was highly significant ($P<0.001$) and explained the interaction pattern better than the subsequent interaction axes. It was further observed that the IPCA1 had larger magnitude contribution than the subsequent IPCAs, However, all the IPCAs were significant meaning that genotypic variation was more important among the hybrids. This is in agreement with report from Crossa (1990) that IPCA1 accounts for genotypic variation and the remaining variation is accounted for by the other IPCAs.

The study revealed that some hybrids exhibited specific adaptation while others demonstrated general adaptation. These results complement previous similar GEI studies conducted (Kang

et al., 2006; Pereira *et al.,* 2009). Specific adaptation of a genotype is associated with large genotypic IPCA1 scores to environments with IPCA1 scores of the same sign or similar magnitude (Romagosa *et al.,* 1993). For example, hybrid G101 (CML544/I-38) had the highest positive IPCA1 score of 0.61 among the 40 hybrids and was specifically adapted to environment E2 with the highest positive IPCA1 score of 0.98.

The result is further confirmed by the existence of genotype G55 (CML202/CK21) among the best four genotypes that AMMI analysis ranked for the environment E2 (Table 4.7). Similar trends also applied to genotypes with negative IPCA1 scores and the majority of the genotypes in this study demonstrated the same relationship.

Stable genotypes are the ones having low ASV and are close to the centre in a biplot (Purchase *et al.,* 2000). In this study, hybrids G134 (I-42/CKDHL0295), G169 (local check), G168 (local check), G102 (CML544/I-42), G12 (CML339/CML442), G160 (U2540W/CKDHL0378), G82 (CML444/CML442), G121 (I-38/CML202), G142 (I-42/CML544) and G26 (CK21/CML444) had low ASV and, therefore, have general adaptation.

4.4.3 GGE biplot analysis

The GGE biplot analysis was used to identify the best hybrids in each environment and assess their stability, to show discriminating ability and representativeness of the environments and to study the interrelationship among the tested environments.

The “which-won-where” biplot helps to envisage the possible existence of mega-environments in multi-environmental studies and indicates the best performing genotype in each environment (Kaya *et al.*, 2006). The genotypes that appeared at the vertex of the polygon formed were either the best or poorest in the sectors and designated environments they fell in (Yan *et al.*, 2007). Hybrids G10 (CB323/U2540W) and G12 (CB339/CML442) won in environments E1 and E2 and for environments E3, E5 and E4, G18 (CK21/CKDHL0089) was the winning genotype. Hybrids G99 (CML544/CK21), G121 (I-38/CML202), G168 (local check), G133 (I-42/CB339) and G9 (CB323/RO549W) had no environment in their sector implying that they performed poorly across all locations. Asfaw *et al.* (2009) also illustrated a similar trend of results. G1 (CB322/CK21), G28 (CK21/CML511) and the others close to the point of origin of the biplot had their mean performance close to the grand mean; hence, their performance across the locations had the same response.

According to Yan (2001), long environment vectors represent a good discriminating ability for a given environment. A discriminant test environment accurately resolves genotype

differences. Representative environments have better capability compared to the environment with low discriminatory ability and without representativeness.

A representative location implies that varieties selected in that location would have high probability to also perform well in other locations of the same region (Xu *et al.,* 2014).

Lack of environment representativeness might lead to misleading results on the performance of the genotypes. Abakemal *et al.* (2016) indicated that lack of discriminating power of the environments is generally attributed to unfavourable seasonal conditions and thus genotypic differences based on environments with low discriminatory ability may not be dependable. The study revealed that the least discriminating environment was E5 which happened to be the low N stressed environment. The results are in agreement with the findings of Yan *et al*. (2007) that stressed environments were also the least in terms of discriminating ability. This confirms that adequate information on performance of the genotypes can hardly be acquired under stressed environments.

In this study, E3 had the longest vector, thus was the most discriminating on hybrids G100 (CML544/CML442), G42 (CKDHL0378/CML442), G45 (CKDHL0378/U2540W) and G66 (CML442/CB339) among others. The most representative environments were E4 followed by E5. The biplot measures representativeness of the environments by identifying an average environment and using it as a reference for comparison. According to Solonechnyi *et al*. (2015), a test environment with a small angle to average environment coordinate is the most representative related to the test environment. The ideal environment is the most discriminating for the genotypes and yet representative of the other test environments. Therefore, environments E3 in this study were the most desirable for selecting the genotypes, hence the best environments for genetic differentiation of experimental hybrids.

Within a single mega-environment, genotypes should be evaluated for both mean performance and stability across the environments. The line passing through the biplot origin from lower left to upper right is the average environmental axis, defined by the average first and second principal components scores for all environments. Closeness to the circle along the average environment axis indicates higher mean yield. Therefore, hybrids G18 (CK21/CKDHL0089), G12 (CB339/CML442), G134 (I-42/CKDHL0295), G24 (CK21/CML216), G169 (local checks), G102 (CML544/I-42), G33 (CKDHL0089/CML442), G101 (CML544/I-38), G26 (CK21/CML444) and G46 (CKDHL0470/CK21) were the top 10 yielding because they appeared on the upper right towards circle. Yan and Wu (2008) reported similar results in the GEI study on *Pinus radiata*. Either direction away from the biplot origin, on the axis

indicate greater GEI and reduced stability (Yan and Hunt 2001). Hybrids G169 (local check), G33 (CKDHL0089/CML442), G26 (CK21/CML444), G102 (CML544/I-42), G134 (I-42/CKDHL0295), G24 (CK21/CML216), G60 (CML216/CML442), G142 (I-42/CML544), G160 (U2540W/CKDHL0378) and G12 (CB339/CML442) exhibited short vectors originating from the AEC and hence were very stable, while hybrids G9 (CB323/RO549W), G99 (CML544/CK21), G18 (CK21/CKDHL0089) etc., were considered highly unstable since they had the longest vectors. For broad adaptation in a practical situation, the ideal genotypes are those that have both high mean yield and high stability and they are defined as group one. These hybrids are close to the origin and they have the shortest vector from the average environmental axis. Hybrids G134 (I-42/CKDHL0295), G12 (CB339/CML442), G24 (CK21/CML216), G33 (CKDHL0089/CML442), and G102 (CML544/I-42) etc., are in this group one. On the other hand, for specific selection, the ideal genotypes are those that have high yield but low stability and respond best to particular environments, provided all the required minimum agronomic standards are followed. Genotypes G18 (CK21/CKDHL0089) and G12 (CB339/CML442) are in this group.

The presence of close associations among the test environments E1 (Potch optimum), E4 (Potch Low N) and E5 (Cedara Low N) suggest that the environment generated redundant information about the genotypes. Similar information about the genotypes could be obtained from fewer test environments, and hence the potential to reduce the testing cost and increases breeding efficiency. This is in agreement with Yan and Tinker (2006). If persistent positive correlation is observed across years, some environments can be dropped without loss of much information about the genotypes under investigation

4.5 Conclusions

The present study revealed that maize yields were significantly affected by genotype, environment and genotype by environment interactions based on the AMMI and GGE biplot analyses results. Ten hybrids G134 (I-42/CKDHL0295), G169 (local check), G168 (local check), G102 (CML544/I-42), G12 (CB339/CML442), G160 (U2540W/CKDHL0378), G82 (CML444/CML442), G121 (I-38/CML202) G142 (I-42/CML544) and G 26 (CK21/CML444) were considered stable across the environments based on their small ASV. GGE biplot analysis identified hybrids G10 (CB323/U2540W) and G12 (CB339/CML442) as the best yielding in environments E1 and E2 while G18 (CK21/CKDHL0089) emerged the best in environments E3, E5 and E4. Genotypes G99 (CML544/CK21), G121 (I-38/CML202), G168 (local check), G133 (I-42/CB339) and G9 (CB323/RO549W) performed poorly across all the locations.

Winning genotypes identified by GGE and those identified by AMMI displayed similar results, notably for hybrid G18 (CK21/CKDHL0089) confirming that these two methods can be effectively utilised for the identification of the suitable genotypes. The top most stable hybrids based on ASV and the top 10 based on GGE biplot analysis across the environments revealed five genotypes in common namely G142 (I-42/CML544), G26 (CK21/CML444), G160 (U2540W/CKDHL0378), G12 (CB339/CML442) and G 102 (CML544/I-42) confirming the reliability of the stability analysis results of this study. Five genotypes G134 (I-42/CKDHL0295), G12 (CB339/CML442), G24 (CK21/CML216), G33 (CKDHL0089/CML442) and G102 (CML544/I-42) have broad adaptation because they were high yielding and stable across the environments. These five genotypes are recommended for further evaluation and possible release. The use of both AMMI and GGE biplot yielded more reliable and informative results than one approach alone.

4.6 References

Abakemal, D., H. Shimelis and J. Derera. 2016. Analyses of combining ability and genotype-by-environment interaction of quality protein maize inbred lines adapted to tropical-highlands. Crop Science 56: 3078-3089.

Akintunde, A.N. 2012. Path Analysis Step by Step Using Excel. Journal of Technology and Science Technology 1:9-15.

Akter, A., M.J. Hasan, U. Kulsum, M. Rahman, M. Khatun and M. Islam. 2015. GGE biplot analysis for yield stability in multi-environment trials of promising hybrid rice (*Oryza sativa L*.). Bangladesh Rice Journal 19: 1-8.

Annicchiarico, P. and M. Perenzin. 1994. Adaptation Patterns and Definition of Macro-environments for Selection and Recommendation of Common-wheat Genotypes in Italy. Plant Breeding 113: 197-205.

Asfaw, A., F. Alemayehu, F. Gurum and M. Atnaf. 2009. AMMI and SREG GGE biplot analysis for matching varieties onto soybean production environments in Ethiopia. Scientific Research and Essays 4: 1322-1330.

Blanche Sr, S.B. 2005. New methods to assess cotton varietal stability and identify discriminating environments. PhD Thesis, Louisiana State University.

Crossa, J. 1990. Statistical analyses of multilocation trials. Advances in agronomy. Elsevier. p. 55-85.

Dagnachew, L., M. Fetene, S. de Villiers and K. Tesfaye. 2014. Additive Main Effects and Multiplicative Interactions (AMMI) and genotype by environment interaction (GGE) biplot analyses aid selection of high yielding and adapted finger millet varieties. Journal of Applied Biosciences 76: 6291–6303.

Edmeades, G.O., Banziger, M., Campos, H. and Schussler, J. (2006). Improving tolerance to abiotic stresses in staple crops. A random or planned process? pp. 293-309. (Eds.). Plant breeding: *The Arnel R. Hallauer International Symposium.* Blackwell Publishing. Ames, Iowa, USA.

Farshadfar, E. 2008. Incorporation of AMMI stability value and grain yield in a single non-parametric index (GSI) in bread wheat. Pakistan Journal of biological sciences 11: 1791-1796

Gauch, H. and R. Zobel. 1989. Accuracy and selection success in yield trial analyses. Theoretical and applied genetics 77: 473-481.

Gauch, H.G. 2006. Statistical analysis of yield trials by AMMI and GGE. Crop Science 46: 1488-1500.

Jeberson, M., L. Kant, N. Kishore, V. Rana, D. Walia and D. Singh. 2017. AMMI and GGE Biplot Analysis of Yield Stability and Adaptability of Elite Genotypes of Bread Wheat *(Triticum aestivum L.)* for Northern Hill Zone of India. Red 80: 19E.p.635-641

Kang, M.S., V.D. Aggarwal and R.M. Chirwa. 2006. Adaptability and stability of bean cultivars as determined via yield-stability statistic and GGE biplot analysis. Journal of Crop Improvement 15: 97-120.

Kaya, Y., Ackura, M. and Taner, S. 2006. GGE Bi-plot analysis of multi-environment yield trials in bread wheat. Turkey Journal of Agriculture 62:199-246.

Kerby, T., Burgess, J., Bates, M., Albers, D. and Lege, K., 2000.. Partitioning variety and environment contribution to variation in yield, plant growth, and fiber quality. 2000 Proceedings Beltwide Cotton Conferences, San Antonio, USA, 4-8 January, 2000: Volume 1., National Cotton Council.

Khodarahmpour, Z. and I. Shoushtar. 2011. Gene action studies of different traits in maize *(Zea mays* L.) under heat stress and normal conditions. Journal of American Science 7: 442-448.

Kroonenberg, P.M. 1995. Introduction to biplots for G x E Tables. Research Report No. 51.

Mehari, M., S. Alamerew and B. Lakew. 2014. Genotype× environment interaction and yield stability of malt barley genotypes evaluated in Tigray, Ethiopia using the AMMI analysis. Asian Journal of Plant Science 13: 73-79.

Pacheco, A., Vargas, M., Alvarado, G., Rodríguez, F., López, M., Crossa, J. and Burgueño, J. 2015. GEA-R (Genotype x Environment Analysis with R for Windows). Version 4.0.

Pereira, H.S., L.C. Melo, L.C. de Faria, J.L. Cabrera Diaz, M.J. Del Peloso, J.G.C. da Costa et al. 2009. Stability and adaptability of carioca common bean genotypes in states of the central South Region of Brazil. Embrapa Arroz e Feijão-Artigo em periódico indexado (ALICE).

Purchase, J., H. Hatting and C. Van Deventer. 2000. Genotype× environment interaction of winter wheat *(Triticum aestivum* L.) in South Africa: II. Stability analysis of yield performance. South African Journal of Plant and Soil 17: 101-107.

Purchase, J.L. 1997. Parametric analysis to describe genotype x environment interaction and yield stability in winter wheat. Ph.D. Thesis, Department of Agronomy, Faculty of Agriculture of the University of the Free State, Bloemfontein, South Africa.

Rad, M.N., M.A. Kadir, M. Rafii, H.Z. Jaafar, M. Naghavi and F. Ahmadi. 2013. Genotype environment interaction by AMMI and GGE biplot analysis in three consecutive generations of wheat *(Triticum aestivum)* under normal and drought stress conditions. Australian Journal of Crop Science 7: 956-958

Romagosa, I. and P. Fox. 1993. Genotype x environment interaction and adaptation. In: Hayward M.D., N.O. Bosemark and I. Romagosa, editors. Plant breeding principles and prospects. Chapman and Hall, London, UK. p. 373 -390.

Romagosa, I., P. Fox, L.G. Del Moral, J. Ramos, B.G. Del Moral, F.R. De Togores et al. 1993. Integration of statistical and physiological analyses of adaptation of near-isogenic barley lines. Theoretical and Applied Genetics 86: 822-826.

Silveira, L.C.I.d., V. Kist, T.O.M.d. Paula, M.H.P. Barbosa, L.A. Peternelli and E. Daros. 2013. AMMI analysis to evaluate the adaptability and phenotypic stability of sugarcane genotypes. Scientia Agricola 70: 27-32.

Solonechnyi, P., Vasko, N., Naumov, A., Solonechnyi, O., Vazhenina, O., Bondareva, O. and Logvinenko, Y. 2015. GGE Bi-plot analysis of genotype by environment interaction of spring barley cultivar. Zemdirbyste-Agriculture 102:431-436.

Worku, M. and H. Zelleke. 2008. Genotype x Environment interaction and yield stability of maize. East African Journal of Sciences 2: 7-12.

Xu, N., M. Fok, G.-W. Zhang, J. Li and Z.-g. Zhou. 2014. The application of GGE biplot analysis for evaluating test locations and mega-environment investigation of cotton regional trials. Journal of Integrative Agriculture 13: 1921-1933.

Yan, W. 2002. Singular-value partitioning in biplot analysis of multienvironment trial data. Agronomy Journal 94: 990-996.

YAN, W. and H. WU. 2008. Application of GGE biplot analysis to evaluate Genotype (G), Environment (E), and G× E interaction on Pinus radiata: a case study. New Zealand Journal of Forestry Science 38: 132-142.

Yan, W. and Hollard, J.B. 2010. A heritability-adjusted GGE Bi-plot for test environment evaluation. Euphytica 171:355-369.

Yan, W. and Kang, M. S. 2003. GGE Bi-plot Analysis: A graphical tool for breeders, geneticists and agronomists. CRC Press, Boca Raton, Florida., USA. pp 288.

Yan, W. and L. Hunt. 2001. Interpretation of genotype× environment interaction for winter wheat yield in Ontario. Crop Science 41: 19-25.

Yan, W. and L. Hunt. 2001. Interpretation of genotype× environment interaction for winter wheat yield in Ontario. Crop Science 41: 19-25.

Yan, W. and Tinker, N. A. 2006. Bi-plot analysis of multi-environment trial data: Principles and applications. Canadian Journal of Plant Science 86: 623-645.

Yan, W., Hunt, L. A., Sheng, Q. and Szlavnics, Z. 2000. Cultivar evaluation and mega-environment investigation based on the GGE Bi-plot. Crop Science 40:597-605.

Yan, W., Kang, M. S., Ma, B., Woods, S. and Cornelius, L.P. 2007. GGE Bi-plot vs. AMMI analysis of genotype by environment data. Crop Science 47:643-655.

4.7 Appendices

APPENDIX 4.1

Mean grain yield (t ha^{-1}), IPCA and ASV for 170 maize hybrids evaluated under five environments in 2017/18

Hybrid Code	Mean	IPCAg1	IPCAg2	IPCAg3	IPCAg4	ASV
G1	6.33	-0.5	-0.02	0.29	0.11	1.04
G10	5.8	-0.35	0.08	0.27	-0.05	0.73
G100	6.14	-0.25	-0.14	-0.12	0.09	0.54
G101	6.62	0.61	0.76	0.16	-0.05	1.49
G102	6.63	-0.06	0.27	-0.18	0.07	0.29
G103	6.22	0.12	0.13	-0.2	-0.04	0.29
G104	5.34	0.17	0.46	0.44	0.17	0.58
G105	5.2	0.3	-0.25	-0.01	0.04	0.68
G106	5.19	-0.1	-0.18	-0.63	-0.09	0.27
G107	5.15	0.07	0	0.2	0.16	0.16
G108	4.4	0.37	0.15	-0.39	0.01	0.79
G109	3.7	0.43	-0.68	-0.05	-0.29	1.13
G11	5.67	-0.05	0.26	-0.56	-0.25	0.28
G110	4.67	0.89	-0.49	-0.04	0.27	1.92
G111	5.75	0.45	-0.01	0.07	-0.12	0.93
G112	5.46	0.03	-0.48	0.32	-0.07	0.49
G113	5.22	-0.35	-0.42	0.02	-0.06	0.84
G114	6.2	0.02	0.12	0.36	0.15	0.12
G115	5.84	-0.53	0.02	0.12	0.19	1.1
G116	5.81	-0.29	-0.31	-0.29	0.02	0.68
G117	5.87	-0.64	0.04	0.02	-0.31	1.34
G118	6.1	-0.22	-0.12	-0.09	0.15	0.46
G119	5.28	0.04	-0.58	0.24	0.09	0.59
G12	6.89	0.13	0.13	0.17	0.07	0.3
G120	5.17	-0.33	0.06	-0.26	-0.43	0.69
G121	4.68	0.19	-0.23	0.14	0.12	0.46
G122	6.33	-0.15	0.34	-0.06	-0.09	0.46
G123	2.83	0.49	-0.17	-0.37	-0.04	1.03
G124	5.76	0.05	0.15	0.05	-0.27	0.18
G125	5.2	0.31	-0.27	0.28	-0.17	0.71
G126	5.38	0.18	-0.15	0.06	-0.4	0.39
G127	5.08	0.62	0.16	-0.29	-0.07	1.31
G128	5.83	0.13	0.2	-0.14	-0.17	0.33
G129	5.81	0.24	0.13	0.13	0.26	0.51
G13	6.27	-0.75	0.01	0.11	0.02	1.56
G130	5.08	-0.15	-0.38	-0.19	0.02	0.5

Hybrid Code	Mean	IPCAg1	IPCAg2	IPCAg3	IPCAg4	ASV
G131	5.52	0.17	-0.18	-0.2	-0.24	0.4
G132	4.43	-0.18	-0.25	-0.34	-0.09	0.45
G133	5.27	-0.4	0.11	0.23	0.21	0.84
G134	6.85	0.04	0.11	0.18	0.08	0.14
G135	6.32	0.18	0.59	0.18	0.04	0.7
G136	6.05	0.12	0.09	0.05	0.29	0.26
G137	5.42	0.4	0.18	-0.15	0.15	0.86
G138	6.3	-0.42	0.28	0.18	-0.21	0.93
G139	5.54	0.02	-0.06	0.27	-0.01	0.08
G14	5.6	-0.24	0.1	0.11	0.13	0.51
G140	5.4	-0.21	-0.07	-0.01	0.15	0.44
G141	5.7	0.22	0.13	0.01	0.17	0.47
G142	5.79	-0.24	0.07	0.03	-0.1	0.51
G143	6.47	-0.31	0.22	-0.34	-0.2	0.69
G144	4.53	0.48	0.22	0.28	0.1	1.02
G145	4.53	0.7	-0.23	0.21	0.08	1.48
G146	5.07	-0.24	-0.39	0.09	-0.17	0.64
G147	5.47	0.33	-0.37	-0.11	0.08	0.79
G148	5.68	-0.26	0.32	-0.07	-0.27	0.63
G149	5.89	-0.55	-0.54	0.02	-0.04	1.26
G15	6.01	-0.39	-0.4	0.16	0.14	0.9
G150	3.93	0.32	-0.54	-0.05	0.58	0.86
G151	5.92	-0.18	0.11	-0.31	-0.08	0.39
G152	5.11	0.39	-0.33	0.14	-0.31	0.88
G153	4.39	0.57	-0.2	-0.36	-0.36	1.21
G154	4.96	0.7	-0.26	0.1	0.33	1.48
G155	5.82	-0.09	-0.05	0.1	-0.43	0.19
G156	5.62	-0.34	0.17	0.25	-0.12	0.72
G157	5.04	-0.13	-0.18	-0.02	0.19	0.33
G158	5.42	0	-0.02	0.03	0.13	0.02
G159	5.43	-0.76	-0.35	0.02	0.06	1.62
G16	5.39	0.13	-0.05	-0.24	0.05	0.28
G160	5.76	-0.12	0.24	-0.04	-0.28	0.35
G161	6.08	-0.4	0.11	0.22	0.45	0.84
G162	4.94	0.32	0.11	-0.13	-0.17	0.68
G163	5.29	0.61	-0.03	0.43	-0.38	1.27
G164	5.94	-0.38	0.11	0.37	-0.18	0.8
G165	2.33	0.88	0.41	-0.9	-0.49	1.89
G166	4.68	0.04	-0.33	-0.06	-0.62	0.34
G167	5.68	-0.11	0.1	0.41	-0.01	0.26
G168	6.09	0.08	-0.14	0.41	0.44	0.22
G169	6.36	-0.08	-0.09	0	-0.02	0.2
G17	5.89	0.03	-0.01	0.07	0.06	0.07

Hybrid Code	Mean	IPCAg1	IPCAg2	IPCAg3	IPCAg4	ASV
G170	5.42	-0.19	0.19	-0.23	0.07	0.43
G18	7.33	-0.59	0.72	-0.45	0.2	1.42
G19	6.04	-0.18	-0.32	-0.07	0.01	0.49
G2	5.61	-0.61	-0.14	-0.16	0.14	1.27
G20	6.22	-0.87	-0.35	0.09	-0.33	1.84
G21	5.84	0.23	0.11	-0.18	-0.13	0.5
G22	5.39	-0.29	-0.3	0.17	0.32	0.68
G23	6.23	-0.46	0.07	0.01	-0.23	0.95
G24	7.02	-0.19	0.52	0.25	0.02	0.65
G25	6.19	0.37	0.24	0.36	-0.05	0.81
G26	6.49	0.17	0.36	-0.09	0.27	0.51
G27	5.2	0.32	-0.33	-0.06	-0.11	0.75
G28	6.35	-0.27	0.08	0.15	0.1	0.57
G29	4.97	0.47	-0.43	0.04	-0.09	1.08
G3	5.91	-0.04	0.18	-0.12	0.42	0.19
G30	4.75	0.71	0.01	-0.03	0.16	1.47
G31	4.42	0.4	-0.43	0.24	-0.01	0.94
G32	6.07	-0.23	0.22	0.36	-0.54	0.53
G33	6.77	-0.37	0.33	0.02	-0.17	0.84
G34	5.73	-0.03	0.89	0.19	0.1	0.89
G35	5.33	-0.3	-0.04	-0.12	0.5	0.63
G36	4.47	-0.09	0.51	-0.57	-0.06	0.55
G37	5.81	0.21	0.11	0.26	0.17	0.46
G38	2.14	0.67	-0.02	-0.47	-0.05	1.39
G39	5.64	0.19	-0.26	0.02	-0.04	0.47
G4	5.92	-0.25	-0.37	0.42	-0.11	0.65
G40	5.68	0.42	-0.4	0.34	-0.16	0.97
G41	5.74	0.32	0.1	0.16	-0.36	0.67
G42	6.32	-0.32	0.11	-0.15	0.06	0.68
G43	5.59	0.08	-0.15	-0.14	-0.08	0.23
G44	5.49	0.21	-0.38	-0.23	-0.13	0.58
G45	6.13	-0.46	-0.24	-0.2	-0.16	0.99
G46	6.76	-0.25	0.44	0.23	-0.27	0.69
G47	5.15	0	0.16	0.06	0.07	0.16
G48	6.37	-0.53	0.15	0.11	-0.48	1.13
G49	5.44	0.07	-0.17	0	0.29	0.23
G5	6.21	-0.3	-0.03	0.15	0.31	0.63
G50	5.17	0.44	0.25	0.18	0.31	0.95
G51	5.31	-0.29	0	-0.08	-0.02	0.6
G52	5.36	0.45	-0.3	-0.07	-0.07	0.98
G53	5.77	-0.37	-0.05	0.01	0.27	0.76
G54	5.72	0.41	-0.42	0.24	-0.25	0.96
G55	6.09	0.51	-0.2	0.17	0.06	1.08

Hybrid Code	Mean	IPCAg1	IPCAg2	IPCAg3	IPCAg4	ASV
G56	5.38	0.35	0.01	-0.13	0.17	0.73
G57	5.39	0.01	0.33	-0.05	0.28	0.33
G58	5.3	0.1	0	-0.22	-0.36	0.21
G59	5.65	0.73	0.47	0.06	-0.07	1.59
G6	6.23	-0.8	0.25	-0.05	0.22	1.68
G60	5.91	-0.41	0.23	0.12	-0.35	0.89
G61	5.8	-0.44	0.06	-0.07	-0.37	0.92
G62	5.95	0.17	-0.18	-0.19	-0.12	0.4
G63	5.29	-0.32	0.04	0.42	-0.17	0.66
G64	5.63	0.01	0.1	0.15	0.18	0.1
G65	6.25	-0.39	0.3	0.11	-0.18	0.86
G66	6.56	-0.47	0.28	0.12	-0.02	1.02
G67	5.69	-0.11	-0.19	-0.04	-0.08	0.3
G68	5.42	-0.15	0.33	-0.16	0.11	0.45
G69	5.72	-0.37	-0.62	-0.01	-0.02	0.99
G7	5.07	-0.52	-0.47	-0.04	0.13	1.19
G70	5.86	0.07	-0.02	0.01	-0.28	0.14
G71	5.83	-0.16	-0.16	0.13	0.17	0.37
G72	5.54	-0.12	0.02	0.06	0.01	0.25
G73	4.89	0.06	0.05	0.01	-0.08	0.14
G74	5.13	-0.14	0.15	0.11	0.27	0.34
G75	5.36	0.27	-0.39	-0.03	-0.24	0.68
G76	5.27	0.03	0.18	-0.41	-0.21	0.19
G77	4.38	0.44	0.04	-0.21	0.33	0.91
G78	5.51	0.42	-0.45	0.49	0.17	0.98
G79	4.89	-0.17	0.21	0.29	0.17	0.41
G8	5.59	-0.18	0.38	-0.1	0.04	0.54
G80	5.28	0.54	0.03	-0.04	-0.35	1.12
G81	6.08	0.05	0.05	0	-0.11	0.11
G82	5.63	-0.19	-0.12	0	0.05	0.42
G83	6.04	-0.05	0.06	-0.11	0.02	0.11
G84	5.63	0.27	0.11	-0.27	0.34	0.57
G85	5.99	-0.29	0.16	0.24	-0.05	0.62
G86	5.72	0.43	0.34	0.07	0.08	0.97
G87	5.73	-0.03	0.13	-0.18	0.45	0.14
G88	5.5	-0.22	0.17	-0.19	-0.2	0.48
G89	3.28	-0.5	-1.02	-1.11	0.52	1.47
G9	6.31	-0.83	0.09	-0.4	0.26	1.73
G90	4.65	-0.07	-0.23	0.03	0.18	0.28
G91	5.95	-0.04	-0.06	-0.07	0	0.11
G92	5.69	0.39	0.19	0.35	-0.03	0.83
G93	5.98	0.11	0.5	-0.35	0.26	0.55
G94	5.88	0.05	0.03	0.13	-0.15	0.1

Hybrid Code	Mean	IPCAg1	IPCAg2	IPCAg3	IPCAg4	ASV
G95	5.03	-0.2	0.12	-0.06	0.05	0.43
G96	4.9	0.25	-0.1	-0.04	0.23	0.53
G97	6.26	0.63	1.23	-0.18	0.29	1.8
G98	4.85	0.28	-0.13	-0.12	0.3	0.6
G99	6.14	0.5	-0.19	0.35	0	1.05

APPENDIX 4.2

Mean grain yield (t ha^{-1}) of 170 hybrids at five environments and across five environments in 2017/18

Hybrid Code	Potch Low N	Potch Opt	Ced Low N	Ced Opt	Val Opt	Mean GY Across Sites
G1	3.242	7.391	4.75	6.195	10.083	6.33
G2	3.295	6.163	4.542	4.337	9.692	5.61
G3	3.24	8.578	5.154	4.789	7.802	5.91
G4	3.575	6.195	4.479	6.903	8.442	5.92
G5	3.206	7.693	5.256	5.79	9.107	6.21
G6	3.023	7.693	4.505	4.576	11.355	6.23
G7	2.948	4.705	4.527	4.579	8.61	5.07
G8	3.229	8.491	3.576	4.383	8.25	5.59
G9	3.799	7.27	5.163	3.894	11.433	6.31
G10	3.105	7.374	3.764	5.768	9.004	5.80
G11	4.917	8.309	3.641	3.76	7.713	5.67
G12	4.539	9.545	5.332	6.929	8.121	6.89
G13	3.427	6.954	4.425	5.475	11.075	6.27
G14	2.922	7.531	4.094	5.108	8.328	5.60
G15	3.57	6.1	5.264	6.062	9.04	6.01
G16	3.97	7.499	4.413	4.623	6.452	5.39
G17	3.781	7.921	4.593	5.755	7.411	5.89
G18	4.635	10.67	5.098	4.364	11.862	7.33
G19	4.374	6.694	5.164	5.706	8.237	6.04
G20	4.272	5.416	4.204	5.899	11.317	6.22
G21	4.597	8.548	4.188	5.266	6.613	5.84
G22	2.596	6.033	4.924	5.317	8.061	5.39
G23	4.229	7.53	3.966	5.566	9.874	6.23
G24	3.951	10.294	4.455	6.56	9.814	7.02
G25	3.809	9.53	4.183	6.91	6.513	6.19
G26	4.044	10.044	5.178	5.507	7.657	6.49
G27	4.198	6.648	4.346	5.475	5.324	5.20
G28	3.654	8.145	4.769	5.985	9.213	6.35
G29	3.975	6.394	4.344	5.746	4.412	4.97
G30	3.222	8.075	4.037	4.93	3.464	4.75
G31	2.856	5.732	3.778	5.583	4.154	4.42
G32	4.038	8.093	2.734	6.543	8.934	6.07
G33	4.462	9.07	4.212	5.875	10.22	6.77
G34	2.404	10.496	2.822	4.817	8.086	5.73
G35	2.491	6.889	4.998	4.121	8.167	5.33
G36	3.113	7.935	2.402	2.099	6.814	4.47
G37	3.216	8.557	4.5	6.108	6.665	5.81
G38	1.791	5.253	1.324	1.332	0.995	2.14

Hybrid Code	Potch Low N	Potch Opt	Ced Low N	Ced Opt	Val Opt	Mean GY Across Sites
G39	4.164	7.098	4.681	5.87	6.364	5.64
G40	4.184	7.065	4.595	7.175	5.371	5.68
G41	4.369	8.429	3.44	6.273	6.201	5.74
G42	4.164	8.115	4.791	5.155	9.388	6.32
G43	4.279	7.212	4.422	5.22	6.818	5.59
G44	4.764	6.601	4.741	5.296	6.026	5.49
G45	4.656	6.485	4.717	5.232	9.566	6.13
G46	4.257	9.575	3.699	6.465	9.819	6.76
G47	2.843	7.653	3.555	4.742	6.933	5.15
G48	4.492	7.676	3.314	5.959	10.428	6.37
G49	3.232	7.153	4.978	5.182	6.672	5.44
G50	2.528	8.839	4.078	5.244	5.161	5.17
G51	3.307	6.78	3.753	4.517	8.173	5.31
G52	4.396	7.151	4.605	5.679	4.971	5.36
G53	3.062	7.059	4.826	4.983	8.9	5.77
G54	4.601	6.978	4.552	7.034	5.445	5.72
G55	4.395	8.363	5.281	6.878	5.507	6.09
G56	3.696	8.104	4.569	4.947	5.576	5.38
G57	2.764	8.553	4.054	4.405	7.183	5.39
G58	4.532	7.308	3.33	4.757	6.575	5.30
G59	3.976	10.369	3.572	5.724	4.612	5.65
G60	3.826	7.727	3.028	5.467	9.487	5.91
G61	4.218	7.049	3.302	5.055	9.37	5.80
G62	4.919	7.624	4.817	5.591	6.795	5.95
G63	2.598	6.742	2.965	5.802	8.361	5.29
G64	3.022	8.009	4.335	5.457	7.338	5.63
G65	3.828	8.409	3.661	5.608	9.767	6.25
G66	3.744	8.593	4.28	5.781	10.375	6.56
G67	4.059	6.842	4.414	5.404	7.712	5.69
G68	4.11	5.083	5.175	5.713	8.532	5.72
G69	4.51	7.778	3.904	5.829	7.276	5.86
G70	3.352	7.09	4.886	5.733	8.063	5.83
G71	3.349	7.385	4.021	5.217	7.721	5.54
G72	3.095	7.111	3.22	4.653	6.345	4.89
G73	2.244	7.456	3.887	4.589	7.479	5.13
G74	4.542	6.463	4.27	5.836	5.694	5.36
G75	4.326	7.812	3.383	3.879	6.925	5.27
G76	2.594	7.416	3.99	3.682	4.203	4.38
G77	3.205	6.843	5.103	7.232	5.161	5.51
G78	1.773	7.272	3.185	4.8	7.417	4.89
G79	4.518	8.152	3.369	5.574	4.772	5.28
G80	4.36	8.273	4.366	5.812	7.606	6.08
G81	3.542	6.905	4.446	5.23	8.009	5.63

Hybrid Code	Potch Low N	Potch Opt	Ced Low N	Ced Opt	Val Opt	Mean GY Across Sites
G82	4.177	8.133	4.623	5.333	7.939	6.04
G83	3.725	8.592	5.1	4.559	6.194	5.63
G84	3.319	7.934	3.884	5.849	8.982	5.99
G85	3.6	9.558	4.074	5.567	5.801	5.72
G86	3.171	8.269	5.169	4.488	7.528	5.73
G87	3.909	7.555	3.388	4.443	8.18	5.50
G88	2.928	1.45	5.313	0.478	6.253	3.28
G89	2.493	5.852	3.978	4.47	6.455	4.65
G90	4.176	7.661	4.664	5.481	7.754	5.95
G91	3.372	8.93	3.824	6.437	5.903	5.69
G92	3.837	9.859	4.569	4.16	7.455	5.98
G93	4.021	7.957	4.021	6.006	7.403	5.88
G94	2.828	7.071	3.477	4.194	7.591	5.03
G95	2.953	7.094	4.327	4.684	5.439	4.90
G96	3.552	13.382	3.788	4.539	6.049	6.26
G97	2.815	7.113	4.725	4.388	5.218	4.85
G98	3.3	7.458	4.139	6.455	4.729	5.22
G99	4.177	7.275	5.142	5.365	8.748	6.14
G100	4.337	12.04	3.974	6.503	6.252	6.62
G101	4.58	9.4	5.006	5.465	8.686	6.63
G102	4.719	8.813	4.696	5.411	7.444	6.22
G103	2.017	9.122	3.251	5.666	6.637	5.34
G104	3.727	6.943	4.48	5.387	5.444	5.20
G105	4.655	6.424	4.295	3.454	7.113	5.19
G106	2.645	7.332	3.988	5.291	6.514	5.15
G107	3.356	7.552	3.217	3.269	4.591	4.40
G108	3.447	4.158	3.117	4.624	3.152	3.70
G109	3.602	6.783	5.178	5.488	2.282	4.67
G110	4.326	8.439	4.269	6.132	5.573	5.75
G111	3.572	5.92	4.512	6.572	6.713	5.46
G112	3.442	5.24	4.202	5.114	8.097	5.22
G113	3.267	8.63	4.667	6.568	7.883	6.20
G114	2.859	7.018	4.448	5.156	9.7	5.84
G115	4.407	6.328	5.036	4.819	8.473	5.81
G116	3.852	6.726	3.372	5.132	10.279	5.87
G117	3.981	7.4	5.158	5.379	8.568	6.10
G118	3.345	5.51	4.914	6.19	6.417	5.28
G119	4.136	6.589	2.753	4.098	8.269	5.17
G120	2.679	6.311	3.928	5.087	5.405	4.68
G121	4.215	9.087	4.085	5.397	8.842	6.33
G122	2.234	5.155	2.142	2.271	2.351	2.83
G123	4.139	8.168	3.491	5.635	7.363	5.76
G124	3.602	6.785	3.858	6.301	5.428	5.20

Hybrid Code	Potch Low N	Potch Opt	Ced Low N	Ced Opt	Val Opt	Mean GY Across Sites
G125	4.376	7.024	3.427	5.797	6.271	5.38
G126	4.216	8.655	3.77	4.514	4.239	5.08
G127	4.387	8.606	3.857	5.164	7.114	5.83
G128	3.288	8.715	4.756	5.726	6.566	5.81
G129	3.692	5.614	4.43	4.53	7.122	5.08
G130	4.722	7.155	4.133	5.23	6.369	5.52
G131	3.342	5.291	3.412	3.419	6.66	4.43
G132	2.08	6.972	3.747	4.891	8.664	5.27
G133	4.386	9.28	5.312	6.833	8.417	6.85
G134	3.604	10.5	3.926	5.936	7.655	6.32
G135	3.531	8.641	5.127	5.667	7.271	6.05
G136	3.698	8.746	4.333	4.83	5.508	5.42
G137	3.782	8.311	3.618	5.85	9.951	6.30
G138	3.226	7.34	4.022	5.998	7.092	5.54
G139	3.104	6.855	4.32	4.829	7.883	5.40
G140	3.531	8.559	4.51	5.348	6.535	5.70
G141	3.706	7.535	3.877	5.297	8.528	5.79
G142	5.032	8.539	4.324	4.863	9.59	6.47
G143	2.14	8.067	2.989	5.075	4.36	4.53
G144	2.95	7.049	3.894	5.634	3.137	4.53
G145	3.433	5.324	3.79	5.28	7.539	5.07
G146	4.258	6.922	5.117	5.548	5.497	5.47
G147	3.826	8.111	3.042	4.767	8.671	5.68
G148	4.007	5.196	5.025	5.724	9.497	5.89
G149	1.849	5.031	4.916	4.03	3.846	3.93
G150	4.439	7.931	4.274	4.579	8.394	5.92
G151	4.162	6.608	3.698	6.1	4.997	5.11
G152	4.472	6.611	3.107	4.176	3.57	4.39
G153	3.132	7.493	5	5.634	3.534	4.96
G154	4.462	7.285	3.471	6.007	7.885	5.82
G155	3.009	7.492	3.282	5.481	8.835	5.62
G156	2.85	6.302	4.282	4.615	7.133	5.04
G157	3.225	7.38	4.283	5.106	7.082	5.42
G158	3.008	5.007	4.355	4.769	10.033	5.43
G159	4.073	8.173	3.3	5.128	8.118	5.76
G160	2.466	7.904	5.064	5.507	9.454	6.08
G161	3.758	7.771	3.223	4.602	5.351	4.94
G162	3.865	8.052	3.116	6.929	4.479	5.29
G163	3.217	7.521	3.492	6.18	9.308	5.94
G164	3.328	7.029	0.258	0.467	0.576	2.33
G165	4.331	5.436	2.58	5.02	6.049	4.68
G166	2.845	7.741	3.727	6.171	7.921	5.68
G167	1.674	6.749	4.429	5.573	6.133	4.91

Hybrid Code	Potch Low N	Potch Opt	Ced Low N	Ced Opt	Val Opt	Mean GY Across Sites
G168	4.211	7.632	4.752	5.792	8.053	6.09
G169	4.372	8.668	4.844	5.013	8.907	6.36
G170	3.138	8.235	3.706	4.113	7.899	5.42
MIN	1.67	1.45	0.26	0.47	0.58	2.14
MAX	5.03	13.38	5.33	7.23	11.86	7.33
MEAN	3.62	7.53	4.13	5.23	7.22	5.55
% cv	4.86	10.53	15.25	12.12	12.26	12.07
SE	0.1761	0.7926	0.6304	0.6333	0.885	0.67
LSD (0.05)	0.3492	1.572	1.25	1.256	1.755	0.59
P VALUE	<.001	<.001	<.001	<.001	<.001	<.001

CHAPTER 5

SNP- BASED ASSESSMENT OF GENETIC PURITY IN MAIZE HYBRID BREEDING AND SEED PRODUCTION

Abstract

Assessment of genetic purity of parental inbred lines and their F1 hybrids is an important quality control component and requirement in hybrid breeding, seed production, variety release as well as intellectual property protection (IP). The aim of the present study was to investigate the genetic purity, parent-offspring relationship and genetic diversity of inbred lines and their hybrids. One hundred and eighty-eight maize genotypes comprising of 30 elite parental inbred lines and 158 single-cross hybrids, were genotyped using 92 Kompetitive Allele Specific Polymerase Chain Reaction (KASP) genotyping assays. The bi-allelic data obtained was analysed for genetic purity and diversity parameters using GenAlex software. In this study, 66.7% of the parental inbred lines genotyped were considered pure with residual heterozygosity of <5%, while the remaining 33.3% had residual heterozygosity levels ranging from 5.43 - 57.61% and hence not pure. The line CK21 (5.43%) requires further purification through ear-to-row selection method, while the remaining nine inbred lines with heterozygosity exceeding 15% should be discarded due to high genetic contamination. Pairwise genetic distances among the tested inbred lines varied from 0.05 to 0.56, with 93% of the pairs falling above 0.40. The maximum genetic distance (0.56) was observed for CKDHL0089, CML443 when either of them paired with CB323 while the lowest (0.05) was between I-42 and I-40. Cluster analysis effectively discriminated the lines into three distinct genetic clusters. The parent-offspring test conducted on the 158 experimental hybrids resulted in the elimination of 38% of the hybrids due to genetic contamination of their parental inbred lines. Incidences of residual heterozygosity exceeding 5% observed in some of the inbred lines suggested the requirement for further purification through inbreeding. Of the 68 hybrids that passed the parent-offspring test, seven hybrids, including SCHP29, SCHP95, SCHP94, SCHP134, SCHP44, SCHP114 and SCHP126, were selected as potential candidates for further evaluation and release due to their outstanding yield performance.

Key words: Genetic purity, hybrid breeding, parent-offspring test, single nucleotide polymorphism, *Zea mays*.

5.1 Introduction

Maize (*Zea mays* L.) remains the principal source of food security and nutrition for the majority of people in sub-Saharan Africa (SSA) and Latin America (Shiferaw *et al.,* 2011). However, adequate production is hampered by poor grain yields due to biotic and abiotic stresses. Hence, there is a need to improve grain yield through hybrid breeding to exploit heterosis.

Assessment of genetic purity of parental inbred lines and parent-offspring test for the resultant F1 hybrids are important quality control functions in maize hybrid breeding programmes. These functions are now more critical due to the stringent intellectual property requirements governing plant breeding and variety registration in many countries (Semagn *et al*., 2012). Additionally, maintenance of high levels of genetic purity is key for robust agronomic performance of the genotype. Parent-offspring test help to prove parentage for a specific hybrid if it is the true derivative of the original parental inbred lines without pollen contamination (Gowda *et al.*, 2017). Inbred lines' genetic purity and parentage can be proved through three approaches namely; grow out test (GOT), use of biochemical markers and use of molecular markers.

The grow out test is a morphologically based approach using a set of descriptors, while the biochemical markers approach analyses the protein/isoenzyme profiles of the genotype and the molecular marker approach detects variation of the genotype directly at DNA level. Unlike GOT and biochemical marker methods which have low polymorphism and high environmental influences, molecular markers are ideal for genotyping since they are: codominant, highly abundant and polymorphic, independent of the environment and reproducible, expressed at all developmental stages, known position in the genome, linked to traits of interest and automation is possible (Gowda *et al.,* 2017).

Semagn *et al*. (2006) highlighted several types of molecular markers that are available for detection of polymorphism. The main ones include; restriction fragment length polymorphism (RFLPs), random amplified polymorphic DNA (RAPD), simple sequence repeats (SSRs), amplified fragment length polymorphism (AFLP) and single nucleotide polymorphism (SNP). In this study, SNP markers were used to determine the genetic purity of the maize parental inbred lines and to prove parentage of the resultant single-cross hybrids. The SNPs are the most widely used markers since they target single nucleotide differences between genotypes, hence showing more polymorphism unlike the other types of markers (Ndhlela, 2012). Recent advances in molecular technology have emphasized the use of single-nucleotide polymorphic markers (Hamblin *et al.,* 2007) because they are cost effective per data point, adequate

genomic abundance, locus–specificity, codominance, simple documentation and potential for high throughput unlike the other markers.

The application of molecular markers, is more efficient, saves time and resources (Ertiro *et al*., 2015) and they are free from environmental influences compared to morphological markers. It is often assumed that the use of a large number of markers results in higher accuracy. In most sequencing based marker systems, the levels of missing data can lead to wrong interpretation, hence, selection of fewer markers with high and repeatable representation across samples is desired and is cost effective. Therefore, there has to be a balance between accuracy and cost. Chan *et al.* (2016) suggested that fewer markers with high excepted heterozygosity, missing value <20%, and observed heterozygosity of <6% are ideal markers for accurate quality control genotyping. Similarly, Semagn *et al*. (2012) suggested that a set of 50–100 single plex assay SNPs are adequate for molecular-based quality control genotyping. It is against this background that in the present study, a set of 92 SNPs were effectively used to genotype 30 parental lines and 158 hybrids and reliable results were realised.

According to Gowda *et al.,* (2017), parental inbred lines are expected to be pure with residual heterozygosity of less than 5%. Inbred lines having residual heterozygosity above 5% are either not pure due to genetic contamination or not fixed unless if they were deliberately maintained at early generation during development. Genetic contamination reduces the genetic and physiological quality of the seeds leading to decreased crop productivity (Salgado *et al.,* 2006). Hence, inbred line genetic purity assessment and parent offspring test are important quality control procedures for a successful hybrid breeding programme.

5.2 Materials and methods

5.2.1 Experimental material

A total of 188 maize genotypes, comprising 26 elite parental inbred lines, four doubled haploid lines and 158 experimental single-cross hybrids were genotyped using 92 single nucleotide polymorphism (SNP) markers presented in Appendix 5.3. These markers chosen are a subset of the 100 SNP markers recommended by CIMMYT for routine quality control genotyping in maize (Gowda *et al.,* 2017). All the genotypes used in this study were sourced from Agriculture Research Council-Grain Crops Institutes (ARC-GCI), Potchefstroom, South Africa. Details of the maize genotypes used in this study are presented in Table 5.1.

Table 5.1 List of 30 parental maize lines and 158 experimental single-cross hybrids sourced from ARC-GCI

N	Name	Type	Pedigree	No	Name	Type	Pedigree
1	CB322	Inbred line		95	SCHP67	Hybrid	CML442 / CML488
2	CB323	Inbred line		96	SCHP68	Hybrid	CML442 / CML511
3	CB339	Inbred line		97	SCHP69	Hybrid	CML442 / CML544
4	CK21	Inbred line		98	SCHP70	Hybrid	CML442 / CML547
5	CKDHL0089	DH line		99	SCHP71	Hybrid	CML442 / CZL068
6	CKDHL0295	DH line		100	SCHP72	Hybrid	CML443 / CK21
7	CKDHL0378	DH line		101	SCHP73	Hybrid	CML443/CML442
8	CKDHL0470	DH line		102	SCHP74	Hybrid	CML443 / I-38
9	CKL05022	Inbred line		103	SCHP75	Hybrid	CML443 / I-42
10	CML202	Inbred line		104	SCHP76	Hybrid	CML444 / CK21
11	CML216	Inbred line		105	SCHP77	Hybrid	CML444 / CML442
12	CML442	Inbred line		106	SCHP78	Hybrid	CML444 / I-38
13	CML443	Inbred line		107	SCHP79	Hybrid	CML444 / I-42
14	CML444	Inbred line		108	SCHP80	Hybrid	CML488 / CK21
15	CML488	Inbred line		109	SCHP81	Hybrid	CML488 / CML442
16	CML511	Inbred line		110	SCHP82	Hybrid	CML488 / I-38
17	CML543	Inbred line		111	SCHP83	Hybrid	CML488 / RO549W
18	CML544	Inbred line		112	SCHP84	Hybrid	CML488 / U2540W
19	CML547	Inbred line		113	SCHP85	Hybrid	CML511 / CK21
20	CZL068	Inbred line		114	SCHP86	Hybrid	CML511 / CML442
21	I-38	Inbred line		115	SCHP87	Hybrid	CML511 / I-38
22	I-42	Inbred line		116	SCHP88	Hybrid	CML511 / I-42
23	RO549W	Inbred line		117	SCHP89	Hybrid	CML511 / RO549W
24	U2540W	Inbred line		118	SCHP90	Hybrid	CML543 / I-38
25	CML540	Inbred line		119	SCHP91	Hybrid	CML543 / RO549W
26	CZL99017	Inbred line		120	SCHP92	Hybrid	CML543 / U2540W
27	CML312	Inbred line		121	SCHP93	Hybrid	CML544 / CK21
28	CZL0718	Inbred line		122	SCHP94	Hybrid	CML544 / CML442
29	CZL0919	Inbred line		123	SCHP95	Hybrid	CML544 / I-38
30	I-40	Inbred line		124	SCHP96	Hybrid	CML544 / I-42
31	SCHP1	Hybrid	CB322 / CK21	125	SCHP97	Hybrid	CML544/RO549W
32	SCHP2	Hybrid	CB322 / I-42	126	SCHP98	Hybrid	CML547 / CML442
33	SCHP3	Hybrid	CB322/RO54W	127	SCHP99	Hybrid	CML547 / RO549W
34	SCHP5	Hybrid	CB323 / CK21	128	SCHP100	Hybrid	CML547 / U2540W
35	SCHP6	Hybrid	CB323/ CML442	129	SCHP101	Hybrid	CZL068 / CK21
36	SCHP7	Hybrid	CB323 / I-38	130	SCHP102	Hybrid	CZL068 / I-38
37	SCHP8	Hybrid	CB323 / I-42	131	SCHP103	Hybrid	CZL068 / I-42
38	SCHP9	Hybrid	CB323/RO54W	132	SCHP104	Hybrid	CZL068 / RO549W
39	SCHP10	Hybrid	CB323/U2540W	133	SCHP105	Hybrid	CZL068 / U2540W
40	SCHP11	Hybrid	CB339/ CML442	134	SCHP106	Hybrid	I-38 / CB322
41	SCHP12	Hybrid	CB339 / I-38	135	SCHP107	Hybrid	I-38 / CB323
42	SCHP13	Hybrid	CB339/U2540W	136	SCHP108	Hybrid	I-38 / CB339
43	SCHP14	Hybrid	CK21 / CB322	137	SCHP109	Hybrid	I-38 / CKDHL0089
44	SCHP15	Hybrid	CK21 / CB323	138	SCHP110	Hybrid	I-38 / CKDHL0295
45	SCHP16	Hybrid	CK21 / CB339	139	SCHP111	Hybrid	I-38 / CKDHL0378
46	SCHP17	Hybrid	CK21/CKDHL089	140	SCHP112	Hybrid	I-38 / CKDHL0470
47	SCHP18	Hybrid	CK21/CKDHL095	141	SCHP113	Hybrid	I-38 / CKL05022
48	SCHP19	Hybrid	CK21 /CKDHL0378	142	SCHP114	Hybrid	I-38 / CML202

N	Name	Type	Pedigree	No	Name	Type	Pedigree
49	SCHP20	Hybrid	CK21 / CML202	143	SCHP115	Hybrid	I-38 / CML216
50	SCHP21	Hybrid	CK21 / CML443	144	SCHP116	Hybrid	I-38 / CML443
51	SCHP22	Hybrid	CK21 / CML444	145	SCHP117	Hybrid	I-38 / CML444
52	SCHP23	Hybrid	CK21 / CML488	146	SCHP118	Hybrid	I-38 / CML488
53	SCHP24	Hybrid	CK21/ CML511	147	SCHP119	Hybrid	I-38 / CML511
54	SCHP25	Hybrid	CK21 / CML544	148	SCHP120	Hybrid	I-38 / CML543
55	SCHP26	Hybrid	CK21 / CML547	149	SCHP121	Hybrid	I-38 / CML544
56	SCHP27	Hybrid	CK21 / CZL068	150	SCHP122	Hybrid	I-38 / CML547
57	SCHP28	Hybrid	CKDHL0089/ CK21	151	SCHP123	Hybrid	I-38 / CZL068
58	SCHP29	Hybrid	CKDHL0089/CML442	152	SCHP124	Hybrid	I-42 / CB323
59	SCHP30	Hybrid	CKDHL0089/RO549W	153	SCHP125	Hybrid	I-42 / CB339
60	SCHP31	Hybrid	CKDHL0089/ U2540W	154	SCHP126	Hybrid	I-42 / CKDHL0295
61	SCHP32	Hybrid	CKDHL0089/ I-38	155	SCHP127	Hybrid	I-42 / CKL05022
62	SCHP33	Hybrid	CKDHL0295/ CK21	156	SCHP128	Hybrid	I-42 / CML202
63	SCHP35	Hybrid	CKDHL0295 / U2540W	157	SCHP129	Hybrid	I-42 / CML216
64	SCHP36	Hybrid	CKDHL0295 / I-38	158	SCHP130	Hybrid	I-42 / CML443
65	SCHP37	Hybrid	CKDHL0295 / I-42	159	SCHP131	Hybrid	I-42 / CML444
66	SCHP38	Hybrid	CKDHL0378 / CML442	160	SCHP132	Hybrid	I-42 / CML488
67	SCHP39	Hybrid	CKDHL0378 / I-38	161	SCHP133	Hybrid	I-42 / CML511
68	SCHP40	Hybrid	CKDHL0378 / I-42	162	SCHP134	Hybrid	I-42 / CML544
69	SCHP41	Hybrid	CKDHL0378 / U2540W	163	SCHP135	Hybrid	I-42 / CML547
70	SCHP42	Hybrid	CKDHL0470 / CK21	164	SCHP136	Hybrid	RO549W/CKDHL0295
71	SCHP43	Hybrid	CKDHL0470 / RO549W	165	SCHP137	Hybrid	RO549W/CKDHL0470
72	SCHP44	Hybrid	CKDHL0470 / I-38	166	SCHP138	Hybrid	RO549W/CKL05022
73	SCHP45	Hybrid	CKDHL0470 / I-42	167	SCHP139	Hybrid	RO549W / CML216
74	SCHP46	Hybrid	CKDHL0470 /U2540W	168	SCHP140	Hybrid	RO549W / CML444
75	SCHP47	Hybrid	CKL05022 / I-38	169	SCHP141	Hybrid	RO549W / CML488
76	SCHP48	Hybrid	CKL05022 / I-42	170	SCHP142	Hybrid	RO549W / CML511
77	SCHP49	Hybrid	CKL05022 / RO549W	171	SCHP143	Hybrid	RO549W / CML543
78	SCHP50	Hybrid	CKL05022 / U2540W	172	SCHP144	Hybrid	RO549W / CML544
79	SCHP51	Hybrid	CML202 / CK21	173	SCHP145	Hybrid	RO549W / CML547
80	SCHP52	Hybrid	CML202 / CML442	174	SCHP146	Hybrid	RO549W / CZL068
81	SCHP53	Hybrid	CML202 / I-38	175	SCHP147	Hybrid	U2540W / CB322
82	SCHP54	Hybrid	CML202 / I-42	176	SCHP148	Hybrid	U2540W / CB323
83	SCHP55	Hybrid	CML216 / CML442	177	SCHP149	Hybrid	U2540W/CKDHL0295
84	SCHP56	Hybrid	CML216 / I-42	178	SCHP150	Hybrid	U2540W/CKDHL0378
85	SCHP57	Hybrid	CML216 / RO549W	179	SCHP151	Hybrid	U2540W/CKDHL0470
86	SCHP58	Hybrid	CML216 / U2540W	180	SCHP152	Hybrid	U2540W / CKL05022
87	SCHP59	Hybrid	CML442 / CB322	181	SCHP153	Hybrid	U2540W / CML216
88	SCHP60	Hybrid	CML442 / CB323	182	SCHP155	Hybrid	U2540W / CML511
89	SCHP61	Hybrid	CML442 / CB339	183	SCHP156	Hybrid	U2540W / CML543
90	SCHP62	Hybrid	CML442/CKDHL0089	184	SCHP157	Hybrid	U2540W / CML547
91	SCHP63	Hybrid	CML442 / CKDHL0378	185	SCHP158	Hybrid	CML540 / CZL99017
92	SCHP64	Hybrid	CML442 / CML216	186	SCHP159	Hybrid	CZL0718 / CZL0919
93	SCHP65	Hybrid	CML442 / CML443	187	SCHP160	Hybrid	CML312 / CML443
94	SCHP66	Hybrid	CML442 / CML444	188	SCHP161	Hybrid	I-40 / CML312

5.2.2 Leaf sampling, hybrids' field evaluation, DNA extraction and genotyping

Maize genotypes were planted at ARC-GCI research farm, Potchefstroom (26°74''S; 27°8'E) during the 2017/18 summer season for leaf sampling for DNA extraction. Leaf sampling was done using supplied LGC sampling kit (LGC Genomics Laboratory, United Kingdom). Five to eight leaf discs were taken per entry five weeks after planting for DNA extraction. Leaf samples from the same entry were placed in a specific 2 x 96-well plate with each well representing an individual genotype. Each well was sealed using perforated trip cap and the desiccant sachet was placed directly on top of the strip cap-sealed well and the plastic lid was replaced on top. The storage rack was secured by using an elastic band and was placed inside a sealable plastic bag. The sealed bag was placed into the plant kit box and the samples were shipped to LGC Genomics Laboratory, United Kingdom for genotyping. DNA extraction, amplification and visualization were done according to the LGC protocol (www.lgcgroup.com). Genomic DNA was extracted from the leaf disc samples and the quality and quantity of the extracted DNA was determined. Genotyping was done using 92 SNP markers, distributed across the 10 pairs of the maize chromosomes, following the Kompetitive Allele Specific Polymerase Chain Reaction (KASP) protocol used by LGC Genomics (www.lgcgroup.com).

Field evaluation of single-cross hybrids was done at three locations namely; Potchefstroom (ARC-GCI) in North West province, Cedara in KwaZulu-Natal province and Vaalharts in the Northern Cape province, South Africa during the 2017/18 summer season. The trial constituted of five production environments, Potchefstroom and Cedara represented by two environments (low N and optimum), while Vaalharts had an optimum environment. The 188 maize genotypes were planted in a 4 x 47 (0,1) alpha lattice design with two replications. Each replicate was made up of 47 incomplete blocks with four entries each.

5.2.3 Statistical analyses

The assessment of genetic purity of the inbred lines and their hybrid were tested using SNP markers. Data filtering for monomorphic SNPs and/or SNPs with minor allele frequency of less than 2% were performed and all the 92 SNPs were polymorphic and of high quality. Genetic purity of the parental inbred lines was calculated as percentage residual heterozygosity using the formula described by Gowda *et al.* (2017). Genetic diversity parameters such as observed heterozygosity (Ho), expected heterozygosity (He), and fixation index/ inbreeding coefficient (F_{IS}) were determined using GenAlex version 6 (Peakall and Smouse, 2016). The formula: $PIC = 1 - \Sigma P_{ij}^2$, where P_{ij} is the frequency of j^{th} allele of the i^{th} locus, were used to calculate Polymorphic information content (PIC).

$$\% \, Heterozygosity = \left(\frac{SNP \; with \; heterozygous \; Loci}{Total \; number \; of \; SNPs}\right) 100$$

Genetic relationships within and among the inbred lines were assayed with a neighbour-joining algorithm, using the unweighted pair group method (UWPGM) in DARwin 6.0 software (Perrier and Jacquemoud-Collet, 2006). Pairwise dissimilarity matrices were obtained from the Jaccarrd's coefficient and a dendrogram was generated. For the node construction, a bootstrap analysis was performed, based on 10000 bootstrap values, using DARwin. The distinctiveness of the clusters was checked, using the cophenetic correlation coefficient (r). The parent-offspring relationship for each parent-hybrid pair was tested according to methods described by Gowda *et al*. (2017). Parameters such as proportion of SNPs from parent A and parent B, SNPs shared by both parents and SNPs that do not belong to either of the parent were estimated.

5.3 Results

5.3.1 SNP characterisation

The distributions of values for polymorphic information content, gene diversity, inbreeding coefficient and minor allele frequencies of the 92 SNPs estimated on the 188 maize genotypes are shown in Figure 5.1. Inbreeding coefficient, displayed contrasting values ranging from -0.17 to 1.00, with a mean of 0.10. About 24% of the SNPs showed negative F_{IS} values. Nearly 39% of the SNPs had F_{IS} values between 0.10 and 0.40 (Figure 5.1a). The SNPs diversity ranged from 0.11 to 0.50, however, the vast majority (92%) fell between 0.30 and 0.50 and eight SNPs revealed moderate gene diversity (Figure 5.1b). Approximately 90% of the markers used in this study had PIC values exceeding 0.30, demonstrating the high discriminatory power of the markers. However, the majority of the values (77%) were between 0.40 and 0.50 and only one marker (PZA03527_3) displayed a PIC value less than 0.2 (Figure 5.1c). The minor allele frequency ranged from 0.06 for the marker PZA03527_3 to 0.50 for the marker sh1_12, with a mean of 0.35 (Figure 5.1d). More than 55% of the SNPs revealed a minor allele frequency exceeding the mean (0.35). Observed heterozygosity (H_o) values ranged from 0.0 to 0.56 with a mean of 0.40 (data now shown). SNPs PZA00793_2 and PHM2350_17 had H_o value of 0.0 indicating the alleles of these SNPs were 100% fixed among the maize genotypes, however, 97% of the SNPs had H_o values exceeding 15%. SNP markers PHM2350_17, and PZA00793_2 showed inbreeding coefficient value of 1.

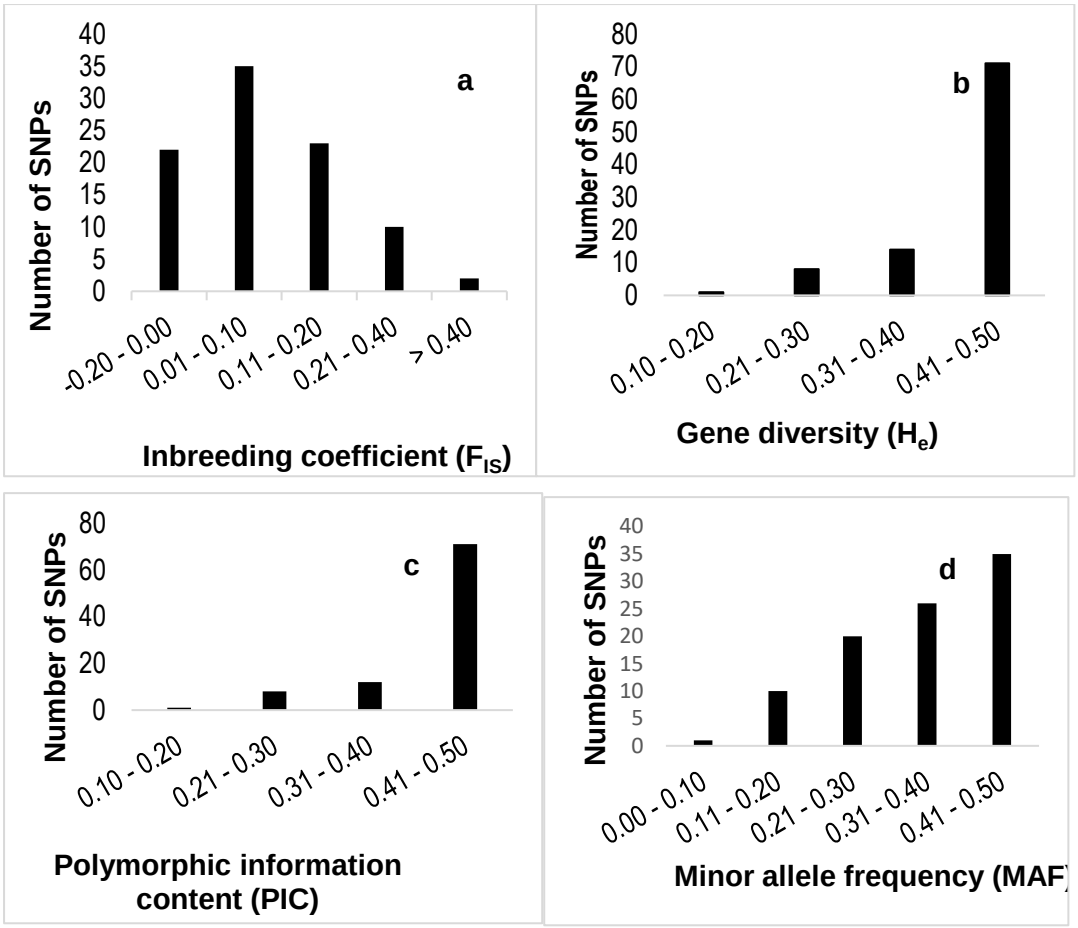

Figure 5.1 Distribution of the 92 SNPs estimated for all 188 maize genotypes for (a) Inbreeding coefficient, (b) Gene diversity, (c) Polymorphic information content and (d) Minor allele frequency

The genetic diversity parameter estimates of the 92 SNPs used in this study summarized per chromosome are presented in Table 5.2. The number of SNPs on each chromosome ranged from six on chromosome 10 to 12 on chromosomes 2 and 5, with a mean of 9.2 SNPs per chromosome. The observed heterozygosity of the SNP loci for the inbred lines ranged from 6% to 11% while the hybrids revealed Ho values ranging from 39% to 49%. The PIC values for the inbred lines varied from 0.30 to 0.49, with a mean of 0.44. The gene diversity values for the inbred lines ranged from 0.42 to 0.49, with a mean gene diversity of 0.45. However, no significant differences were observed in PIC and gene diversity values among the ten chromosomes. The mean inbreeding coefficient (F_{IS}) was significantly higher for inbred lines ranging from 0.75 to 0.85, with a mean value of 0.80 suggesting the majority of the inbred lines were considered to be fixed. The hybrids on the other hand, revealed very low F_{IS} values ranging from -0.02 to 0.17, with a mean value of -0.05 validating an excess of heterozygotes, which is expected for hybrids.

Table 5.2 Summary of genetic diversity parameters of 92 SNPs per chromosome measured in a set of 188 maize genotypes

Chromosome	No. SNPs	Genetic parameters							
		Maize Inbred lines				Hybrids			
		Ho	He	F_{IS}	PIC	Ho	He	F_{IS}	PIC
1	9	0.07	0.42	0.82	0.42	0.41	0.40	-0.03	0.40
2	12	0.11	0.45	0.75	0.44	0.44	0.42	-0.05	0.42
3	11	0.08	0.45	0.80	0.44	0.47	0.44	-0.07	0.43
4	9	0.07	0.45	0.84	0.44	0.45	0.45	-0.02	0.45
5	12	0.11	0.46	0.76	0.45	0.51	0.45	-0.13	0.45
6	9	0.09	0.45	0.79	0.44	0.44	0.42	-0.05	0.42
7	7	0.10	0.42	0.75	0.30	0.49	0.44	-0.12	0.44
8	9	0.08	0.49	0.84	0.49	0.39	0.43	0.17	0.43
9	8	0.06	0.44	0.85	0.43	0.46	0.42	-0.08	0.42
10	6	0.09	0.46	0.81	0.45	0.47	0.44	-0.06	0.44
Overall mean		0.09	0.45	0.80	0.44	0.45	0.43	-0.05	0.43
SE		0.00	0.01	0.01	0.01	0.01	0.01	0.02	0.01

N= Number of individuals tested; H_o= observed heterozygosity; H_e= expected heterozygosity; F_{IS}= inbreeding coefficient; PIC= polymorphic information content; SE= Standard error

5.3.2 Genetic purity of parental maize inbred lines

Table 5.3 presents the residual heterozygosity among 26 maize inbred lines and four doubled haploid lines. The percentage of missing data per SNP in this study was below 3% and varied from 0 to 2.17% with the overall mean of 1.16%. Based on the 92 SNPs, genetic purity among the 30 inbred lines varied from 0.0 to 57.6, with an overall mean of 10.43. All the 92 SNP loci tested in this study were fixed in 60% of the inbred lines demonstrating the high genetic purity of the lines. Out of the 18 genotypes that showed 100% genetic purity, four lines (CKDHL0089, CKDHL0295, CKDHL0378 and CKDHL0470) were doubled haploids. Inbreds CKL05022 and CB323 had heterozygous percentage of less than 5% and these inbred lines were considered to be fixed. However, 33.3% of the inbred lines had residual heterozygosity ranging from 5.43 to 57.61%.

Table 5.3 Genetic purity of 26 maize inbred lines and four doubled haploid lines based on 92 SNPs

Name	% of missing alleles	% of heterozygote alleles
CKDHL0089	2.17	0.00
CKDHL0295	0.00	0.00
CKDHL0378	1.09	0.00
CKDHL0470	0.00	0.00
CML202	1.09	0.00
CML216	1.09	0.00
CML442	1.09	0.00
CML443	1.09	0.00
CB322	2.17	0.00
CML544	0.00	0.00
CML511	0.00	0.00
CZL068	0.00	0.00
I-38	2.17	0.00
I-42	2.17	0.00
CML540	2.17	0.00
CZL99017	1.09	0.00
CML312	2.17	0.00
I-40	1.09	0.00
CKL05022	1.09	2.00
CB323	2.17	4.35
CK21	1.09	5.43
RO549W	2.17	15.22
U2540W	1.09	16.30
CML444	2.17	18.48
CB339	0.00	19.57
CML543	2.17	39.13
CZL0919	1.09	42.39
CML488	0.00	45.65
CML547	0.00	48.91
CZL0718	1.09	57.61
Mean	1.16	10.43
SE	0.16	3.23

5.3.3 Genetic relationship among 30 parental lines

Population structure for the parental lines was assessed using distance-based cluster analyses. Cluster analysis based on Jaccarrd's genetic distance values classified the 30 parental lines into three distinct clusters (Figure 6.2). The distinctiveness of the clusters was

confirmed by the high cophenetic correlation coefficient for SNPs (r = 0.93). Using SNP markers, the highest genetic distance between the parental lines was 0.56 and the lowest was 0.05, while the mean was 0.47 (data not shown). The highest genetic distance (0.56) was found between parental lines CKDHL0089, CML443 and CB323. The lowest genetic distance (0.05) was found between inbred lines I-42, and I-40. The majority (92%) of the genetic distance values fell between 0.40 and 0.60 suggesting the genotypes were moderately and distantly related (Figure 6.3). Cluster I consisted of 10 parental lines and further sub-divided into two sub-clusters. Cluster II also had two sub-clusters comprising of 15 parental lines, while cluster III consisted of five parental lines. Overall, the cluster analysis was effective in discriminating the parental lines into groups and in providing genetic information for breeding and conservation. In this analysis three sets of parental lines with different genetic backgrounds were included. The clustering patterns of the parental lines was according to their genetic background. All the CB inbred lines (CB323, CB322 and CB 339) were grouped in Cluster II sub-cluster 1, while all the I inbred lines (I-40, I-42, I-38) were clustered in Cluster I sub-cluster 1. Similarly, all the CZL lines (CZL068, CZL0718 and CZL99017) except CZL0919 were clustered in Cluster I. The two CML heterotic tester lines, CML444 and CML202, which belong to heterotic groups A and B, respectively, were assigned in different clusters.

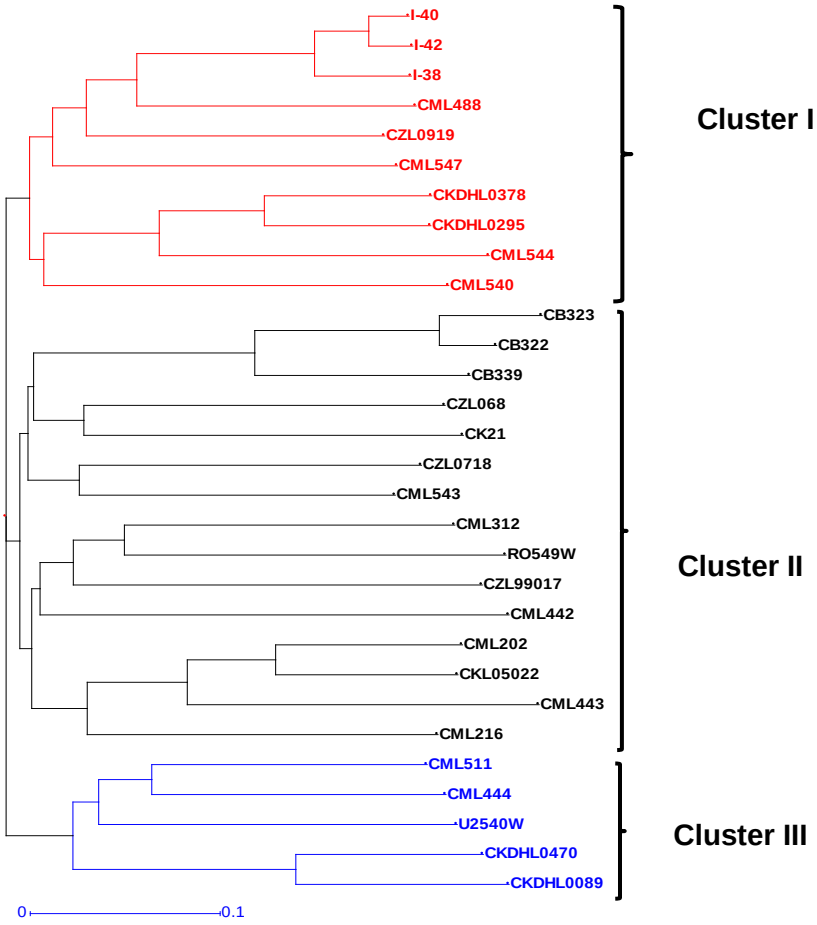

Figure 5.2 Neighbor-joining dendograms based on UPGMA genetic dissimilarity depicting genetic relationship between 30 maize parental lines based on 92 SNPs

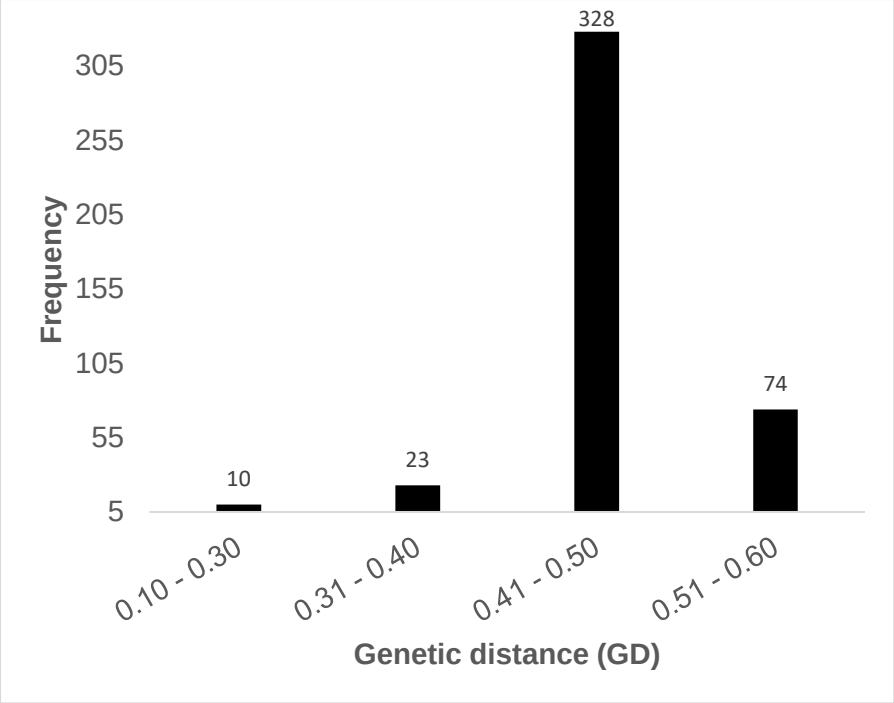

Figure 5.3 Genetic distance data summary of 30 maize parental lines using 92 SNPs

5.3.4 Parent-offspring test and grain yield performance for the selected maize hybrids (the top and bottom 10%)

Parent offspring verification test revealed that out of 158 single-cross hybrids tested,16 hybrids had 0% contamination, 96 hybrids registered contamination level within the range of 0.54% to 4.89% (<5%) and 46 hybrids had contamination greater than 5% (Appendix 5.1). Further quality analysis revealed that some of the ten contaminated lines were used for 90 single-cross hybrids. The test also confirmed that the remaining 68 single-cross hybrids were generated using pure parental inbred lines with acceptable levels of genetic contamination (<5%).

The hybrids were evaluated for grain yield performance and the top and bottom 10% performing hybrids are presented in Table 5.3. Grain yield observed from 158 single-cross hybrids ranged from 2.83 t ha^{-1} for SCHP115 to 7.33 t ha-1 for SCHP17, with a mean yield of 5.55 t ha^{-1}. The parent-offspring test was performed based on the criteria of < 5% genetic contamination on at least one of the parents and their hybrids. Based on the above criteria, of the 15 top performing hybrids, seven hybrids (SCHP29, SCHP95, SCHP94, SCHP134, SCHP44, SCHP114 and SCHP126) passed the test and represented 47% of the hybrids. Similarly, among the bottom 15 performing hybrids, five hybrids (SCHP113, SCHP102, SCHP32, SCHP71, and SCHP15) fulfilled the requirement. Notably, hybrids SCHP29 and

SCHP115 among the top 10% and bottom 10% performing hybrids, respectively, exhibited genetic contamination of 0%.

Table 5.3 Parent-offspring test for top and botton 10% performing hybrids based on grain yield

Hybrid	Pedigree	% SNPs parent A	% SNPs parent B	% alleles shared by both	% contamination	GY (t ha-^{1})
Top 10 %						
SCHP17	CK21/CKDHL0089	25.54	28.26	43.48	1.63	7.33
SCHP11	CB339/CML442	22.83	23.91	51.63	0.54	6.89
SCHP125	I-42/CB339	26.09	26.63	46.20	1.09	6.85
SCHP29	CKDHL0089/CML442	23.91	25.54	49.46	0.00	6.77
SCHP42	CKDHL0470/CK21	28.80	27.17	39.67	2.17	6.76
SCHP95	CML544/I-38	21.74	18.48	57.07	1.63	6.63
SCHP94	CML544 CML442	26.09	24.46	46.20	1.09	6.62
SCHP61	CML442/CB339	25.00	23.37	51.63	0.00	6.56
SCHP22	CK21/CML444	30.98	13.59	41.85	13.59	6.49
SCHP134	I-42/CML544	17.39	22.83	54.89	3.80	6.47
SCHP44	CKDHL0470/I-38	26.09	22.28	50.00	0.54	6.37
SCHP24	CK21/CML511	21.74	21.20	55.43	1.63	6.35
SCHP1	CB322/CK21	25.00	22.28	47.83	4.89	6.33
SCHP114	I-38/CML202	22.28	24.46	53.26	0.54	6.33
SCHP126	I-42/CKDHL0295	20.11	28.80	49.46	1.63	6.32
Bottom 10%						
SCHP26	CK21 / CML547	22.28	22.28	54.35	1.09	4.75
SCHP155	U2540W / CML511	11.96	23.91	50.00	10.87	4.68
SCHP113	I-38 / CKL05022	20.11	21.20	57.07	0.54	4.68
SCHP102	CZL068 / I-38	23.91	22.28	52.72	1.09	4.67
SCHP84	CML488 / U2540W	30.43	11.96	45.11	12.50	4.65
SCHP135	I-42 / CML547	14.67	21.20	60.87	2.17	4.53
SCHP32	CKDHL0089/ I-38	26.09	26.09	45.65	1.09	4.47
SCHP27	CK21 / CZL068	20.65	19.02	58.70	0.54	4.42
SCHP100	CML547 / U2540W	23.37	22.83	38.04	10.33	4.40
SCHP142	RO549W / CML511	20.11	29.35	44.02	5.43	4.39
SCHP71	CML442 / CZL068	23.37	21.74	51.09	0.54	4.38
SCHP139	RO549W / CML216	17.39	31.52	42.39	8.70	3.93
SCHP101	CZL068 / CK21	16.85	17.39	54.35	0.54	3.70
SCHP83	CML488 / RO549W	29.89	23.37	42.39	4.35	3.28
SCHP115	I-38 / CML216	19.57	21.74	57.61	0.00	2.83
Mean		23.73	23.48	48.05	3.6	5.55
SE		0.46	0.45	0.55	0.31	0.67

5.4 Discussion

5.4.1 SNP characterisation

In this study the percentage of missing data was lower than 3% and 82% of the SNPs had a minor allele frequency of >0.25. The PIC value of 90% of the SNPs was above 0.30 and 77% of the SNPs had PIC values falling between 0.40 and 0.50, with a mean PIC value of 0.44 confirming that the markers were effective in discriminating the genotypes. The PIC range of 0.18 to 0.50 and mean value of 0.44 reported in this study is on the higher side than the PIC values reported by other scientist who conducted similar studies. Hao *et al.* (2011) reported PIC values within the range of 0.01 to 0.38 using 1536 SNP markers on 95 parental inbred lines in maize. Correspondingly, Yang *et al.* (2011) reported PIC value range of 0.27 to 0.38 with a mean of 0.34 while using 884 SNP markers. The mean PIC value observed in this study was comparable to the one reported by Adeyemo and Omidiji (2013) of 0.43 using 122 tropical yellow endosperm maize inbred lines using 66 SSR markers. However, PIC values obtained in this study are by far higher than those reported in previous studies (Lu *et al*., 2009; Dao *et al.,* 2014; Nyombayire *et al*., 2016). Abakemal *et al*. (2015) indicted that PIC gives an estimate of the discriminatory ability and effectiveness of a particular marker with respect to the number of alleles that are expressed and their relative frequencies. Lander and Botstein (1989) described PIC mean value of >0.50 as highly informative, 0.25-0.50 moderately informative and <0.25 is slightly informative. Hence, the mean PIC value of 0.44 in this study confirms that the markers were reasonably informative and of high quality. This is attributed to the fact that the 92 SNPs used in this study were a subset of the 100 SNPs distributed across the 10 chromosomes in the maize genome, which were carefully selected and recommended by CIMMYT for quality control genotyping.

5.4.2 Cluster analysis of the maize parental lines

In this study, three sets of inbred lines were included (CB, I and CZL lines). Using the 92 SNP markers, the inbred lines were clustered in three distinct genetic groups (r=0.93). The clustering pattern clearly classified and correctly, differentiated three sets of lines according to their genetic background suggesting that the SNPs used in this particular study were discriminatory. Cluster analysis also revealed the presence of sufficient diversity among the inbred lines tested. Clustering of inbred lines within the same genetic cluster suggest that the lines share a similar genetic background and same ancestral lines while those inbred lines grouped in different clusters are distantly related. This implies that, when selecting parents for

crossing, it is recommended to involve parents originating from different genetic clusters since they are distantly related genetically, hence capable of expressing higher heterosis.

5.4.3 Genetic purity of the parental lines

In cross-pollinated crops such as maize, genetic purity is an important quality control criterion in hybrid breeding and seed production. Assessment of the genetic purity of parental inbred lines is vital before starting hybrid breeding programmes. The main purpose of routine quality control genotyping is to identify contamination during hybrid development, seed increase or seed distribution (Semagn *et al.,* 2012). According to Semagn *et al.* (2012), inbred lines are regarded pure or fixed if the proportion of heterozygous SNP loci does not go beyond 5%. Inbred lines are also expected to maintain all the genetic characters that the breeder selected them for. Significant change in genetic constitution of the inbred lines affects both the quality of hybrid seed and development of new hybrids. In the current study, the genetic purity ranged from 0.0 to 57.61% with a mean 10.43%. Majority of the tested parental lines (67%) exhibited a residual heterozygosity less than 5%, of which 18 parental lines attained 100% homozygosity, of which four lines are doubled haploids.

The fact that all the doubled haploid (DH) lines (CKDHL0089, CKDHL0295, CKDHL0378 and CKDHL0470) used in this study exhibited 100% homozygosity indicates the advantages of using the DH approach in line development. The DH approach enhances breeding efficiency through rapid generation of homozygous lines which are more reliable and predictive than those developed using conventional methods (Forster and Thomas 2005; Geiger and Gordillo 2009; Chang and Coe, 2009). This approach significantly shortens the breeding cycle though rapid development of fixed lines in two to three generations unlike the conventional approach that requires six to eight generations of inbreeding with approximately 99% homozygosity. In this study, it was confirmed that 14 of the inbred lines (CB323, CML202, CML216, CML442, CML443, CML511, CML544, CZL068, I-38, 1-42, CML 540, CZL99017, CML312 and I-40) generated through the conventional system also exhibited 100% homozygosity. This affirms that the maintenance of these inbred lines was carefully done for several generations of selfing for seed increase. Similar findings were reported by Dao (2013) in his study using 1237 SNPs, where the majority of the inbred lines tested exhibited 100% homozygosity. However, Ertiro *et al*. (2017) reported that of the 265 inbred lines tested using 22,787 SNPs, only 22% of the inbred lines had 99.9% genetic purity. The higher level of homogeneity observed in most of the inbred lines used in this study indicates how strong and efficient the ARC maize breeding programme is in terms of inbred line development and maintenance.

Inbred line CK21 had residual heterozygosity of 5.43%, which is slightly higher than the threshold of 5%. It is currently used as a parent of a recently released hybrid and should therefore be purified using ear-to-row selection methods. Lines with more than 15% residual heterozygosity are likely to have been contaminated with pollen from unrelated genetic materials and should be discarded (Gowda *et al.,* 2017). The higher level of heterogeneity observed in some of the inbred lines may be attributed to either that the inbred line is in the early generation of inbreeding or there was pollen contamination and/or seed admixture during maintenance breeding. An additional generation of inbreeding and extensive selection is highly recommended in order to fix these inbred lines. Some reports suggest that due to the strong inbreeding depression, higher levels of residual heterozygosity may have been deliberately maintained at early breeding level. Ertiro *et al.* (2017) also reported higher levels of residual heterozygosity among the inbred lines tested from Ethiopian Institute of Agricultural Research (EIAR) due to use of early generation inbred lines (S4). Heckenberger *et al*. (2002) and Warburton *et al.* (2010), on the other hand, suggested that high level of residual heterozygosity may occur due to pollen contamination and/or seed mixture during seed regeneration, maintenance and bulking.

5.4.4 Parent-offspring test and grain yield performance of the tested hybrids

The rationale for doing parent-offspring test is to confirm if the particular hybrid is the true resultant F1 hybrid derived from the original inbred lines with no pollen contamination or within acceptable contamination levels (Gowda *et al.*, 2017). The test provides a means to check if the pollination exercise was done correctly during hybrid development. During hybridisation, there is a possibility of pollen contamination arising from self-pollination or cross-pollination from undesired neighbouring crops due to inadequate isolation distance. Therefore, parent-offspring test is important to ensure production of genuine quality hybrid seed.

The results showed that out of 158 experimental hybrids tested, 90 failed the test due to higher percentage of foreign contamination of greater than 5%. This could be attributed partly to the use of genetically impure parental inbred lines or partly due to pollen contamination during hybrid production. Our results revealed that the resultant hybrids derived from segregating inbred lines exhibited higher levels of genetic contamination of greater than 5%. In addition, it was evident that there was genetic contamination due to lack of pollen control between the crossing block and the neighbouring field since hybrids developed from pure inbred lines revealed high level of contamination. Daniel *et al*. (2012) reported a similar trend, where inbred lines with higher percentage of residual heterozygosity resulted in hybrids with higher

contamination percentage. The results clearly confirm that the purity level of the parental inbred lines determines the purity of the hybrids.

Parent inbred line and hybrid seed purity are critical parameters for high agronomic performance (Bonan 1991). In maize hybrid breeding, technical competency in plant and ear selection is vital during growing in the field and at harvest to eliminate off-type plants (Craig 1977).

To produce high quality pure hybrid seed, isolation distance is key to avoid pollen contamination. Ipsilandis *et al*. (2005) recommended seed purity and germination tests to ensure the physical quality of seeds. The main source of genetic impurity of hybrid seed comes from incomplete detasseling of the mother inbred line (Moreiral *et al.,* 2010). However, this kind of impurity is usually tolerated at a level of 3-5%, without any effect on the yield performance. In the current study 90 experimental hybrids were discarded due to high contamination of the parental inbred lines. The low level of hybrid performance detected in this study may be attributed to the use of the genetically impure inbred parental lines. Among the 15 top performing hybrids, only seven hybrids passed the parent-offspring test. Ipsilandis *et al.* (2005) reported that agronomic performance of different genetic materials in terms of yield was significantly influenced by the seed purity conditions. It further justified that pure hybrid seed had better competitive ability and yielded better than low purity seed.

5.5 Conclusion

This study showed that the set of SNP markers recommended for quality control test were effective and reliable in assessing genetic purity. The results of study will be helpful in the verification of genetic purity of maize hybrid seed. The inbred lines used in the present study were expected to be genetically pure with not more than 5% residual heterozygosity, but 33% of inbred lines showed residual heterozygosity of greater than 5% which requires additional generations of purification. Parent-offspring test conducted on 158 experimental hybrids led to the elimination of 60% hybrids since at least one of their parental inbred lines failed the genetic purity test. Of the 30% of the hybrids that passed the quality control test, seven high yield potential hybrids were identified for further evaluation and release. Failure of some genotypes to pass inbred line genetic purity test and parent-offspring test suggests the need for further quality improvement by ARC-GCI maize-breeding programme in the breeding nurseries and during pollinations for hybrid production.

5.6 References

Abakemal, D., S. Hussein, J. Derera and K. Semagn. 2015. Genetic purity and patterns of relationships among tropical highland adapted quality protein and normal maize inbred lines using microsatellite markers. Euphytica 204: 49-61.

Adeyemo O. and O. Omidiji 2013. SSR-based and carotenoid diversity assessment of tropical yellow endosperm maize inbred lines. Plant Genetic Resources 12: 67-73. doi:10.1017/S1479262113000294.

Bonan G.B. 1991. Density effects on the size of annual plant populations: An indication of neighbourhood competition. Annals of Botany 68: 341-347.

Chang, M.-T. and E.H. Coe. 2009. Doubled haploids. Molecular genetic approaches to maize improvement. Springer. p. 127-142.

Chen J., C. Zavala, N. Ortega, C. Petroli, J. Franco, J. Burgueño, D.E. Costich and S.J. Hearne 2016. The development of quality control genotyping approaches: A case study using elite maize lines. PLoS ONE 11(6):e0157236. doi:10.1371/journal.pone.0157236.

Craig W.F. 1977. Production of Hybrid Corn Seed. In: Sprague G.F. (Ed.) Corn and Corn Improvement. American Society of Agronomy, Madison, WI., pp: 305-362.

Daniel, I., J. Adetumbi, O. Oyelakin, S. Olakojo, M. Ajala and S. Onagbesan. 2012. Application of SSR markers for genetic purity analysis of parental inbred lines and some commercial hybrid maize *(Zea mays L.).* American Journal of Experimental Agriculture 2: 597-606

Dao A., J. Sanou, S.E. Mitchell, V.Gracen and E.Y. Danquah 2014. Genetic diversity among INERA maize inbred lines with single nucleotide polymorphism (SNP) markers and their relationship with CIMMYT, IITA, and temperate lines. BMC Genetics 15: 127. doi:10.1186/s12863-014-0127-2.

Dao, A. 2013. Genetic characterization, association mapping and evaluation of feterotic patterns of tropical maize *(Zea mays* L*.)* inbred lines under drought and non-drought conditions. PhD thesis,University of Ghana

Ertiro, B.T., K. Semagn, B. Das, M. Olsen, M. Labuschagne, M. Worku et al. 2017. Genetic variation and population structure of maize inbred lines adapted to the mid-altitude sub-humid maize agro-ecology of Ethiopia using single nucleotide polymorphic (SNP) markers. BMC Genomics 18: 777-779

Ertiro, B.T., V. Ogugo, M. Worku, B. Das, M. Olsen, M. Labuschagne et al. 2015. Comparison of Kompetitive Allele Specific PCR (KASP) and genotyping by sequencing (GBS) for quality control analysis in maize. BMC genomics 16: 908-910

Forster, B.P. and W.T. Thomas. 2005. Doubled haploids in genetics and plant breeding. Plant Breed Reviews 25: 57-88.

Geiger, H. and G. Gordillo. 2009. Doubled haploids in hybrid maize breeding. Maydica 54: 485-499.

Gowda, M., M. Worku, S.K. Nair, N. Palacios-Rojas, G. Huestis and B. Prasanna. 2017. Quality Assurance/Quality Control (QA/QC) in Maize Breeding and Seed Production: Theory and Practice. CIMMYT

Hamblin, M.T., M.L. Warburton and E.S. Buckler. 2007. Empirical comparison of simple sequence repeats and single nucleotide polymorphisms in assessment of maize diversity and relatedness. PloS one 2: e1367.

Hao, Z., X. Li, C. Xie, J. Weng, M. Li, D. Zhang et al. 2011. Identification of functional genetic variations underlying drought tolerance in maize using SNP markers. Journal of Integrative Plant Biology 53: 641-652.

Heckenberger, M., M. Bohn, J.S. Ziegle, L.K. Joe, J.D. Hauser, M. Hutton et al. 2002. Variation of DNA fingerprints among accessions within maize inbred lines and implications for identification of essentially derived varieties. Molecular Breeding 10: 181-191.

Ipsilandis C.G., B.N. Vafias , A. Karagiozopoulou and C.K. Goulas. 2005. F1 Single-cross maize hybrid performance under low purity conditions. Asian Journal of Plant Sciences 4: 75-82.

Lander, E.S. and D. Botstein. 1989. Mapping mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121: 185-199.

LGC Genomics. www.lgcgroup.com (accessed 17/02/2018).

Lu Y., J.Yan, C.T. Guimarães, S. Taba, Z. Hao, S. Gao, S. Chen, J. Li, S. Zhang, B.S. Vivek, C. Magorokosho, S. Mugo, D. Makumbi, S.N. Parentoni, T. Shah, T. Rong, J.H. Crouch and Y. Xu. 2009. Molecular characterization of global maize breeding germplasm based on genome-wide single nucleotide polymorphisms. Theoretical and Applied Genetics 120: 93-115. doi:10.1007/s00122-009-1162-7.

Makongwana, M.P. 2016. GGE-Biplot and genetic diversity analysis of maize hybrids and inbred lines from the breeding programme at UKZN.

Moreiral J.N., P.S.L. Silval, K.M.B. SilvaII, J.L.D. DombroskiI and R.S. CastroI. 2010. Effect of detasseling on baby corn, green ear and grain yield of two maize hybrids. Horticultura Brasileira 28: 406-411.

Ndhlela, T. 2012. Improvement strategies for yield potential, disease resistance and drought tolerance of Zimbabwean maize inbred lines. PhD desertation ,University of the Free State.

Nyombayire A., J. Derera, , J. Sibiya, E. Gasura and C.N. Aboyisonga. 2016. Genetic diversity among maize inbred lines selected for the mid- altitudes and highlands of Rwanda. Maydica 61(M17): 1-7.

Nzuve, F., S. Githiri, D. Mukunya and J. Gethi. 2013. Analysis of genotype x environment interaction for grain yield in maize hybrids. Journal of Agricultural Science 5: 75-85

Peakall, R and Smouse P. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes 6: 288-295.

Perrier, X. and J. Jacquemoud-Collet. 2006. DARwin software: Dissimilarity analysis and representation for windows. Website http://darwin. cirad. fr/darwin [accessed 1 September 2018].

Salgado, K.C.P.d.C., d.G.G.C. Vieira, É.V.d.R. Von Pinho, C.T. Guimarães, R.G. Von Pinho and L.V.d. Souza. 2006. Genetic purity certificate in seeds of hybrid maize using molecular markers. Revista Brasileira de Sementes 28: 169-175

Semagn, K., Å. Bjørnstad and M. Ndjiondjop. 2006. An overview of molecular marker methods for plants. African Journal of Biotechnology 5:2540–2568

Semagn, K., Y. Beyene, D. Makumbi, S. Mugo, B. Prasanna, C. Magorokosho, G. Atlin 2012. Quality control genotyping for assessment of genetic identity and purity in diverse tropical maize inbred lines. Theoretical and Applied Genetics 125: 1487-1501.

Shiferaw, B., B.M. Prasanna, J. Hellin and M. Bänziger. 2011. Crops that feed the world 6. Past successes and future challenges to the role played by maize in global food security. Food Security 3: 307-327

Warburton, M.L., P. Setimela, J. Franco, H. Cordova, K. Pixley, M. Bänziger et al. 2010. Toward a cost-effective fingerprinting methodology to distinguish maize open-pollinated varieties. Crop Science 50: 467-477

Yang, X., Y. Xu, T. Shah, H. Li, Z. Han, J. Li et al. 2011. Comparison of SSRs and SNPs in assessment of genetic relatedness in maize. Genetica 139: 1045.

5.7 Appendices

APPENDIX 5.1

Parent-offspring test for the evaluated 158 maize hybrids

Hybrid	Pedigree	% SNPs parent A	% SNPs parent B	% SNPs shared by both	% SNPs belong to neither parents
SCHP7	CB323 / I-38	26.09	26.09	47.83	0.00
SCHP19	CK21 / CKDHL0378	20.11	23.91	55.98	0.00
SCHP20	CK21 / CML202	20.65	22.28	57.07	0.00
SCHP28	CKDHL0089 / CK21	29.35	26.63	44.02	0.00
SCHP29	CKDHL0089/ CML442	23.91	25.54	49.46	0.00
SCHP33	CKDHL0295/ CK21	23.91	20.11	55.98	0.00
SCHP52	CML202 / CML442	27.17	25.00	47.83	0.00
SCHP60	CML442 / CB323	27.72	25.54	46.74	0.00
SCHP61	CML442 / CB339	25.00	23.37	51.63	0.00
SCHP65	CML442 / CML443	24.46	24.46	48.91	0.00
SCHP68	CML442 / CML511	28.8	26.63	44.57	0.00
SCHP73	CML443 / CML442	24.46	25.54	48.91	0.00
SCHP86	CML511 / CML442	27.17	28.26	44.57	0.00
SCHP93	CML544 / CK21	27.72	25.54	45.65	0.00
SCHP115	I-38 / CML216	19.57	21.74	57.61	0.00
SCHP133	I-42 / CML511	2.17	29.89	66.85	0.00
SCHP6	CB323 / CML442	25.00	28.26	46.20	0.54
SCHP11	CB339 / CML442	22.83	23.91	51.63	0.54
SCHP12	CB339 / I-38	24.46	21.20	52.72	0.54
SCHP27	CK21 / CZL068	20.65	19.02	58.70	0.54
SCHP36	CKDHL0295 / I-38	21.20	17.93	60.33	0.54
SCHP39	CKDHL0378 / I-38	24.46	20.65	54.35	0.54
SCHP44	CKDHL0470 / I-38	26.09	22.28	50.00	0.54

Hybrid	Pedigree	% SNPs parent A	% SNPs parent B	% SNPs shared by both	% SNPs belong to neither parents
SCHP47	CKL05022 / I-38	22.28	19.57	57.61	0.54
SCHP51	CML202 / CK21	21.20	19.57	56.52	0.54
SCHP53	CML202 / I-38	24.46	21.74	53.26	0.54
SCHP64	CML442 / CML216	24.46	23.91	50.00	0.54
SCHP67	CML442 / CML488	20.65	26.09	50.54	0.54
SCHP69	CML442 / CML544	25.54	26.63	47.28	0.54
SCHP70	CML442 / CML547	25.00	23.37	51.09	0.54
SCHP71	CML442 / CZL068	23.37	21.74	51.09	0.54
SCHP81	CML488 / CML442	27.17	22.28	50.00	0.54
SCHP82	CML488 / I-38	25.54	7.07	65.76	0.54
SCHP98	CML547 / CML442	22.28	23.91	51.09	0.54
SCHP101	CZL068 / CK21	16.85	17.39	54.35	0.54
SCHP110	I-38 / CKDHL0295	17.93	21.2	60.33	0.54
SCHP111	I-38 / CKDHL0378	20.65	24.46	54.35	0.54
SCHP112	I-38 / CKDHL0470	23.91	25.54	50.00	0.54
SCHP113	I-38 / CKL05022	20.11	21.20	57.07	0.54
SCHP114	I-38 / CML202	22.28	24.46	53.26	0.54
SCHP117	I-38 / CML444	26.09	26.09	46.2	0.54
SCHP119	I-38 / CML511	6.52	24.46	67.39	0.54
SCHP123	I-38 / CZL068	20.11	21.20	58.15	0.54
SCHP160	CML312 / CML443	21.74	25.00	48.37	0.54
SCHP18	CK21 / CKDHL0295	20.11	23.91	54.89	1.09
SCHP23	CK21 / CML488	19.57	27.72	51.63	1.09
SCHP26	CK21 / CML547	22.28	22.28	54.35	1.09
SCHP32	CKDHL0089/ I-38	26.09	26.09	45.65	1.09
SCHP38	CKDHL0378 / CML442	23.91	22.28	52.72	1.09
SCHP45	CKDHL0470 / I-42	28.26	22.83	47.83	1.09
SCHP48	CKL05022 / I-42	22.28	20.65	53.80	1.09

Hybrid	Pedigree	% SNPs parent A	% SNPs parent B	% SNPs shared by both	% SNPs belong to neither parents
SCHP55	CML216 / CML442	24.46	23.91	50.54	1.09
SCHP63	CML442 / CKDHL0378	22.83	23.91	52.17	1.09
SCHP74	CML443 / I-38	27.17	26.63	45.11	1.09
SCHP75	CML443 / I-42	28.80	25.00	44.02	1.09
SCHP78	CML444 / I-38	22.28	25.00	51.63	1.09
SCHP80	CML488 / CK21	28.26	20.11	50.54	1.09
SCHP90	CML543 / I-38	21.74	23.37	53.8	1.09
SCHP94	CML544 / CML442	26.09	24.46	46.20	1.09
SCHP102	CZL068 / I-38	23.91	22.28	52.72	1.09
SCHP107	I-38 / CB323	26.09	23.91	46.74	1.09
SCHP108	I-38 / CB339	21.20	23.37	53.26	1.09
SCHP109	I-38 / CKDHL0089	25.00	26.09	46.74	1.09
SCHP116	I-38 / CML443	25.00	39.67	46.20	1.09
SCHP121	I-38 / CML544	23.37	21.20	54.35	1.09
SCHP122	I-38 / CML547	18.48	20.65	57.61	1.09
SCHP124	I-42 / CB323	22.28	23.91	52.72	1.09
SCHP125	I-42 / CB339	26.09	26.63	46.20	1.09
SCHP127	I-42 / CKL05022	17.39	21.20	59.24	1.09
SCHP158	CML540 / CZL99017	22.28	22.28	52.17	1.09
SCHP15	CK21 / CB323	27.17	26.09	44.02	1.63
SCHP17	CK21 / CKDHL0089	25.54	28.26	43.48	1.63
SCHP21	CK21 / CML443	20.65	26.63	51.09	1.63
SCHP24	CK21/ CML511	21.74	21.20	55.43	1.63
SCHP40	CKDHL0378 / I-42	23.91	17.39	55.98	1.63
SCHP59	CML442 / CB322	30.43	22.83	45.11	1.63
SCHP66	CML442 / CML444	28.26	22.83	46.20	1.63
SCHP72	CML443 / CK21	25.00	22.83	50.54	1.63
SCHP87	CML511 / I-38	22.83	22.28	53.26	1.63

Hybrid	Pedigree	% SNPs parent A	% SNPs parent B	% SNPs shared by both	% SNPs belong to neither parents
SCHP95	CML544 / I-38	21.74	18.48	57.07	1.63
SCHP103	CZL068 / I-42	23.91	19.02	52.17	1.63
SCHP126	I-42 / CKDHL0295	20.11	28.80	49.46	1.63
SCHP130	I-42 / CML443	16.85	23.37	33.15	1.63
SCHP131	I-42 / CML444	24.46	31.52	42.39	1.63
SCHP5	CB323 / CK21	25.54	28.26	44.02	2.17
SCHP16	CK21 / CB339	23.37	23.91	50.54	2.17
SCHP25	CK21 / CML544	27.72	23.91	44.02	2.17
SCHP30	CKDHL0089 / RO549W	40.22	20.11	37.5	2.17
SCHP42	CKDHL0470 / CK21	28.80	27.17	39.67	2.17
SCHP85	CML511 / CK21	21.20	20.65	55.98	2.17
SCHP120	I-38 / CML543	21.74	21.20	52.72	2.17
SCHP129	I-42 / CML216	21.74	30.43	45.65	2.17
SCHP135	I-42 / CML547	14.67	21.20	60.87	2.17
SCHP8	CB323 / I-42	29.89	23.91	43.48	2.72
SCHP37	CKDHL0295 / I-42	22.28	15.22	58.70	2.72
SCHP56	CML216 / I-42	27.72	20.11	49.46	2.72
SCHP96	CML544 / I-42	23.37	17.93	55.98	2.72
SCHP54	CML202 / I-42	30.43	21.20	45.11	3.26
SCHP99	CML547 / RO549W	34.24	19.02	40.22	3.26
SCHP106	I-38 / CB322	23.37	20.65	50.54	3.26
SCHP14	CK21 / CB322	22.83	24.46	48.91	3.80
SCHP128	I-42 / CML202	17.39	27.72	50.00	3.80
SCHP134	I-42 / CML544	17.39	22.83	54.89	3.80
SCHP136	RO549W / CKDHL0295	20.11	30.98	32.07	3.80
SCHP144	RO549W / CML544	23.91	32.61	35.33	3.80
SCHP57	CML216 / RO549W	25.54	19.57	49.46	4.35
SCHP83	CML488 / RO549W	29.89	23.37	42.39	4.35

Hybrid	Pedigree	% SNPs parent A	% SNPs parent B	% SNPs shared by both	% SNPs belong to neither parents
SCHP161	I-40 / CML312	20.65	23.37	49.46	4.35
SCHP1	CB322 / CK21	25.00	22.28	47.83	4.89
SCHP2	CB322 / I-42	23.91	21.20	50.00	4.89
SCHP88	CML511 / I-42	22.83	18.48	50.54	4.89
SCHP145	RO549W / CML547	21.20	34.24	39.67	4.89
SCHP89	CML511 / RO549W	28.80	21.74	44.02	5.43
SCHP97	CML544 / RO549W	35.87	23.37	35.33	5.43
SCHP138	RO549W / CKL05022	17.93	26.63	48.91	5.43
SCHP142	RO549W / CML511	20.11	29.35	44.02	5.43
SCHP49	CKL05022 / RO549W	28.80	16.85	48.37	5.98
SCHP137	RO549W / CKDHL0470	18.48	32.61	41.85	5.98
SCHP9	CB323 / RO549W	23.37	23.91	45.11	6.52
SCHP43	CKDHL0470 / RO549W	36.41	13.59	42.39	6.52
SCHP77	CML444 / CML442	13.04	38.04	40.22	6.52
SCHP92	CML543 / U2540W	34.78	10.33	47.28	6.52
SCHP146	RO549W / CZL068	14.67	32.07	45.65	6.52
SCHP152	U2540W / CKL05022	16.85	33.15	36.96	6.52
SCHP50	CKL05022 / U2540W	33.70	18.48	40.22	7.61
SCHP104	CZL068 / RO549W	32.61	16.30	40.22	7.61
SCHP150	U2540W / CKDHL0378	15.22	35.33	40.76	7.61
SCHP159	CZL0718 / CZL0919	21.74	13.04	54.35	7.61
SCHP3	CB322 / RO549W	23.37	20.11	48.37	8.15
SCHP10	CB323 / U2540W	35.33	21.20	34.24	8.15
SCHP13	CB339 / U2540W	35.87	11.96	42.93	8.15
SCHP35	CKDHL0295 / U2540W	35.33	11.96	44.57	8.15
SCHP58	CML216 / U2540W	33.15	18.48	39.13	8.15
SCHP147	U2540W / CB322	18.48	36.96	36.41	8.15
SCHP46	CKDHL0470 / U2540W	30.43	12.50	47.28	8.70

Hybrid	Pedigree	% SNPs parent A	% SNPs parent B	% SNPs shared by both	% SNPs belong to neither parents
SCHP91	CML543 / RO549W	27.72	15.22	48.37	8.70
SCHP118	I-38 / CML488	33.15	15.22	41.85	8.70
SCHP139	RO549W / CML216	17.39	31.52	42.39	8.70
SCHP141	RO549W / CML488	15.76	35.87	39.67	8.70
SCHP153	U2540W / CML216	15.76	35.87	37.50	8.70
SCHP41	CKDHL0378 / U2540W	33.15	16.30	39.13	9.24
SCHP143	RO549W / CML543	13.04	27.72	47.83	9.24
SCHP149	U2540W / CKDHL0295	15.76	30.43	41.30	9.24
SCHP156	U2540W / CML543	17.93	33.70	39.13	9.24
SCHP157	U2540W / CML547	14.13	30.43	42.93	9.24
SCHP31	CKDHL0089/ U2540W	25.54	14.13	49.46	9.78
SCHP151	U2540W / CKDHL0470	17.39	26.63	45.11	9.78
SCHP76	CML444 / CK21	17.39	29.89	42.39	10.33
SCHP100	CML547 / U2540W	23.37	22.83	38.04	10.33
SCHP148	U2540W / CB323	17.93	39.13	32.61	10.33
SCHP62	CML442 / CKDHL0089	39.67	11.96	37.5	10.87
SCHP155	U2540W / CML511	11.96	23.91	50.00	10.87
SCHP105	CZL068 / U2540W	27.72	26.09	32.61	11.41
SCHP84	CML488 / U2540W	30.43	11.96	45.11	12.50
SCHP132	I-42 / CML488	26.09	15.76	40.76	13.04
SCHP22	CK21 / CML444	30.98	13.59	41.85	13.59
SCHP79	CML444 / I-42	17.93	27.17	36.41	18.48
SCHP140	RO549W / CML444	18.48	19.57	42.39	19.57
Mean		23.73	23.48	48.05	3.60
SE		0.46	0.45	0.55	0.31

APPENDIX 5.2

Genetic parameter estimates measured on the 188 maize genotypes

SNPs	N	Ho	He	F_{IS}	PIC
PZA00175_2	30	0.03	0.44	0.92	0.43
PZA00495_5	30	0.13	0.50	0.73	0.49
PZA03409_1	30	0.03	0.49	0.93	0.49
PZA00643_13	30	0.10	0.51	0.80	0.50
PZA00981_3	30	0.10	0.51	0.80	0.50
PZA01427_1	30	0.07	0.45	0.85	0.44
PZA02462_1	30	0.13	0.49	0.72	0.48
PZA02480_1	30	0.13	0.43	0.68	0.42
PZA00214_1	30	0.13	0.45	0.70	0.44
PZA01462_1	28	0.07	0.42	0.83	0.41
PZA00084_2	30	0.10	0.49	0.79	0.49
PZA03645_1	30	0.13	0.47	0.71	0.46
PZA00770_1	29	0.10	0.51	0.79	0.50
PZA01062_1	30	0.03	0.46	0.93	0.46
PZB01899_1	30	0.03	0.50	0.93	0.50
PHM13687_14	28	0.00	0.34	1.00	0.34
PZA03603_1	28	0.18	0.51	0.64	0.50
PHM5181_10	30	0.07	0.51	0.87	0.50
PZA01791_2	30	0.03	0.44	0.92	0.43
PZA00218_1	30	0.10	0.41	0.75	0.41
PZA01477_3	29	0.07	0.48	0.85	0.47
PZA00527_10	30	0.10	0.51	0.80	0.50
PZA01447_1	30	0.00	0.47	1.00	0.46
PZA02742_1	30	0.03	0.50	0.93	0.50
PZA02358_1	29	0.03	0.51	0.93	0.50
PZA00866_2	30	0.07	0.49	0.86	0.48
IAC1_3	30	0.13	0.51	0.73	0.50
PZA03211_6	25	0.08	0.51	0.84	0.50
PZA02164_16	30	0.07	0.45	0.85	0.44
PZA00352_23	29	0.17	0.51	0.66	0.50
PHM3457_6	30	0.10	0.50	0.80	0.50
PZA00664_3	30	0.03	0.49	0.93	0.49
PZB01403_1	30	0.17	0.41	0.59	0.41
PZA02436_1	30	0.13	0.51	0.73	0.50
PZA00413_20	30	0.13	0.49	0.72	0.48
PZB01658_1	30	0.10	0.30	0.67	0.30

SNPs	N	Ho	He	F_{IS}	PIC
PZA03629_1	30	0.07	0.28	0.76	0.28
PHM3466_69	30	0.10	0.41	0.75	0.41
Ae1_8	29	0.03	0.51	0.93	0.50
sh1_12	30	0.10	0.50	0.80	0.50
PZB01109_1	30	0.07	0.33	0.79	0.32
PHM3078_12	30	0.07	0.36	0.81	0.36
PZA02090_1	30	0.17	0.51	0.67	0.50
PHM2749_10	28	0.11	0.46	0.76	0.45
PZA01919_2	30	0.07	0.45	0.85	0.44
PZA02187_1	29	0.03	0.50	0.93	0.49
PZA03536_1	30	0.07	0.50	0.86	0.49
PHM4134_8	30	0.10	0.50	0.80	0.50
PHM4080_15	30	0.13	0.33	0.58	0.32
PHM2350_17	29	0.00	0.49	1.00	0.49
PHM2770_19	30	0.10	0.51	0.80	0.50
PHM5572_19	30	0.10	0.44	0.77	0.43
PHM12706_14	29	0.14	0.50	0.72	0.49
PZA01607_1	30	0.10	0.41	0.75	0.41
PZA00793_2	29	0.00	0.51	1.00	0.50
wx1_1	30	0.03	0.21	0.84	0.21
PHM13639_13	28	0.11	0.36	0.70	0.36
PHM3922_32	30	0.13	0.45	0.70	0.44
PZA01933_3	30	0.07	0.36	0.81	0.36
Ae1_7	30	0.07	0.28	0.76	0.28
PZA02378_7	30	0.10	0.50	0.80	0.50
PHM2343_25	30	0.10	0.50	0.80	0.50
PZA02325_4	30	0.07	0.40	0.83	0.39
PHM11114_7	30	0.07	0.50	0.86	0.49
PHM1968_22	30	0.07	0.49	0.86	0.48
PHM10621_29	30	0.00	0.28	1.00	0.28
PZA02737_1	30	0.07	0.28	0.76	0.28
PZB01062_3	30	0.07	0.50	0.86	0.49
PHM6111_5	29	0.10	0.47	0.78	0.46
PHM3668_12	30	0.10	0.49	0.79	0.49
PHM3626_3	29	0.14	0.37	0.62	0.37
PZD00022_5	30	0.13	0.43	0.68	0.42
PHM13440_13	29	0.10	0.49	0.78	0.48
PHM13360_13	29	0.10	0.31	0.66	0.31
PHM17210_5	29	0.10	0.50	0.79	0.49

SNPs	N	Ho	He	F_{IS}	PIC
PZA00667_2	30	0.13	0.45	0.70	0.44
PHM5502_31	30	0.07	0.51	0.87	0.50
PZA02779_1	28	0.04	0.51	0.93	0.50
PZA00726_10	30	0.13	0.43	0.68	0.42
PZA03322_5	30	0.03	0.30	0.89	0.30
PHM662_27	30	0.20	0.51	0.60	0.50
PHM4165_14	30	0.13	0.50	0.73	0.49
PZA00440_1	30	0.03	0.46	0.93	0.46
PZA00355_2	29	0.07	0.51	0.86	0.50
PZA03527_3	30	0.07	0.18	0.63	0.18
PZA03154_2	30	0.03	0.51	0.93	0.50
PZA01715_2	27	0.15	0.49	0.69	0.48
PZA02174_2	30	0.07	0.49	0.86	0.48
PZA01533_2	30	0.10	0.50	0.80	0.50
PZA03182_5	30	0.13	0.50	0.73	0.49
PZA00498_5	30	0.10	0.49	0.79	0.49
PZA02269_3	30	0.10	0.41	0.75	0.41
Overall mean	29.62	0.09	0.45	0.80	0.44
SE	0.08	0.00	0.01	0.01	0.01

APPENDIX 5.3

List of 92 SNPs markers used in this study

No	SNP Name	Chrome #	Physical Position(BP)	No	SNP NAME	Chrome #	Physical Position(BP)
1	PZA00175_2	1	8,510,027	56	PZA00214_1	6	91,704,092
2	PZB01062_3	1	56,846,728	57	PZB01658_1	6	102,953,833
3	PHM10621_29	1	101,421,468	58	lac1_3	6	120,230,802
4	PHM1968_22	1	183,647,544	59	PZA02187_1	6	139,106,115
5	PHM12706_14	1	212,356,401	60	PZA02436_1	6	149,251,173
6	PZA00664_3	1	227,542,649	61	PZA01462_1	6	155,546,716
7	PZA02269_3	1	252,722,026	62	PHM3466_69	6	167,148,384
8	PZB01403_1	1	285,273,845	63	PHM3078_12	7	5,963,009
9	PZA02737_1	1	69(Cm)	64	PHM4080_15	7	20,240,404
10	PHM13440_13	2	2,527,344	65	PZA00084_2	7	43,948,264
11	PZA03629_1	2	72 (Cm)	66	PZA01607_1	7	68,051,112
12	PHM6111_5	2	21,990,814	67	PZA03645_1	7	73,892,322
13	PZA02378_7	2	35,040,818	68	PZA01933_3	7	98,070,498
14	PHM3457_6	2	62,804,122	69	PZA01533_2	7	162,381,818
15	PHM13360_13	2	107,146,579	70	PZA02174_2	8	4,101,256
16	PHM3626_3	2	125,642,617	71	PHM2350_17	8	23,985,819
17	PZA03211_6	2	148,837,605	72	PZA00498_5	8	48,775,713
18	PZA00495_5	2	170,377,814	73	PZA00793_2	8	64,421,988
19	PHM3668_12	2	195,555,350	74	PHM11114_7	8	70,899,841
20	PZA00527_10	2	216,833,071	75	PHM4134_8	8	105,795,742
21	PZD00022_5	2	233,128,511	76	PZA00770_1	8	134,140,609
22	PZA02090_1	3	4,138,512	77	PZA03182_5	8	152,155,087
23	PHM2343_25	3	27,981,649	78	PHM2749_10	8	171,703,522
24	PZA01447_1	3	53,549,251	79	sh1_12	9	11,340,882
25	PHM5502_31	3	67,284,067	80	PHM5181_10	9	15,582,065
26	PZA02742_1	3	97,441,783	81	PZA01791_2	9	77,467,426
27	PZA00413_20	3	125,192,432	82	PZA01062_1	9	88,057,320
28	PZA00667_2	3	161,516,227	83	PZB01899_1	9	98,502,843
29	PHM17210_5	3	178,229,653	84	PZA02325_4	9	117,870,773
30	PZA03154_2	3	109 (Cm)	85	wx1_1	9	40 (cM)
31	PZB01109_1	3	194,643,731	86	PZA01715_2	9	142,948,449
32	PZA03527_3	3	22(cM)	87	PHM3922_32	10	17,722,938
33	PZA02358_1	4	11,329,241	88	PHM13687_14	10	47 (cM)
34	PHM5572_19	4	35,384,118	89	PZA03603_1	10	75 (cM)
35	PZA00726_10	4	60,768,063	90	PHM2770_19	10	72,565,410
36	PZA00218_1	4	78,946,415	91	PZA01919_2	10	111,260,278
37	PZA03536_1	4	107,751,353	92	PZA00866_2	10	124,203,565
38	PZA03409_1	4	128,632,208				
39	PZA01477_3	4	172,301,064				
40	PZA02779_1	4	207,114,208				
41	PZA03322_5	4	242,019,440				
42	PZA02462_1	5	6,820,571				
43	Ae1_8	5	79(cM)				
44	PHM13639_13	5	152(cM)				
45	PZA01427_1	5	23,135,578				
46	PZA00981_3	5	37,030,384				
47	PHM4165_14	5	65,741,535				
48	PZA00643_13	5	91,096,945				
49	PZA02164_16	5	112,179,855				
50	PHM662_27	5	135,569,668				
51	ae1_7	5	167,873,309				
52	PZA00352_23	5	191,075,557				

No	SNP Name	Chrome #	Physical Position(BP)	No	SNP NAME	Chrome #	Physical Position(BP)
53	PZA02480_1	5	214,953,055				
54	PZA00440_1	6	22,404,308				
55	PZA00355_2	6	78,756,133				

CHAPTER 6
Overview of the Research Findings

6.1 Introduction

Maize (*Zea mays* L.) is a strategic food, feed and industrial crop in sub-Saharan Africa (SSA) and the crop accounts for 40% of the total cereal production within the region. Among the aspects that have received intensified research attention in the last three or four decades is the genetic enhancement for hybrid tolerance to abiotic stresses including low nitrogen (N). Improved hybrids that are tolerant to low N stress are also now available in South Africa (SA) and this study complemented the low N research effort through evaluation of 170 single-cross maize hybrids developed by Agricultural Research Council-Grain Crops Institute (ARC-GCI). The research further assessed the genetic purity of parental inbred lines and proved parentage for their resultant F1 hybrids as a quality control check to ensure production of high quality low N tolerant hybrids. This chapter highlights the main findings of the study and their implications for hybrid maize breeding.

6.2 Summary of research findings

6.2.1 Grain yield performance, estimation of genetic parameters, correlation and path coefficient analyses across low nitrogen and optimum environments.

- The analysis of variance revealed highly significant (P<0.001) differences among the experimental hybrids for all the traits studied in each environment.
- Differences in grain yield performance among the experimental hybrids were observed under low N implying that the experimental hybrids exhibited different levels of low N stress tolerance.
- Based on grain yield and low N selection index, 13 experimental low N tolerant hybrids with better performance than the checks were identified. These hybrids are CB339/CML442, CK21/CKDHL0089, CML216/RO549W, I-42/CB339, CML202/CK21, CML544/I-38, CK21/CKDHL0295, CKDHL0378/I-42, CKDHL0378/U2540W, I-42/CML544, CML544/CK21, CK21/CML444 and CKLO5022/I-42.
- Across the low N environments, high broad-sense heritability estimates for days to anthesis, days to silking and anthesis-silking interval were observed. Ear height, grain yield and plant height had moderate broad-sense heritability while field weight, ears per plant and leaf senescence had low broad-sense heritability.

- Across the optimum environments, high broad-sense heritability estimates were observed for ear height, plant height and days to silking while moderate broad-sense heritability was observed for grain yield, field weight and ears per plant. Low broad-sense heritability was observed for anthesis-silking interval only.
- Higher values for PCV than values for GCV were observed among all the studied traits under low N and optimum conditions implying that the environment had greater influence on the expression of the traits.
- Under low N conditions, grain yield was weakly (r = 0.141 to 0.349), but positively correlated (P<0.01) to days to anthesis, plant height, ear height, and ears per plant and strongly (r = 0.853) correlated to field weight. On the other hand, it was weakly (r = -0.069 to -0.310) and negatively correlated to days to silking, anthesis-silking interval and leaf senescence.
- Grain yield under optimum conditions was highly significantly (P<0.01) and positively correlated to field weight and ears per plant, (r= 0.887** and r=0.307**) and significant but negatively correlated to days to anthesis, days to silking, anthesis-silking interval, plant height and ear height (r=-0.628**, r=-0.615**, r= -0.124**, r= 0.469** and 0.473**, respectively).
- Under low N conditions, path coefficient analysis revealed that days to silking exhibited the highest direct effect on grain yield followed by field weight, plant height and ear height. On the other hand, days to anthesis, anthesis-silking interval, ears per plant and leaf senescence had negative direct effect on grain yield.
- Under optimum conditions, path coefficient analysis revealed that field weight, exhibited the highest direct effect on grain yield followed by days to anthesis, anthesis-silking interval and ears per plant. On the other hand, days to silking, plant height and ear height had negative direct effect on grain yield.
- Significant correlations for field weight, days to anthesis, days to silking, anthesis-silking interval and leaf senescence with grain yield under low N signifies the importance of these secondary traits for consideration during selection for low N stress tolerance.
- Higher positive direct effect for days to anthesis, field weight and anthesis-silking interval on grain yield under low N and optimum environments confirm that they are very important secondary traits and should be included as selection criteria for yield improvement under low N.

6.2.2 Assessment of the magnitude of GEI on grain yield of single-cross maize hybrids across low N and optimum conditions

Assessment of genotype by environmental interaction for grain yield of single-cross hybrids was done using AMMI and GGE biplot models across the five environments in three locations. The results were as follows:

- Grain yield was significantly affected by genotype, environment and genotype by environment interaction as revealed by the general combined ANOVA and AMMI analyses.
- GGE biplot using the 'which-won-where' identified hybrids CML544/I-38 and CB339/CML442 as highest yielding in environments E1 (Potchefstroom optimum) and E2 (Cedara optimum) while CK21/CKDHL0089 was the highest yielder for environments E3 (Vaalharts Optimum), E5 (Cedara Low N) and E4 (Potchefstroom Low N).
- Hybrids I42/CKDHL0295, CB339/CML442, CK21/CML216,CKDHL0089/CML442 and CML544/I-42 were considered as high yielding and stable across the environments with higher yields of 6.32 t ha^{-1}, 6.89 t ha^{-1}, 7.01 t ha^{-1}, 6.77 t ha^{-1} and 6.22 t ha^{-1}, respectively, above the grand mean of 5.55 t ha^{-1}. These are the ideal hybrids recommended for further evaluation and release.
- The least discriminating environment was E5 and the most discriminating one was E3..
- GGE biplot analysis also grouped the five environments into two groups, with one group made up of E4, E2 and E1 and the other group comprised of E3 and E5.
- The presence of close associations among the test environments (E3 versus E5, E5 versus E4, E1 versus E4 and E1 versus E2) suggest that the same information about the genotypes could be obtained from fewer test environments, and hence the potential to reduce the testing cost.

6.2.3 Assessment of the genetic purity of maize parental lines and parent-offspring test for their F1 hybrids using SNPs markers

Assessment of the genetic purity, diversity of the 30 parental lines, and parent-offspring test for the resultant 158 F1 hybrids was done using 92 SNP markers recommended by CIMMYT. The results of the study are as follows:

- The overall mean genetic distance among the parental lines was 0.47, with a minimum and maximum value of 0.05 and 0.56, respectively.

- Polymorphic information content (PIC) ranged from 0.18 to 0.50 with 90% of the makers bearing a PIC value of greater than 0.3 confirming that the markers were effective.
- The maximum genetic distance (0.56) was found among CKDHL0089, CML443 when each paired with CB323 while lowest genetic distance (0.05) was found between I-42 and I-40.
- The study also revealed genetic diversity among the 30 parental lines by clustering them in three main genetic clusters each having sub-clusters.
- Ten of the 30 parental lines showed heterozygosity greater than 5%, which requires additional generations of purification for them to be fixed.
- Sixty-eight hybrids out of the 158 tested passed the parent-offspring test and these were derived from pure lines and genetic contamination during actual crossing was less than 5%.
- The top seven performing hybrids observed were: SCHP29, SCHP95, SCHP94, SCHP134, SCHP44, SCHP114 and SCHP126. These hybrids were derived from genetically pure lines and had genetic contamination levels of less than 5%, hence they are potential candidates for further evaluation and release.

6.3 General recommendations based the findings

The following are the general recommendations and breeding implications derived from the results of this study:

Thirteen hybrids that showed tolerance to low N stress based on their grain yield and low N selection index value entails that these are the potential candidates for further evaluation and release as low N tolerant hybrids. Moderate heritability values for grain yield under low N implies that under practical field condition the heritability for grain yield will be very low. Hence, indirect selection for grain yield using secondary traits with significant positive correlation to grain yield is highly recommended when selecting genotypes under low N.

High yielding hybrids with broad and specific adaptation, and environments which are informative and closely associated to each other were identified in this study. This entails that when conducting multi-location trials, the same information about the genotypes could be obtained from fewer test environments, and hence the potential to reduce the testing cost. Those genotypes which showed inferior yield performance but with great stability across the environments are recommended for genetic enhancement in terms of grain yield through crossing them with genotypes which are high yielding but not stable.

Continued use of the identified parental inbred lines which are not pure in hybrid development would result in release and commercialisation of wrong hybrids and of compromised productivity. Hence it is recommended that these inbred lines should either be discarded from the ARC-GCI maize breeding programme or purified using ear-to-row selection methods. Hybrids, which are high yielding and passed the parent-offspring test were indeed derived from their parental inbred lines and are recommended for further evaluation, release and commercialisation as low N tolerant hybrids.