DEVELOPMENT OF HIGH-YIELDING AND STABLE MAIZE (*Zea mays* L.) HYBRIDS TOLERANT TO LOW SOIL NITROGEN

By

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DECLARATION

I hereby declare that except for references to work of other researchers, which have been duly cited, this work is my original research and that neither part nor whole has been presented elsewhere for the award of a degree.

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ABSTRACT

Maize is Ghana's most important cereal crop and is grown by the vast majority of rural households. It is widely consumed throughout the country, and is the second most important staple food in Ghana, after cassava. Low soil nitrogen (N) impedes maize production in the small-scale farming sector in Ghana. Development of improved maize varieties that are tolerant to low soil N will reduce the need for nitrogen inputs and enhance production. The objectives of this study were to (1) assess maize production constraints of Ghanaian maize farmers, and their perceptions and knowledge on soil fertility, (2) determine heterotic groups and combining ability of grain yield for intermediate maturing maize inbred lines under low and high soil N environments, (3) determine the mode of gene action conditioning grain yield under low N, (4) evaluate the testcrosses (single cross hybrids) for high yield, stability and tolerance to low and high soil N and (5) identify and map Quantitative Trait Loci (QTL) for grain yield (GY) and secondary traits under high N and low soil N.

A Participatory Rural Appraisal (PRA) was conducted among 120 farmers in six communities in the forest savanna transition agro-ecological zone of Ghana using Focus Group Discussion (FGD) and semi-structured interview. Thirty-two inbreds received from Institute of Agricultural Research for Development (IRAD), International Institute of Tropical Agriculture (IITA) and International Maize and Wheat Improvement Center (CIMMYT) were crossed to three elite testers (87036, 1368 and 9071) in a line x tester scheme to generate 96 F_1 hybrids. The 96 F_1

hybrids along with 4 checks were evaluated under low N (30 kg ha-1) and high N (90 kg ha-^{1} N) soil environments at three locations in Ghana in 2013 and 2014. One hundred and fifty BC_2F_1 families in a mapping population (CML 444 x CML 494) were used to identify SNP markers associated with quantitative trait loci (QTLs) for yield and yield related traits under low N and high N environments.

The PRA revealed low soil fertility, drought, pests and diseases as the major maize production constraints. Most farmers grow improved varieties but, have very little knowledge about hybrids. Farmers preferred low N and drought tolerant varieties with good storability that are disease and insect resistant and require low inputs. They also indicated preference for slender cobs, light in weight with lots of grains. Evaluation of hybrids and inbreds showed genetic variability for grain yield and secondary traits. Significant GCA and SCA effects for grain yield and most measured traits were detected with predominance of GCA effects over SCA effects, indicating that most traits were controlled predominantly by additive gene action. Hybrids CLWN 247 x 9071, ZM523B-29-2-1-1-B*6 x 9071, TZD II 68 x 1368, and P43SCRq Fs100-1-1-8 x 9071 were identified as high yielding, and low N tolerant. These are recommended for further testing for potential release to farmers in low soil N environments. Seven hybrids (CZL 00001 x 9071, LapostaseqC7-F18-3-2-1 x 9071, CLWN 364 x 9071, CLWN 247 x 9071, CLWN 247 X 87036, TZD II 68 x 1368, and CML 395/CML 444 x 9071) were among the 20 best yielding hybrids across environments. These are candidates for further testing for commercialization. Based on SCA of grain yield, HGCAMT and HSGCA methods for heterotic classification, the lines were classified into three heterotic groups for each

environment. The inbreds in each heterotic group may be recombined to form populations which could be improved through recurrent selection. Subsequently, inbred lines could be extracted from each population for the production of superior hybrids and synthetics by selfing and crossing onto an inbred tester of opposing heterotic group. The GGE biplot analysis revealed CML 395/ CML 444 x 9071 and TZDII 68 x 1368 as the most high yielding and stable hybrids. These hybrids should also be further tested in multi-location trials and promoted for release. A total of 13 QTLs were identified with 158 SNP markers for six different traits (grain yield, days to silking, stay green characteristic, ears per plant, anthesis silking interval and plant height) under low N environment (9 QTLs) and high N environment (4 QTLs). Five QTLs (*qgy-1, qts-1, qsg-1, qsg-4 and qasi-6*) for GY, DTS, SG, ASI and EPP, respectively, were close to their adjacent markers, with an interval of 0.7 to 5.2 cM between them and explained phenotypic variance of 9% to 21%.

DEDICATION

To my father in-law (Elder Moses Kofi Francisco Ribeiro) and my darling son Jenedis Paa Kwesi Francisco Ribeiro.

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TABLE OF CONTENTS

DECLARATION i

ABSTRACT ii

DEDICATION v

ACKNOWLEDGEMENT vi

TABLE OF CONTENTS viii

LIST OF FIGURES xii

LIST OF TABLES xiv

LIST OF ABBREVIATIONS xvii

CHAPTER ONE 1

1.0 GENERAL INTRODUCTION 1

1.1 Specific objectives 7

CHAPTER TWO 9

2.0 LITERATURE REVIEW 9

2.1 Biology of maize as a crop 9

2.2 History of maize breeding in Ghana 9

2.3 Constraints to maize production in sub-Saharan Africa 12

2.4 Nitrogen fertilizers for maize production 13

2.5 The problem of low soil N in maize 14

2.6 Genetics of tolerance to low N 18

2.7 Breeding and selection for Tolerance to Low-Nitrogen 20

2.8 Hybrid development and heterosis 22

2.9 Heterotic Groups / Patterns in Tropical Maize Germplasm 24

2.10 Combining ability 28

2.11 Genotype x environment (G x E) interaction 30

2.12 Molecular breeding of maize tolerant to low soil nitrogen 32

2.13 Farmer participation in breeding/selection 34

CHAPTER THREE 36

3.0 Farmers' perceptions on low soil N and maize hybrids and their implications in plant breeding 36

3.1 Introduction 36

3.2 Materials and Methods 38

3.2.1 Study Area 38

3.2.2 Selection of farmers 39

3.2.3 Survey procedure and data analysis 39

3.3 Results 40

3.3.1 Demographic Characteristics 40

3.3.2 Cropping systems and crop production 42

3.3.3 Constraints to maize production and soil fertility 45

3.3.4 Management of low soil fertility (Copping strategies) 46

3.3.5 Fertilizer use 47

3.3.6 Source of information about fertilizer 49

3.3.7 Farmers' perception of hybrids 50

3.3.8 Willingness to use low N tolerant maize varieties 51

3.4 Discussion 52

3.5 Conclusions 56

CHAPTER FOUR 58

4.0 Combining ability, hybrid performance and heterotic grouping of intermediate maturing maize inbreds under low, high and across soil N environments 58

4.1 Introduction 58

4.2 Materials and Methods 60

4.2.1 Genetic materials 60

4.2.2 Experimental sites and field layout 64

4.2.3 Depletion of N in experimental sites 65

4.2.4 Determination of the nutrient status of experimental sites 65

4.2.5 Low soil Nitrogen treatment 66

4.2.6 Data collection 67

4.2.7 Statistical Analysis 68

4.3 Results 76

4.3.1 Performance of hybrids and inbred parents under low N and high N environments 76

4.3.2 Performance of hybrids 76

4.3.3 Performance of the inbred lines under contrasting environments 89

4.3.4 Relationship between performance of parental inbred lines and their hybrids 98

4.3.5 Correlation between grain yield and secondary traits 101

4.3.6 Genetic analysis of intermediate maturing maize inbred lines under low and high N environments 102

4.3.7 Relative contributions of combining ability effects (Mode of gene action) 107

4.3.8 General combining ability effects (GCA effects) 109

4.3.9 Specific combining ability 118

4.3.10 Heterotic groupings, relationship among different grouping methods and grouping efficiencies 122

4.3.11 Stability Analysis of Grain Yield of Intermediate Maturing Inbreds and Hybrids 133

4.4 Discussion 143

4.5. Conclusions 155

CHAPTER FIVE 158

5.0 Identification of Quantitative Trait Loci (QTL) for yield and yield related traits under high and low soil nitrogen environments 158

5.1 Introduction 158

5.2 Materials and Methods 161

5.2.1 Germplasm 161

5.2.2 Field Evaluation (Phenotyping) 161

5.2.3 Data collection 162

5.2.4 Data Analysis 163

5.2.5 SNPs genotyping, Construction of genetic linkage map and QTL mapping 164

5.3 Results 165

5.3.1 Distribution of grain yield and secondary traits in the BC_2F_1 population 165

5.3.2 Evaluation of BC_2F_1 population for yield and yield related traits 168

5.4 Discussion 179

5.5 Conclusions 183

CHAPTER SIX 185

6.0 Conclusions and recommendations 185

6.1 Recommendations 190

BIBLIOGRAPHY 191

APPENDICES 231

LIST OF FIGURES

Figure 3. 1 Local Variety – Kwoappiah.....43

Figure 3. 2 Constraints to maize production in the various villages.....45

Figure 3. 3 Causes of low soil fertility.....46

Figure 3. 4 Ways of managing low soil fertility by farmers.....47

Figure 3. 5 Types of fertilizer use.....48

Figure 3. 6 Sources of fertilizer information.....49

Figure 4. 1 Proportion of total genotypic sum of squares of grain yield and other agronomic traits of intermediate maturing inbred lines attributable to GCA_l, GCAt and SCA across environments.....108

Figure 4. 2 Proportion of total genotypic sum of squares of grain yield and other agronomic traits of intermediate maturing inbred lines attributable to GCA_l, GCAt and SCA across low N environments.....108

Figure 4. 3 Proportion of total genotypic sum of squares of grain yield and other agronomic traits of intermediate maturing inbred lines attributable to GCA_l, GCAt and SCA across high N environments.....109

Figure 4. 4 Classification of intermediate maturing maize inbreds into heterotic groups based on HGCAMT method under low N environment. Note: tester 1- 1368, tester 2= 9071, tester 3=87036.....128

Figure 4. 5 Classification of intermediate maturing maize inbreds into heterotic groups based on HGCAMT method under high N environments. Note: tester 1- 1368, tester 2= 9071, tester 3=87036.....129

Figure 4. 6 Classification of intermediate maturing maize inbreds into heterotic groups based on HGCAMT method across low and high N environments. Note: tester 1- 1368, tester 2= 9071, tester 3=87036.....130

Figure 4. 7 An entry/tester genotype main effect plus genotype x environment biplot of grain yield of 36 intermediate maturing maize inbreds across low and high N environments in 2013 and 2014.....135

Figure 4. 8 A 'which won where' GGE biplot of grain yield of 36 intermediate maturing maize inbreds evaluated across low N and high N environments in 2013 and 2014.....136

Figure 4. 9 An entry/tester genotype main effect plus genotype x environment biplot of grain yield of 29 intermediate maturing maize hybrid across low and high N environments in 2013 and 2014..... 140

Figure 4. 10 A 'which won where' GGE biplot of grain yield of 29 intermediate maturing maize hybrids evaluated across low N and high N environments in 2013 and 2014..... 141

Figure 4. 11 The 'discriminating power and representativeness' view of GGE biplot on a genotype X environment yield data of 29 intermediate maturing maize hybrids evaluated in 12 environment 142

Figure 5. 1 Frequency distribution of eight traits in BC_2F_1 population under high N environment 165

Figure 5. 1 (continued) Frequency distribution of eight traits in BC_2F_1 population under high N environment 166

Figure 5.2 Frequency distribution of eight traits in BC_2F_1 population under low N environment 167

Figure 5.2 (Continued) Frequency distribution of eight traits in BC_2F_1 population under low N environment 168

Figure 5.3 Linkage map showing QTL on chromosomes 1, 4,5,6,8 and 10 for six traits (GY, ASI, DTS, SG, EPP and PHT 178

LIST OF TABLES

Table 3. 1: Demographic characteristics of farmers from the six villages 41

Table 3. 2 Crop Production Systems by Percentage of Farmers 44

Table 3. 3 Prices of fertilizers- (Ghana Cedis, 2014) 48

Table 3. 4 Perception on hybrid seed 51

Table 4. 1: List of inbred lines and testers used in the study 62

Table 4. 2 Soil chemical properties of experimental sites 66

Table 4. 3 Mean squares of intermediate maturing maize hybrids evaluated across low N environments in Fumesua, Ejura and Kwadaso in 2013 and 2014 78

Table 4. 4 Mean squares of intermediate maturing maize hybrids evaluated across high N environments in Fumesua, Ejura and Kwadaso in 2013 and 2014 78

Table 4. 5 Mean squares of intermediate maturing maize hybrids evaluated across environments in Fumesua, Ejura and Kwadaso in 2013 and 2014 79

Table 4. 6 Proportions of the total variance attributable to the sources of variation for grain yield of maize hybrids evaluated in low, high and across environments in 2013 and 2014 80

Table 4. 7 Grain yield and other agronomic traits of intermediate maturing maize hybrids (best 20 and worst 10) evaluated under low N environments in Fumesua, Ejura and Kwadaso in 2013 and 2014 83

Table 4. 8 Grain yield and other agronomic traits of intermediate maturing maize hybrids (best 20 and worst 10) evaluated under high N environments in Fumesua, Ejura and Kwaadaso in 2013 and 2014 85

Table 4. 9 Grain yield and other agronomic traits of intermediate maturing maize hybrids (best 20 and worst 10) evaluated across environments in Fumesua, Ejura and Kwadaso in 2013 and 2014 87

Table 4. 10 Mean squares of intermediate maturing maize inbred lines evaluated across low N environments in Fumesua, Kwadaso in 2013 and 2014 and Ejura in 2014 90

Table 4. 11 Mean squares of intermediate maturing maize inbred lines evaluated across high N environments in Fumesua, Kwadaso in 2013 and 2014 and Ejura in 2014 90

Table 4. 12 Mean squares of 36 intermediate maturing maize inbred lines across environments in Fumesua, Kwadaso in 2013 and 2014 and Ejura in 2014 91

Table 4. 13 Grain yield and other agronomic traits of intermediate maturing maize inbreds (best 20 and worst 10) evaluated under low N environments in Fumesua, Kwadaso in 2013 and 2014 and Ejura in 2014 94

Table 4. 14 Grain yield and other agronomic traits of intermediate maturing maize inbreds (best 20 and worst 10) evaluated under high N environments in Fumesua, Kwadaso in 2013 and 2014 and Ejura in 2014 96

Table 4. 15 Mid-parent and better parent heterosis for grain yield under low N environments in Fumesua and Kwadaso in 2013 and 2014 99

Table 4. 16 Mid-parent and better parent heterosis for grain yield under high N environments in Fumesua and Kwadaso in 2013 and 2014 100

Table 4. 17 Pearson correlation coefficient between grain yield and other agronomic traits under low, high and across N environments 101

Table 4. 18 Line x tester analysis of grain yield and other agronomic traits across low N environments at Fumesua, Ejura and Kwadaso in 2013 and 2014 104

Table 4. 19 Line x tester analysis of grain yield and other agronomic traits across high N environments at Fumesua, Ejura and Kwadaso in 2013 and 2014 105

Table 4. 20 Line x tester analysis of grain yield and other agronomic traits across N environments at Fumesua, Ejura and Kwadaso in 2013 and 2014 106

Table 4. 21 General combining ability effects of lines and testers for grain yield and other agronomic traits under low N environments 112

Table 4. 22 General combining ability effects of lines and testers for grain yield and other agronomic traits under high N environments 114

Table 4. 23 General combining ability effects of lines and testers for grain yield and other agronomic traits across environments 116

Table 4. 24 Specific combining ability effects for grain yield under low N environments 119

Table 4. 25 Specific combining ability effects for grain yield under high N environments 120

Table 4. 26 Specific combining ability effects for grain yield across environments 121

Table 4. 27 Classification of the 15 inbreds into heterotic groups based on SCA effects of grain yield under low, high and across N environments 124

Table 4. 28 Classification of intermediate maturing maize inbreds into heterotic groups based on HSGCA effects of grain yield under low N, high N and across N environments 126

Table 4. 29 Number of hybrids within the best 33 arranged in descending order of their yield (group 1), from 34th to 66th (group 2) and from 67th to 99th (group 3) 132

Table 4. 30 Breeding efficiency (%) of HSGCA, HGCAMT and the SCA heterotic grouping methods under low-N, high-N and across research environments. 133

Table 5. 1 Mean squares of BC_2F1 population evaluated across low N environments 169

Table 5. 2 Mean squares of BC_2F_1 population evaluated across high N environments 169

Table 5. 3 Correlation among traits under high N environment 170

Table 5. 4 Correlation among traits under low N environment 171

Table 5. 5 Genetic map from 158 SNP markers for 150 BC_2F_1 population for CML 444 x CML 494 172

Table 5. 6 QTLs identified based on BC_2F_1 population from CML 444 x CML 494 across two nitrogen (N) environments 175

LIST OF ABBREVIATIONS

ANOVA: Analysis of Variance

ATA: Average Tester coordinate Abscissa

ATO: Average Tester coordinate Ordinate

CIMMYT: International Maize and Wheat Improvement Center

DIVA: Diffusion of Improved Crop Varieties in Africa

FAOSTAT: Statistical Database of the Food and Agriculture of the United Nations

FGD: Focus Group Discussion

GCA: General Combining Ability

GGE: Genotypes plus Genotypes by environments interactions

GEI: Genotypes by environments interactions

HGCAMT: Heterotic grouping based on GCA of Multiple Traits

HSGCA: Heterotic groups' Specific and General Combining Ability

IITA: International Institute of Tropical Agriculture

METs: Multi-environment trials

MOFA: Ministry of Food and Agriculture

NUE: Nitrogen Use Efficiency

NUtE: Nitrogen Utilization Efficiency

OPV: Open Pollinated Varities

PRA: Participatory Rural Appraisal

QTL: Quantitative Trait Loci

QPM: Quality Protein Maize

SCA: Specific Combining Ability

SSA: Sub-Saharan Africa

WECAMAN: West Africa Collaborative Research Network

CHAPTER ONE

1.0 GENERAL INTRODUCTION

Maize (*Zea mays* L.) is the most important cereal crop in sub-Saharan Africa (SSA) and provides food for more than 1.2 billion people in the sub-region. Throughout SSA, small-scale farmers grow maize, mostly for subsistence, as part of agricultural systems that feature several crops and sometimes livestock production (IITA, 2009).

In Ghana, maize is the largest staple crop and contributes significantly to consumer diets (Fening *et al*., 2011). It is also the number one crop in terms of area planted and accounts for 50-60% of total cereal production (Aidoo *et al.*, 2014). Maize demand has been projected to grow at annual compound rate of 2.6% between 2010 and 2015 (MiDA, 2010). Rising population, urbanization, and growing poultry and fish sectors in Ghana have contributed to increased demand for maize. Per capita consumption, mainly of white maize, grew only marginally from 38.4 kg in 1980 to 43.8 kg in 2011 (MoFA, 2012). Furthermore, the poultry industry's demand for maize, used as feed in Ghana was estimated to have grown by 10 percent annually between 2000 and 2009 and would surpass 540,000 mt if chickens were fed a proper ration (Hurelbrink and Boohene, 2011). Without productivity improvements, Ghana's Ministry of Food and Agriculture (MoFA) estimates that 267,000 mt of maize will have to be imported in 2015 to meet domestic demand (FAO, 2012). Ghana is not self-sufficient in this most important staple crop, as Ghana has experienced average shortfalls in domestic maize supplies of 12% (MiDA, 2010). Maize yields in Ghana averaged 1.2–1.8 metric tons (mt) per hectare (ha), far

below the potential yield of 4–6 mt/ha achieved in on-station trials (Ragasa *et al.*, 2014) and over 8 mt/ha in the US (FAOSTAT, 2012).

Overall, maize production in the country has remained relatively static both in terms of area planted and volume harvested because of reliance on traditional farming methods (MiDA, 2009).

This phenomenon persists because production is limited by biotic and abiotic factors. Several biotic and abiotic factors limit maize production and productivity across countries in sub-Saharan Africa (Badu-Apraku *et al*., 2003). Among the abiotic production constraints, drought and low soil fertility, especially Nitrogen (N) deficiency, are the main factors that most frequently limit maize production and productivity. The latter is the most challenging constraint (Banziger and Lafitte, 1997; Banziger and Cooper, 2001, Sanchez 2010, USAID/EAT, 2012). Continuous farming without adequate use of fertilizer has resulted in the depletion of essential soil nutrients required to support plant growth in SSA (Sanchez, 2010). The estimated annual loss of maize grain yield due to low N stress alone varies from 10 to 50% (Wolfe *et al*., 1988; Logrono and Lothrop, 1997).

Low soil nitrogen (low N) is common in farmers' fields in SSA. The high cost of nitrogen fertilizer and poor weed control increase the incidence of nitrogen stress in many instances (Lafitte and Edmeades, 1994). Due to the high prices of inorganic fertilizer, most farmers apply nitrogen at sub-optimal levels (Mccown *et al*., 1992). In Africa, the rate of fertilizer application is low (8 kg ha^{-1}), which is far below the 50 kg ha^{-1} target set by the Africa Fertilizer Summit (2006) as reported by Vanlauwe *et al*., (2010). In order to

increase grain yield, the use of 120 kg N ha^{-1} is recommended for maize production in SSA. Low fertilizer application rates in the sub-region are due to high prices of inorganic fertilizer and the inability of resource poor farmers to purchase these fertilizers as well as the non-availability of some of the fertilizers when they are most needed by farmers

In Ghana, the most limiting factors of maize production in all the agro ecological zones, especially the savanna zone, are erratic rainfall pattern and low soil fertility (Logah *et al*., 2010). Low soil fertility causes serious yield loss in farmers' fields. The major causes of the low soil fertility are low application of external inputs, poor soil fertility, poor management practices, continuous cropping on a piece of land and poor nature of soils. The soils of the major maize growing areas in Ghana are low in organic carbon (<1.5%), total nitrogen (< 0.2 %), exchangeable potassium (< 100 mg/kg) and available phosphorus (Benneh *et al*., 1990; Adu, 1995). Many farmers are unaware of the appropriate time to apply fertilizer in order to maximize maize production and this is not an effective solution for small-scale farmers lacking financial resources to purchase fertilizers. The value-cost ratio for fertilizer application on maize, a rough measure of the profitability of using fertilizer, is much higher in Ghana than in other countries (Jayne and Rashid, 2013) but its use is half the recommended rate (47 kilograms/hectare of nitrogen on average for those who apply fertilizer, compared with the recommended 90 kilograms/hectare) and this is in spite of a national subsidy program that encourages more users and greater rates of application for maize. Increasing dependence on chemical fertilizer and the continuous loss of organic matter in the soil, however, may lead to a declining maize fertilizer response, as other countries have experienced (Jayne and Rashid, 2013). Smallholder, resource poor farmers in Ghana are aware of the benefits of

inorganic fertilizers but high price and unavailability influence the regularity and the quantities they apply. Furthermore, due to high price of imported fertilizers at farm gate as well as delays in delivery due to poor road infrastructure, smallholder farmers often apply very low rates of inorganic fertilizer late in the growing season, leading to poor crop-yield responses (Heisey and Mwangi, 1996).

Low nitrogen effects can be mitigated in Ghana through the application of organic manure and inorganic fertilizer, compost and the use of legumes capable of fixing atmospheric nitrogen. As an option, farmers could resort to using compost and green manure to increase the nitrogen supply in the soil but compost making is very demanding and may require addition of some nitrogen source to ensure that it is rich in N. Consequently, very few farmers can afford to make enough good manure and apply it (Snapp *et al*., 2002; Rufino *et al*., 2006). Another promising alternative that may be considered is the use of nitrogen-fixing legumes in rotation. The challenge with this alternative is the length of time required to grow the legumes. A full season may be required to grow the legumes as an improved fallow and this may not be possible in the highly populated farming areas where the land is used all the time (Kaya *et al*., 2000). In addition, nitrogen fixation is also dependent on many factors including appropriate legume species, presence of nodulating Rhizobia and favorable climatic conditions.

One effective strategy available to reduce fertilizer cost is to develop maize genotypes with combined high nitrogen use efficiency and high yield potential. Genotypes with high yield potential under low N are also needed to support the rapidly growing population and provide incentives to farmers who mostly apply modest amounts of N in their maize

fields. Improved maize varieties that tolerate low N will help maize farmers in N stress-prone areas to obtain better harvests (Zaidi *et al*., 2003; CIMMYT, 2007). In addition to improved yield under severely N-deficient conditions, these cultivars will be more responsive to the small N doses that Ghanaian farmers apply. The development of maize genotypes tolerant to low N stress, therefore, is crucial to increase maize production and productivity in Ghana and SSA as a whole.

Until recently, adoption of maize hybrids was insignificant in Western Africa due to limited seed production and marketing by existing and emerging seed companies and restrictive Seed Laws in most countries. However, during the last couple of years, seed companies have emerged in Ghana, Mali, and Nigeria setting the stage for large scale hybrid production in these countries (Badu-Apraku *et al*., 2011a). Hybrid development and promotion in maize is a promising strategy to appreciably increase maize production and productivity in WCA. Several years of maize improvement in Ghana has resulted in the development, release and wide adoption of open-pollinated varieties (OPVs) and hybrids that have increased maize production and productivity in the country (Morris *et al*., 1999). Exploitation of hybrid vigour in maize has gained high acceptance because of the potential of hybrids to provide substantial yield increases. Hybrids can be continuously improved to develop new products that are superior to the released hybrids in yield and quality and better meet farmers' preferences. There is tremendous potential for increased maize production to supplement local production to feed Ghana's growing population and meet the needs of the poultry and the livestock industries. Breeding maize hybrids that are tolerant to low soil N would increase maize production since only few low N tolerant maize hybrids have been released in Ghana thus far.

The development of parental inbred lines with good combining abilities is essential to producing superior hybrids. This is because lines *per se* performance has little relationship to the performance of hybrids (Hallauer and Miranda, 1988). Maize inbred lines with good specific combining abilities are needed to develop superior hybrids tolerant to low soil N. Identification of lines with superior specific combining ability can be done most efficiently using a line by tester mating scheme when efficient testers are available.

Inbreds that combine specifically with other inbreds can be grouped based on their heterotic groups (Badu-Apraku, and Lum, 2007). Studies have shown that the environment under which inbred lines and populations are evaluated influences the heterotic groups but superior combining pairs of inbreds can be found that produce good hybrids across environments (Menkir *et al.*, 2003; Badu-Apraku *et al*., 2006). However, there is the need to determine the heterotic groups of inbred lines under low and high N environments. This will allow the selection of lines that combine well both under stressed and non-stressed environments. Hybrids derived from inbred lines with complementary heterotic groups have superior grain yield performance than hybrids formed from parental lines of the same heterotic type.

Due to low heritability under stress conditions (Badu-Apraku *et al.*, 2004, 2005) use of secondary traits has been proposed for selecting for yield improvement. The difficulty in measuring some of these secondary traits quickly and precisely, however, has limited their application in some breeding programs (Monneveux and Ribaut, 2006). Therefore, the identification and characterization of quantitative trait loci (QTL) will help to identify

genomic regions associated with the expression of complex traits and their precise genetic contribution at target loci (Ribaut *et al.*, 2007). But then, less attention has been paid to understanding the genetic response of segregating populations to field soil deficiencies like low P (Reiter *et al.*, 1991) or low N (Agrama *et al.*, 1999; Hirel *et al.*, 2001). The development of molecular marker technologies offers powerful alternative methods to examine the relationships between physiological traits controlling maize response to N, and could contribute to a better understanding of metabolic pathways and physiological processes (Limami and de Vienne, 2001). Although some QTLs associated with low N tolerance have been mapped, the results reported are specific to the mapping populations used. The use of different parental lines, segregation in populations or ecological conditions could lead to different results in other mapping studies including QTL number and location or effect. Therefore, it is imperative that different parental lines and populations or environmental conditions are used for identifying the QTLs controlling low N tolerance.

This study is justified because improvement of the productivity, income and livelihood of maize farmers who lack the ability to purchase and apply adequate quantities of fertilizers to improve on their maize yields is essential.

The general objective of this research is to increase maize productivity and farmers' income by developing maize hybrids tolerant to low N.

1.1 Specific objectives

The Specific objectives of this research are to:

- Assess maize production constraints of Ghanaian maize farmers, their perceptions and knowledge of soil fertility.
- Determine heterotic patterns and combining ability for grain yield of selected intermediate maturing maize inbred lines under low and high soil nitrogen environments.
- Determine the mode of gene action conditioning grain yield under low N.
- Evaluate the testcrosses (single cross hybrids) for high yield, stability and tolerance to low and high N as well as across research environments.
- Identify and map Quantitative Trait Loci (QTL) for grain yield (GY) as well as for secondary traits under high and low N.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Biology of maize as a crop

Maize (*Zea. mays* L.) belongs to the family *Graminae* (*Poaceae*), commonly known as the grass family. It is a tall, monecious, annual grass with overlapping sheaths and broad conspicuously distichous blades. Plants have staminate spikelets in long spike-like racemes that form large spreading terminal panicles (tassels) and pistillate inflorescences in the leaf axils, in which the spikelets occur on a thickened, almost woody axis (cob). The whole structure (ear) is enclosed in numerous large foliaceous bracts and a mass of long styles (silks) protrude from the tip as a mass of silky threads (Hitchcock and Chase, 1971). Pollen is produced entirely in the staminate inflorescence and eggs entirely in the pistillate inflorescence. Maize is wind pollinated and both self and cross pollinations are usually possible. Shed pollen usually remains viable for 10 to 30 minutes, but can remain viable for longer periods under favorable conditions (Coe *et al*., 1988). Cultivated maize is presumed to have been derived from teosinte (*Z. mexicana*) and is thought to have been introduced into the old world in the sixteenth century.

2.2 History of maize breeding in Ghana

While maize was introduced into Ghana in the 16th century, actual maize breeding programmes began in the early 1930s. A lot of research has been done in maize breeding in Ghana over the last two decades. The focus of maize breeders has been to develop stable and high yielding maize varieties with the capacity to perform well in all the agro-

ecologies in Ghana (GGDP, 1986). T.L Williams developed local maize germplasm and introduced a yellow variety called 'Tsolo' from South Africa and he developed the C50 variety between 1939-1942 (GGDP, 1984; Sallah, 1986). Nyankariwana Number 1 and Number 2, both yellow varieties in Northern Ghana, were released by J. McEwen between 1954 and 1961. W.K Agble released Synthetics 1, 2, and 3 as (GS1, GS2 and GS3) from parental inbred lines between 1956 and 1960, (GGDP, 1984; Sallah, 1986). Efforts by some local Ghanaian breeders, especially M. K. Akposoe, led to the development of three composite varieties: composites1, 2 and 3, in addition to La Posta CRI, and Golden Crystal between 1968 and 1972. Other varieties such as Composite 4, Dobidi and Okomasa were also released between 1972 and 1988 (Sallah, 1998).

The Ghana/CIDA Grains Development Project aided the intensification of maize breeding efforts in 1979 (Sallah, 1986). The project, a maize improvement programme, developed and released white and yellow varieties with various maturity periods ranging from 80 to 120 days to suit the different agro-ecological zones of Ghana. By 1985, high yielding but streak susceptible open-pollinated maize varieties, Dobidi, Aburotia, Safita-2 and Kawanzie corresponding to the major varietal types required in Ghana for sustainable maize production had been released by the team of maize breeders, B. Badu-Apraku, S. Twumasi-Afriyie and P.Y.K. Sallah for production in Ghana. To date, several varieties of maize, extracted from breeding populations from the International Maize and Wheat improvement Center (CIMMYT) in Mexico and International Institute for Tropical Agriculture (IITA) in Nigeria have been improved, and released in Ghana and have been widely adopted by Ghanaian farmers. For example, following the severe outbreak of the streak virus disease in Ghana in 1982 and 1983, the national maize scientists intensified

the effort to replace all the existing streak susceptible maize varieties in Ghana at that time with streak resistant versions. The effort culminated in the release of "Okomasa", a normal, full-season open pollinated maize variety (OPV) derived from CIMMYT Population 43-SR in 1988 with a yield potential of 5.5 t ha^{-1} (Sallah, 1986; Badu-Apraku, 1990, 1991). Other streak resistant varieties developed and released in Ghana during this period to combat the streak virus disease included Abeleehi, and Dorke SR (Badu-Apraku, 1990, 1991).

Quality Protein Maize (QPM) breeding programme in Ghana was initiated in 1989 which led to the release of 'Obatanpa' in 1992 (Sallah, 1998; Badu-Apraku *et al*., 2006; Badu-Apraku and Fakorede, 2013). The first QPM cultivar released in WCA was Obatanpa GH (Salla, 1998; Badu-Apraku *et al*. 2006; Badu-Apraku and Fakorede, 2013). This cultivar has been widely adopted by farmers and consumers in Ghana. It covers more than 50% of the maize hectarage (650,000 ha) in Ghana (Dankyi *et al*. 2005). It has also been released formally or informally in several other African countries including Benin (as Faaba), Burkina Faso, Cameroon, Côte d'Ivoire, Ethiopia, Guinea, Malawi, Mali (as Debunyuman), Mozambique (Susuma), Nigeria (as SAMMAZ 14), Senegal, South Africa, Swaziland, Togo, Uganda, and Zimbabwe (Badu-Apraku *et al*. 2006). Obatanpa GH also serves as a source of inbred lines for the development of QPM hybrids and synthetic cultivars in several maize breeding programs in Africa. Between 2007 and 2010, extra early maturing QPM varieties such as 'Akposoe (TZEE-W STR)' and 'Abontem (TZEE-Y Pop STR QPM C0), early maturing varieties 'Omankwa (TZE-W Pop DT STR QPM C4)', and 'Aburohemaa (EVDT-W99 STR QPM C0)' with combined resistance/tolerance to the parasitic weed, *Striga hermonthica* and drought were released

by the team of maize breeders, M. Ewool, K. Obeng-Antwi, Marshack and H. Alidu through collaboration with the West Africa Collaborative Research Network (WECAMAN) and the IITA Maize Improvement Program. Other varieties released by the national program scientists include the intermediate maturing varieties, 'Etubi', 'Enibi', 'Golden Jubilee' and 'Aziga' (National Variety Release Committee, 2007 & 2010). The QPM hybrid development programme, commenced in 1991 resulted in the development and release of intermediate maturing hybrids such as 'Mamaba', 'Dadaba' and 'CIDA-ba' in 1996 with high yield potential of 7.5 t ha^{-1} on experimental stations. Also, high-yielding, intermediate maturing QPM hybrids with improved tolerance to drought such as 'Etubi'and 'Enibi' were released in 2007 and 2010 respectively. The first generation of early [Suhudoo (TZE-W Pop DT STR C4 x TZEI 7); Kunjor-wari (TZE-Y Pop DT STR C4 x TZEI 17)] and intermediate maturing normal endosperm hybrids 'Warikamana' and' Kpari-faako' with combined resistance to *Striga* and tolerance to drought were released in 2014 by the national maize program in collaboration with the Drought Tolerant Maize for Africa (DTMA) Project of IITA (National Variety Release Committee, 2014).

2.3 Constraints to maize production in sub-Saharan Africa

Maize production and productivity in Sub-Saharan Africa (SSA) is severely constrained by several biotic and abiotic factors. The biotic factors include downy mildew, rust, leaf blight, stalk and ear rots, leaf spot, and maize streak virus. Insect pests, including stem and ear borers, armyworms, cutworms, grain moths, beetles, weevils, grain borers, rootworms, and white grubs also constitute a great threat to the survival of maize in SSA.

The parasitic weed, (*Striga hermonthica*), is also a major pest in SSA and causes cereal grain yield losses of up to US$7 billion. This adversely affects the lives of about 300 million people (M'Boob., 1986). The most important abiotic stress factors in SSA are drought and low soil nitrogen (Low N). Low soil nitrogen results from limited use of nitrogenous fertilizers and the declining soil fertility. Periodic drought caused by irregular rainfall distribution reduces maize yields by an average of 15% each year (Edmeades *et al*., 1995). This is equivalent to about US$200 million (Edmeades *et al*., 1995). Under field conditions, drought, *Striga*, and low N can occur simultaneously and when this happens the combined effect can be devastating (Cechin and Press, 1993; Kim and Adetimirin, 1997; Badu-Apraku *et al*., 2010). In Ghana, maize production and productivity is also constrained by several biotic and abiotic factors. Among the abiotic factors, drought and low soil fertility are the major factors that limit maize production and productivity (Logah, 2010).

2.4 Nitrogen fertilizers for maize production

The doubling of worldwide agricultural food production has been associated with a 7-fold increase in N fertilizer use (Mosier *et al.*, 2004). Maize is the third most important food crop and the one receiving the largest amount of N fertilizer (Fixen & West, 2002). About 85 to 90 million tons (Mt) of N fertilizers are added to the soil worldwide annually (Peoples *et al*., 1995) with maize receiving around 16.8 Mt of N annually (Fixen & West, 2002) or 19% of the total N applied. Nitrogen is often not only the most limiting and expensive nutrient for maize production but also one of the best input investments that a

farmer can make in terms of economic returns (Pikul *et al*., 2005). Therefore, N has been applied in higher quantities compared to other key nutrients necessary for high maize yields (FAO, 2008).

Although the N fertilizer use has increased in maize, it is estimated that high application levels led to a lower efficiency of nutrient usage at the plant level. Nitrogen Use Efficiency (NUE) of maize is estimated at only 33%, which means that out of 100 units of N applied, only 33 end up in the grain (Raun & Johnson, 1999). Consequently, approximately 70% of the applied N is lost from the plant-soil system raising economic and environmental concerns. The results of various fertilizer experiments carried out in developing countries have led to fertilizer recommendations that gave blanket nutrient requirements for maize in agro-ecologies having varying soil conditions and under varying levels of soil management (FPDD, 1990). For example, hybrid maize cultivation was found to require high fertilizer rate for optimum yield. Findings from research work conducted by Sobulo (1980) indicated that maize responded to nitrogen better in the savanna than in the forest agro-ecology. It was suggested that 60-70 kg N ha^{-1} is an economic rate for maize in the rainforest and over 100 kg N ha^{-1} in the savanna. The difference between the two zones was, however, attributed to the presence of higher insulation in the savanna zones (Sobulo, 1980).

2.5 The problem of low soil N in maize

Nitrogen is essential for agricultural crops and yet, on the average, African farmers apply just one-fifth of the nitrogen needed to maintain adequate soil and crop health. In fact, African farmers use less than 10% of the world average amount of fertilizer. This has led

to lower crop yields and poor soil fertility. African farmers understand the benefits of proper fertilization, but many factors prevent widespread use. African farmers tend to pay two to six times more for fertilizer, when it is available, than farmers in the rest of the world. Poor transportation infrastructure, lack of local nitrogen production, and low trade contribute to high prices and lower usage rates (CIMMYT, 2012). In Ghana, the most limiting factors for maize production in all the agro- ecological zones, especially the savanna agro-ecological zone, are erratic rainfall and low soil fertility (Logah *et al*., 2010). Soil fertility is a major constraint to crop production, because minimal application of fertilizers or none are applied due to high cost (Bationa *et al.*, 1997). Smallholder, resource poor farmers in Ghana realize the advantages of using inorganic fertilizers but high prices and unavailability influence the quantities applied.

Nitrogen is the most limiting nutrient in maize production in the humid and sub-humid tropics. It is the mineral element required in the greatest quantity by maize, thus efficient N uptake and use by the maize plant is of fundamental importance to maize production systems in Africa (Muza *et al*., 2004). Ma and Dwyer (1998) identified N fertilizer as the most energy consuming component of maize grain production.

With increasing economic and environmental costs of excessive N, research aimed at sustainable agricultural production places emphasis on selection of varieties with greater N use efficiency or cultivars with better grain yield under low-N conditions (Ma and Dwyer 1998; Sattelmacher *et al*., 1994). The poor performance of many commercial maize hybrids and varieties under low N in small holder conditions is because they were

developed under very high N levels and on good soils found on research stations (Muza *et al*. 2004).

In Sub-Saharan Africa, inorganic fertilizer use is constrained by high cost, inaccessibility and lack of credit faced by small scale farmers even in the high yield potential mid-altitude zones (Kamara *et al*., 2004; Diallo *et al*., 2004; Banziger *et al*., 2006). Recommended N fertilizer rates are often lower where there is a high risk of crop failure, especially due to drought than N rates that give maximum yields under optimum conditions (McCown *et al*., 1992). Poor weed control also increases the incidence of N stress. Collectively, these constraints result in low N being a frequent characteristic of maize-growing environments in the tropics (Banziger and Lafitte, 1997).

McCullough *et al.* (1994) reported that variation in N supply affects both growth and development of maize plants. The onset of grain filling requires an adequate N supply within the maize plant (Christensen *et al*., 1981). During grain filling, translocation of carbohydrates to the roots is reduced and N uptake decreases (Monneveux *et al*., 2005). N affects photosynthetic rate, leaf area, size of the sink and thus yield (Dass *et al*., 1997). When N supply is limiting, leaves become the main source of remobilized N to the ear (Below, 1997). Chlorophyll concentration reduction and leaf yellowing are good indicators of N remobilization (Dwyer *et al*., 1995). N deficiency accelerates leaf senescence by reducing chlorophyll concentration (Monneveux *et al*., 2005). Stay green characteristic can reflect N balance in cereals during grain filling (Borrell *et al*., 2001), though greenness can also be superficial rather than functional (Thomas and Howarth, 2000). Lack of N enhances kernel abortion (Pearson and Jacob, 1987) and reduces final

grain number (Lemcoff and Loomis, 1986; Uhart and Andrade, 1995; Monneveux *et al*., 2005) and grain yield (Monneveux *et al*., 2005). Reduction of grain weight under low N conditions is more attributable to reduction in the grain filling period than in growth rate (Monneveux *et al*., 2005). Cultivars yield differently in different N environments due to genetic differences (Smalberger and Toit, 2004). Relative grain yield reduction due to N stress also depends on the type of variety and N-stress intensity (Banziger and Lafitte, 1997). Smalberger and du Toit (2004) recorded grain yield ranging from 0.65 - 3.85 t ha^{-1} under low N and 4.17 - 6.93 t ha^{-1} under optimum N for South African maize cultivars. They observed that the cultivar that gave the best yield (6.93 t ha^{-1}) under optimum N gave a low yield of 0.98 t ha^{-1} under low N. Ifie.(2013) compared yield under low and high N environments and found that grain yield in the low N environment was 27% of grain yield in the high N environments. Mafouasson (2014) observed a significant reduction in plant height, ear height, ears per plant, and grain yield under low N. The authors also observed an average of less than one ear per plant, which indicates barreness. Monneveux *et al.* (2005) reported that, on average, anthesis-silking interval (ASI) increased from 0.33 days under optimal to 2.42 days under low N conditions, which is more than seven fold. Low N stress also increases the incidence of ear rot disease (Banziger *et al*., 2006).

In low-N environments, ear height, ear aspect, plant aspect, stay green characteristic, and days to silking had significant direct effects on yield, contributing 58% of the total variation in grain yield (Badu Apraku *et al.,* 2012). Days to silking (–0.48) and ear aspect (–0.47) had the highest direct effects; stay green characteristic had the least direct effect (–0.15). Only ear height had a significant positive direct effect (0.17) on yield. It was

concluded that husk cover contributed to grain yield through plant aspect while ear aspect and ear height contributed indirectly to grain yield through plant aspect (0.46).

2.6 Genetics of tolerance to low N

Nitrogen Use Efficiency (NUE) for grain maize has been defined as the grain yield per unit of nitrogen available from the soil, including nitrogen fertilizer (Moll *et al.,* 1987). It is the product of nitrogen uptake efficiency (N-uptake/N from soil), and nitrogen utilization efficiency (NUtE, i.e. yield/N-uptake). For NUE, genetic variability and genotype x nitrogen fertilization level interactions reflecting differences in responsiveness have been observed in several studies in maize (Pollmer *et al.,* 1979; Reed *et al.,* 1980; Moll *et al.,* 1987; Landbeck, 1995; Bertin and Gallais, 2000). In addition, correlations among various agronomic traits such as grain protein yield and its components vary depending on the level of nitrogen fertilization (Balko and Russell, 1980b; Di Fonzo *et al.,* 1982; Rizzi *et al.,* 1993; Bertin and Gallais, 2000). At high N-input, genetic variation in NUE is explained by variation in N-uptake, whereas at low N-input, NUE variability is attributed mainly to differences in nitrogen utilization efficiency. This suggests that the limiting steps in N-assimilation may be different when plants are grown under high or low levels of nitrogen fertilization. Differences in N-uptake are likely to be related to the quantity and the quality of the root system. However, experiments have shown variability in the architecture of the root system (HeÂbert *et al*., 1992), but this has not been related to variability in N-uptake.

Nitrogen uptake at silking determines kernel number (Di Fonzo *et al*., 1982; Muruli and Paulsen, 1981; Sherrard *et al*., 1986). This may be explained by the high demand for

nitrogen by embryos just after fertilization (Czyzewicz and Below, 1994). As a consequence, kernel number is more sensitive to N stress than kernel weight (Uhart and Andrade, 1995; Reed *et al*., 1988; Below, 1995). Di Fonzo *et al.* (1982) and Moll *et al.* (1987) showed that the role of post-anthesis N-uptake in grain filling can be related to leaf senescence. Indeed, by increasing leaf longevity, thus prolonging the capacity of the plant to absorb mineral nitrogen, better yields were obtained in modern hybrids (Tollenaar, 1991; Ma and Dwyer, 1998; Racjan and Tollenaar, 1999a, b).

There is some information available regarding gene action governing the inheritance of different agronomic traits under low N conditions. Reports in literature vary for the type of gene action important for grain yield under low N conditions. For instance, while Ifie (2013) reported that additive gene action was more important than the non-additive, Mafouasson (2014) found that it was the non-additive gene action that was more important than the additive which was in agreement with Betran *et al*. (2003). Messeka *et al.* (2006) found that non-additive action was slightly higher than additive gene action under low N conditions. Jointly, these studies have shown that many N use traits are under genetic control and that physiological processes limiting yield differ according to the level of N available in the soil. Therefore, genotypes that tolerate low N could be identified and improved. In another study, Beck and Willcox (1997) reported significant crossover type interaction effects between general combining ability (GCA) effects of lines under low N, when compared to those under high N conditions. This implies that genotypes differ in their response to low and high N conditions.

2.7 Breeding and selection for Tolerance to Low-Nitrogen

Breeding for low-N stress tolerance is simpler than breeding for drought tolerance because N deficiency usually affects plant growth more evenly over time compared to random drought spells (Banziger *et al*. 2000). Thus, testing lines under one level of relatively severe low-N stress should suffice to select for low-N stress tolerance for various levels of N deficiency. Studies carried out by CIMMYT have shown that genotypes selected for drought tolerance also perform well under low-N conditions. For example, Edmeades *et al*., (1995) reported that selection for drought tolerance at flowering simultaneously improved tolerance to low-N stress. Also, at IITA, studies have shown that genotypes selected under low soil nitrogen for *Striga* resistance are also tolerant to low N but not necessarily vice versa (Badu-Apraku *et al.,* 2009).

Approaches used in the development of improved maize cultivars for tolerance to low N environments include selection for improved yield under high N and specific mechanisms expected to confer tolerance to low N (Lafitte and Bänziger, 1996). Selection gains under low N are predicted to be higher when selection is conducted under both low and high N (Bänziger and Lafitte, 1997).

The use of secondary traits could improve selection efficiency for grain yield under low N stress conditions because heritability of grain yield is low under low N (Banziger and Lafitte, 1997; Badu-Apraku *et al.,* 2011a, 2012). Moll *et al*., (1987) found that selection for ears per plant improved the identification of superior genotypes under low N. Banziger and Lafitte (1997) used ears per plant and leaf senescence to select superior genotypes under low N.

Increased stress tolerance is considered the primary cause of increased grain yielding ability of US corn belt maize (Tollenaar and Lee, 2002). Alleles related to stress tolerance are present in most elite maize populations at a relatively low frequency and selection under controlled low N conditions was effective in developing varieties that tolerated low N (Vasal *et al.,* 1997). Since yield is controlled by a large number of major and minor genes, its improvement under low N environments will depend on how the respective genes respond to the stress. To maximize selection gains under low N, direct selection (i.e. selection environment similar to target environment) should be employed as it was superior to indirect selection (Banziger *et al.,* 1997). However, when selecting for grain yield (GY) under low N, a number of secondary traits with significant correlations to it should be taken into account. A good secondary trait is genetically associated with GY under stress and its heritability is high and it is easy and cheap to measure (Banziger *et al.,* 2000).

At IITA, a base index that integrates increased grain yield under drought stress and well-watered environments with short anthesis– silking interval, increased ears per plant, good stay green characteristic, and good scores for plant aspect and ear aspect under drought stress has been used since 2001 in selecting for drought tolerant early, intermediate, and late maturing maize genotypes (Menkir and Akintunde, 2001; Badu-Apraku *et al.,* 2004a). Badu-Apraku *et al.* (2011) reported that the most reliable traits for selection for improved grain yield under low N were plant height, days to silking, days to anthesis, ears per plant, anthesis–silking interval, stay green characteristic, ear aspect, and plant aspect.

The relative importance of heredity in determining phenotype is the heritability (Falconer and Mackay, 1996). High heritability implies that the genetic variation for a trait can be precisely assessed from phenotypic observations (Banziger and Cooper, 2001). Heritability of GY generally decreases under stress conditions (Banziger *et al.,* 1997; Banziger and Cooper, 2001). When information on secondary traits was combined with that of GY in a selection index, selection efficiency improved by 14% over selection based on GY alone under low N (Banziger and Lafitte, 1997).

2.8 Hybrid development and heterosis

Maize hybrid development began in the early 1900s (Hallauer *et al*., 1988). The hybrid maize concept (Shull, 1909) was developed in the public sector and is still considered one of the greatest achievements in crop breeding. In maize, sorghum, rice, millet, and many vegetables, hybrid breeding is the method of choice for attaining maximum genetic gain from the effect of heterosis. Food and feed supplies would undoubtedly be greatly reduced if hybrids were not available to the producer (Stuber, 1994). According to Singh (2005), most of the commercial hybrid varieties are F1's from two inbreds. An inbred is defined as an essentially homozygous line obtained through continuous inbreeding of cross pollinated species (Singh, 2005). The success of hybrid maize development depends on the ability of the breeding program to rapidly identify lines that combine well in hybrid combinations and to identify appropriate heterotic combinations to maximize the vigour of the hybrid (Kim and Ajala, 1996). The importance of an inbred is related to its combinability. In hybrid breeding, combinability is much more important than

heritability. Falconer and Mackay (1996) noted that crossing of inbred lines to produce hybrids plays a major role in crop improvement, most particularly maize. Furthermore, they indicated that, in order to attain heterosis, the candidate lines for crosses need to be derived from different base populations; a cross between two unrelated base populations provides heterosis. The general process to develop maize hybrids starts with the creation of a segregating breeding population, source population, from which inbred lines are extracted through inbreeding and selection by crossing early selfed generations onto an inbred tester that has superior specific combinability to the source population. (Betran *et al*., 2004). Selected tester hybrids are then evaluated across locations to select superior hybrids.

Heterosis is defined as the difference between the hybrid value of one trait and the mean value of the two parents for the same trait (Falconer and Mackay, 1996). According to Miranda (1999), heterosis (usually considered to be synonymous with hybrid vigor) is the genetic expression of the superiority of a hybrid in relation to its parents. The two types of heterosis used in the public sector are the mid-parent or average heterosis, which is the increased vigor of the F1 over the mean of two parents; and high-parent or better parent heterosis, which is the increased vigor of the F1 over the better parent (Sinha and Khanna, 1975; Jinks, 1983). In the private sector, heterosis of a new hybrid must be about 10% greater that the best commercial hybrid grown by farmers. Heterosis is the basis of the commercial maize industry (Stuber, 1994). Even though several economically important crops benefit from the manifestation of heterosis, both the genetic and physiological mechanisms underlying this phenomenon are still unexplained (Hallauer

and Miranda, 1988; Tollenaar *et al*., 2004). Three major theories: dominance, over dominance and epistasis have been proposed to explain mechanisms underlying the phenomena of heterosis (Hallauer and Miranda, 1988; Singh, 2005). The amount of heterosis is dependent on the degree of specific combinability of the two inbreds involved in a cross. Van Oosterom *et al*. (1996) noted that the non-additive genetic effects causing heterosis occur under stress conditions as they do under optimal conditions. Heterosis is a constitutive trait and does not require stress in order to be expressed (Blum, 1997). The expression of heterosis is dependent on genes that are expressed in genotypes irrespective of the stress conditions. The manifestation of heterosis depends on genetic divergence of two parental lines for those epistatic genes (Moll *et al*., 1965; Hallauer and Miranda, 1988). Low grain yield heterosis is observed for crosses among genetically similar germplasm and for crosses among broad genetic base germplasm (Hallauer and Miranda, 1981; Beck *et al*., 1990; Crossa, 1990; Beck *et al*., 1991; Vasal *et al*., 1992). Higher levels of heterosis are characterized by increased divergence within a certain range, but that heterosis declined in extremely divergent crosses (Moll *et al*., 1965)

2.9 Heterotic Groups / Patterns in Tropical Maize Germplasm

The concept of heterotic groups and patterns has been widely used to simplify maize breeding (Tracy and Chandler, 2006). A heterotic group, as defined by Melchinger and Gumber (1998), "is a group of related or unrelated genotypes from the same or different populations which show similar combining ability or heterotic response when crossed with genotypes from other genetically distinct germplasm groups and by comparison

heterotic pattern refers to a specific pair of two heterotic groups which express high heterosis and consequently high hybrid performance in their cross". The establishment of heterotic patterns among varieties is important in selecting inbred lines as parental seed stocks in hybrid production (Romanus *et al.*, 2007).

The concept of heterotic patterns requires the subdivision of the germplasm available in a hybrid breeding program into at least two groups, which are then improved with inter-population selection methods. Two populations of a specific heterotic pattern are typically improved as follows: Progenies are generated within the same heterotic pool. The progenies are then evaluated for their yield performance when test-crossed with a tester from the opposite heterotic pool. Lines showing superior testcross performance are inter-mated to form the next cycle of selection. Cycles of selection are repeated and the latest cycle populations are used to produce hybrids (Bernardo, 2001). Heterotic patterns have a strong impact in crop improvement because they predetermine to a large extent the type of germplasm used in a hybrid breeding program over a long period of time (Melchinger and Gumber, 1998). Also, they help breeders in choosing parents of crosses for line development as well as testers to evaluate combining ability of newly developed inbreds. This helps in simplifying germplasm management and organization.

Heterotic patterns can be analyzed either by crossing the germplasm in question with common testers which are known to be of different heterotic patterns, or by crossing the germplasm in a diallel mating system. To assign germplasm into different heterotic patterns, Reif *et al.* (2005) suggests two strategies to be used:(i) a higher mean heterosis and hybrid performance and (ii) a reduced specific combining ability variance and a

lower ratio of specific combining ability to general combining ability variance (δ2SCA:δ2GCA).

A number of approaches have been used to assign germplasms into different heterotic groups. The classical SCA method relates the heterosis observed in crosses with the origin of the parents involved in the crosses and/or field hybrid-yield information (Melchinger and Gumber, 1998, Fan *et al*., 2009). Another method employs various molecular markers to compute genetic similarity or genetic distance to assign maize lines to different heterotic groups (Menkir *et al*., 2004, Aguiar *et a*l., 2008, Fan *et al*., 2009, Badu-Apraku *et al*., 2013a, Akinwale *et al*., 2014). Results were not always consistent with the ones of the classical approach which therefore is still used as a major method for maize heterotic group classification. A major method to develop heterotic groups is to use elite inbred testers to cross onto newly developed inbreds. Inbreds whose tester hybrids that show performance better than the best check varieties are classified as being opposite heterotically from the tester (Prof. Vernon Gracen, personal communication). Fan. (2009) proposed another alternative method that uses both GCA and SCA to assign inbred lines to known maize heterotic groups. The method designated as heterotic group's specific and general combining ability (HSGCA) is reported to explain more variation in maize hybrid yield and produce more predictable yield (Fan *et al*., 2009, Akinwale *et al*., 2014). Badu-Apraku *et al*. (2013) proposed another method designated as heterotic grouping based on GCA of multiple traits (HGCAMT) which could be more effective in classifying inbred lines into appropriate heterotic groups. The advantage of the HGCAMT method particularly in breeding for stress environments is that it uses

GCA of multiple traits rather than GCA of yield alone which has low heritability in those environments.

Much has been done in classifying temperate maize germplasm into heterotic groups such as European flint x US Lancaster (commonly used in Europe) and Stiff Stalk Synthetic (SSS) x Non-SSS (used in the US). In tropical maize germplasm, several studies have been conducted to establish heterotic patterns (Vasal *et al.,* 1999). Some of the studies identified Tuxpeno, ETO, Tuson, Cuban flints and Suwan 1 as the distinct groups of tropical maize. The studies also established Tuxpeno x ETO, Tuson x Tuxpeno, Cuban flint x Tuxpeno, and Suwan 1 x Tuxpeno as the best combinations for hybrid production.

In studies to determine the combining ability and heterotic patterns of tropical inbreds developed at CIMMYT using four line testers, Vasal *et al*. (1992a) identified and formed two divergent tropical heterotic groups (THGA and THGB). Lines showing negative SCA with Tester 1 "Pop 21" (Tuxpeño-1) and positive SCA with Tester 3 "Pop 25" (Blanco Cristalino) were classified under Tropical Heterotic Group "A". Those showing positive SCA with Tester 1 and negative with Tester 3 were classified under Tropical Heterotic Group "B". Studies show that the heterotic patterns of inbred lines and populations can change depending on the test environment under which evaluation is made (Kim and Ajala, 1996; Vasal *et al.*, 1993). The grouping of the inbred lines is not consistent across environments due to genotype x environment interaction. Consequently, determining the stability of heterotic patterns of inbred lines under stress and non-stress conditions would be useful for the development of an efficient hybrid breeding strategy

that can cater for the variable growing conditions in West African Countries (Menkir *et al.*, 2003).

2.10 Combining ability

In designing hybrid breeding programmes, the concept of combining ability is very important. It is especially useful to study and compare the performance of lines in hybrid combinations (Alkuddsi *et al*., 2013, Romanus *et al.*, 2007). Sprague and Tatum (1942) introduced the concepts of general combining ability (GCA) and specific combining ability (SCA). A clear understanding and definition is GCA is the average contribution an inbred makes to hybrid performance in a series of hybrid combinations in comparison to other inbreds in the same series of hybrid combinations. SCA is the contribution of an inbred to hybrid performance in a cross with a specific other inbred in relation to its contributions in crosses with an array of other inbreds

Rawlings and Thompson (1962) used line x tester analysis to estimate GCA and SCA of inbred parents. Since the development of new cultivars through hybridization is a continuous process, information on combining ability of new cultivars is very important.

GCA and SCA effects, especially SCA, are important indicators of the potential value of inbred lines in hybrid combinations (Sprague and Tatum, 1942). Combining ability of inbred lines is the ultimate factor determining future usefulness of the lines for hybrid development (Hallauer and Miranda, 1988). Using the concept of combining ability, genetic variance is partitioned into two components: variance due to GCA and variance due to SCA (Hallauer and Miranda, 1988; Sughroue and Hallauer, 1997). GCA is recognized primarily as a measure of additive gene action and SCA as an estimate of

non-additive gene action such as dominance and epistasis (Sprague and Tatum, 1942; Gowen, 1964; Kambal and Webster, 1965). Combining ability studies allow classification of selected parental materials with respect to breeding behavior (Sprague and Tatum, 1942; Hallauer and Miranda, 1988; Poehlman and Sleper, 1995). According to Hallauer and Miranda (1988), characterization of genetic variance and type of gene action operative in crosses of inbreds are interpreted relative to GCA and SCA of inbred lines. The proportion of additive and non-additive components of genetic variance depends on the genetic structure of the crosses analyzed and the environmental conditions in which they were grown (Khotyleva and Trutina, 1973). Kebede (1989) reported that additive gene effects were more important in determining traits in the populations while non-additive gene actions were important in determining heterosis of inbred line crosses. Younes and Andrew (1978) reported that additive gene action is more important than non-additive components for most traits in previously unselected material. GCA is predominant for parents that have been developed through selection for GCA and for parents that have not been separated into heterotically complementary groups during their development (Pixley and Bjarnason, 1993). On the other hand, Kambal and Webster (1965) reported the importance of non-additive gene action for some traits, including grain yield, in materials that were previously selected for GCA. Betran *et al*. (2003) found negative SCA for hybrids involving inbred lines with the same germplasm origin or related by pedigree and greater SCA for hybrids involving inbred lines of different source germplasm origin. Kim and Ajala (1996) studied combining ability among tropical and temperate maize inbred lines and reported that a major proportion of crosses sum of squares for grain yield is explained by GCA. The stability of GCA and SCA effects are

key in identifying parents and hybrids with improved homeostasis to cater for environmental variations (Dehghanpour and Ehdaie, 2013)

2.11 Genotype x environment (G x E) interaction

Genotype–environment interactions are important to agriculture and animal breeding generally because the genetic architecture for traits, and thus evolutionary dynamics, vary with environmental conditions (Ouborg *et al*., 2010). Genotype by environment interaction occurs when differences between genotypes are not the same in all locations within and across years. Environmental factors have a greater effect on quantitative traits than on qualitative traits. Consequently, performance tests of potential cultivars are conducted in multiple years and locations (Bernardo, 2002). In addition to genotype and environment main effects, performance of cultivars is also determined by the G x E interactions which are the differential responses of cultivars to environmental changes (Hallauer *et al*., 1988; Crossa *et al*., 1990; Vargas *et al*., 1999). Some biotic and abiotic stresses have been identified to cause G x E interaction. G x E interactions in African maize growing environments, for example, result from factors related to temperature, rainfall, season length, within-season drought, sub-soil pH and socio-economic factors that result in sub-optimal input application (Banziger *et al*., 2006). Multi-environment trials (METs) are systematic approaches to increase yield stability of new crop varieties in stress prone environments (Shakhatreh *et al*., 2001). The relative magnitude of G x E provides information concerning the likely area of adaptation of a given genotype. It is also useful in determining efficient methods for using time and resources in a breeding

program (Ceccarelli, 1989; Kang, 1998). In an experiment conducted by Sallah *et al*. (2002) on the potential of elite maize composites for drought tolerance in stress and non-drought environments effects, G x E interaction were highly significant for grain yield, 50% silk emergence, plant height, lodging, ears per plant, and ear rating in both drought and non-drought stressed environments. From their stress environments, grain yields of the varieties ranged from 2.21 to 3.12 t ha^{-1}, while in the favorable environments, yields for the same varieties ranged from 4.17 to 5.96 t ha^{-1}. METs in West and Central Africa usually show significant genotype x environment interaction (GEI) due to the differential response of cultivars to varied growing conditions (Fakorede and Adeyemo, 1986; Badu-Apraku *et al.*, 1995, 2003, 2007, 2008, 2009). METs are routinely conducted by the IITA Maize Program in Nigeria. Consequently, improving genotype resistance/tolerance to different stresses to which they would likely be exposed might minimize G x E interaction (Kang, 1998). Large G x E interaction is expected when genotypes are grown under a wide range of environments and outside their normal zone of adaptation (Beck *et al*., 1991).

Selection of multi-environment sites to sample stresses adequately, where G x E and genotype-by-year interaction are major sources of variation, is a critical step in a successful breeding program (Edmeades *et al*., 2006). The extent of performance testing depends on the magnitude of G x E, which occurs when genotypes differ in their relative performance across environments (Bernardo, 2002). Yield trials frequently have both significant main effects and a significant G x E interaction (Zobel *et al*., 1988). The GGE biplot graphically displays genotype main effect plus G×E of a MET in a way that

facilitates visual evaluation of cultivars and mega-environment identification (Yan *et al.*, 2000). Badu-Apraku *et al.* (2008) and Badu-Apraku and Lum (2010) used the GGE biplot analysis to decompose the G×E in WCA and to obtain information on the early maturing maize cultivars that were suitable for *Striga*-infested and *Striga*-free environments and to investigate stability of cultivars in the various environments. The existence of G x E interaction necessitates that breeders evaluate genotypes in more than one environment to obtain repeatable rankings of genotypes (Hallauer *et al*., 1988). However, G x E is of practical significance only when crossover interactions occur (Baker, 1988; Crossa and Cornelius, 1997). Crossover interactions occur in evaluation trials when ranks of cultivars change across environments (Russell *et al*., 2003).

2.12 Molecular breeding of maize tolerant to low soil nitrogen

The potential for genetic improvement in NUE depends on the magnitude and the nature of differences among genotypes (Moll *et al.*, 1982). Selection for yield in environments having low N status should be more effective than selection for yield potential under high N alone; however, such environments are not normally favored by maize breeders because of increased environmental variability and reduced heritability of grain yield under low N conditions (Blum,1988). In practice, low fertility effects are usually minimized in breeding nurseries by applying N at levels required to saturate the response of maize to N. As a result, the N response of maize populations has been altered by selection for yield in these environments (Lafitte *et al.*, 1994; Ta *et al.*, 1992).The efficiency of selection for yield in low-N environments may be improved by selection for

correlated secondary traits (Banzinger *et al.,* 2001). Although such traits are usually less affected by environmental variation than yield, the difficulty of measuring them quickly and precisely has limited their use in breeding programs.

Genotypic differences in response of maize to nitrogen fertilizers have been reported (Bertin and Gallais, 2000). Genetic studies evaluating N response in breeding lines and hybrids suggest polygenic inheritance (Pollmer *et al.,* 1979). Molecular markers can be used to study the inheritance of complex traits and identify specific loci associated with the expression of these traits. Once a desired trait has been identified in a segregating population, specific chromosome segments controlling variation for the trait can be localized using RFLPs or some other type of genetic marker system (Beavis *et al.,* 1991). Markers linked to specific genes may be used to facilitate selection of desired genotypes through marker-assisted selection (MAS). The identification and characterization of quantitative trait loci (QTL) will help to identify genomic regions associated with the expression of complex traits and their precise genetic contribution at target loci. In maize, the genetic dissection of complex traits for abiotic stress responses has focused primarily on drought tolerance (Agrama *et al*., 1996; Ribaut *et al*., 1996, 1997; Tuberosa *et al*., 2002). Morris *et al.* (2003) evaluated the benefits of molecular markers in breeding programs and Ribaut and Ragot (2007) reported marker-assisted selection experiments used to improve grain yield under water limited conditions and low temperature. Less attention has been paid to understanding the genetic response of segregating populations to field soil deficiencies like low P (Reiter *et al*., 1991) or low N (Agrama *et al*., 1999; Hirel *et al*., 2001).

Ribaut *et al*. (2007) identified eight quantitative trait loci (QTL) for GY under low N. Of these, two were also detected under high N which could be used in laboratories to identify genotypes that tolerate low N stress and also have high yield under optimal conditions. The development of molecular marker technologies offers powerful alternative methods to examine the relationships between physiological traits controlling maize response to N, thereby contributing to a better understanding of metabolic pathways and physiological processes (Limami and de Vienne, 2001).

2.13 Farmer participation in breeding/selection

Farmers have preferences that are key factors when selecting varieties for production. In developing new cultivars and extending them to farmers, the formal breeding sector has often encountered two setbacks (De Groote *et al.*, 2002). First, many new cultivars have been unacceptable to farmers (Witcombe *et al.*, 2003). Secondly, breeders have necessarily discarded many crosses because of traits considered undesirable yet these may be of interest to farmers (De Groote *et al*., 2002). This is because the breeders are not often well informed of the needs and preferences of farmers (De Groote *et al.*, 2002). Farmers in Africa have special preferences for maize varieties such as taste, cooking qualities and high biomass for animal feed. Generally, farmers are heterogeneous in their needs, priorities, and preferences. Failure to consider these could result in rejection of otherwise promising new varieties. Close collaboration between farmers and breeders can promote yield increase or other improvements in marginal environments where modern varieties have not been adopted for agronomic, social, or economic reasons. Engaging

farmers would help breeders understand their needs and preferences which in turn would help in selecting appropriate genetic materials (Witcombe *et al*., 1996). It has been reported that, in most cases, breeders do not have a clear understanding of the farmers' requirements; hence breeding programmes might not have sufficiently considered the needs and preferences of farmers (Toomey, 1999; Banziger and Cooper, 2001; Banziger and de Meyer, 2002). This impedes the adoption of new cultivars. In fact, in developing countries most cultivars grown by farmers are old and only a few released cultivars are grown (Witcombe *et al*., 1996).

CHAPTER THREE

3.0 Farmers' perceptions on low soil N and maize hybrids and their implications in plant breeding

3.1 Introduction

Soil fertility decline is a major biophysical factor challenging crop production in Ghana (Logah *et al*., 2010). The estimated annual loss of maize grain yield due to low N stress alone varies from 10 to 50% (Wolfe *et al*., 1988; Logrono and Lothrop, 1997). Reducing the losses caused by low soil N could increase maize productivity in Ghana and significantly contribute towards national food security and poverty alleviation. Understanding the production constraints that farmers face, low soil fertility in particular, could greatly assist in the design of an effective breeding programme that not only incorporates tolerance to low soil N but improves other agronomic traits as well. The inclusion of farmers' perceptions about low soil N varieties in breeding programs is therefore essential.

More than 30 maize varieties have been released in Ghana from 1942-2012 (CRI library, maize manual). They have been made available to farmers through agricultural extension personnel, but there is slow productivity growth in maize and is caused by low adoption of productivity-enhancing technologies, including improved varieties and management practices, and low use of purchased inputs, especially fertilizer and also partly due to lack of appropriate government policies, lack of credit, inputs etc.

Meanwhile, the studies on technology adoption and impact on maize production in Ghana are outdated. The nationwide maize technology adoption and impact study was

conducted in 1997 (Morris, Tripp, and Dankyi 1999), The 2010–12 Diffusion of Improved Crop Varieties in Africa (DIVA) project sought to measure adoption of improved maize and other varieties but relied on scientists' opinions. Such opinions tend to be overestimates or otherwise unreliable, especially in agricultural research and development systems where adoption studies are not done regularly or researchers are not well connected with farmers, extension agents, and other innovation system actors. This situation impedes the adoption of new cultivars (Witcombe *et al.*, 1996). Engaging farmers in consultation and collaboration will help to exploit their knowledge and preferences in developing new varieties which will gain wider acceptability. Low adoption of improved varieties contributes to low yields and calls for maize breeders to put more emphasis on farmers' criteria of selection and preferences when developing maize varieties. Collaboration between farmers and scientists in problem identification and variety development will enhance breeders' understanding of the priority needs of farmers. This should result in farmers accepting the varieties and increase the adoption rate and on farm yields.

Participatory rural appraisal (PRA) tools are used to capture farmers' perceptions and preferences (Odendo *et al.*, 2002). Formal plant breeding approaches in the public sector have been less effective in doing so, as is evident in both the slow adoption rate of improved varieties by farmers, and the poor performance of adopted varieties under low input conditions (Bänziger and Cooper, 2001). Farmers have extensive knowledge of their environments, crops, and cropping patterns acquired over many seasons and generations (Bänziger *et al.*, 2000). Therefore, an assessment of attributes of maize

varieties preferred by farmers, and the socio-economic environment under which the farmers operate, is important.

PRA, which involves local people in gathering and analyzing information, allows identification of insights about local people and their actual circumstances, and fosters dialogue among scientists and farmers (De Groote and Bellon, 2000). By integrating farmers' concerns and circumstances into agricultural research, researchers would develop technologies that would be more widely adopted, resulting in more productive, stable, equitable and sustainable agricultural systems. The objectives of the study were to:

i) Examine maize production constraints of farmers in Ghana

ii) Assess farmers' perceptions of the problem of low soil fertility in maize production and coping strategies that farmers' use for the control of low soil fertility

iii) Investigate the opportunities for breeding new maize varieties with enhanced tolerance to low soil fertility and other important traits

iv) Evaluate farmers' perceptions on adoption of hybrids

3.2 Materials and Methods

3.2.1 Study Area

The participatory rural appraisal was carried out in two districts; Wenchi in the Brong Ahafo region and Ejura in the Ashanti region of Ghana. The two districts are in the Forest-Savanna transition agro-ecological zone. The Ejura district is located in the Northern part of Ashanti Region and the Wenchi district in the Western part of Brong Ahafo Region. The average annual rainfall is about 1,140 - 1,270 mm at Wenchi and

1,200 - 1,500 mm in Ejura. Three communities (villages) were chosen in each district with the help of extension officers based on the production volumes of maize, accessibility and presence of research activities. The three villages in the Wenchi district were Akrobi (07.743N, 082.129W), Awisa (07.81N, 0.02.11W) and Amposahkro (07.86N, 002.08W) while the villages in the Ejura district were Adiembra (07.43N, 001.49W), Aframso (07.31N, 001.39W) and Teacherkrom (07.33N, 001.43W). The two districts are characterized by a bimodal rainfall pattern (the major season is April –July whereas the minor season is September -November) and hence have two cropping seasons. The temperatures in the districts range from 21^0C -30^0C. The major occupation in these districts is farming and maize is one of the staple crops grown.

3.2.2 Selection of farmers

One hundred and twenty small-scale farmers were involved in the PRA study. They were identified through the local extension agents. The participants were randomly selected regardless of age, gender, experience in farming, or status in the community.

3.2.3 Survey procedure and data analysis

PRA tools such as Focus Group Discussions (FGD) and Key Informant (KI) interviews were conducted using semi-structured questionnaire to collect data. Focus group discussions were carried out in one community in each district. Each group consisted of 12 farmers. Prior to the FGDs the farmers were not informed that the focus of the study was low soil fertility (N) in order to avoid any possible biases in their responses. All of

the farmers in the focus groups were maize growers. The check list used in the discussions covered broad issues on crop environment and general farmer perceptions. The issues discussed included cropping systems, production constraints, soil fertility and seed attributes. To obtain comprehensive information on specific issues covered under the FGD, a formal survey followed in which 120 individual farmers, drawn from similar areas selected for the PRA (60 per district), (20 farmers per community) were interviewed using a semi-structured questionnaire. Data was analyzed using Statistical Package for Social Sciences (SPSS) version 16. Descriptive statistics such as frequency counts, percentages, and charts were used to describe the attributes of the variables collected.

3.3 Results

3.3.1 Demographic Characteristics

The results revealed that majority (57.5%) of the respondents fell between 36 and 65 years of age, 37.5% were between 16 and 35 years with only 5% of the respondents above 65years (Table 3.1). This suggests that most of the respondents were in the middle age and were thus expected to be active and contribute to maize production.

More than half (69.2%) of the respondents were males while 30.8% were females. About a third (36.7%) of the respondents had no formal education, 43.3% had primary education and 17.5% had secondary education. Very few (2.5%) of the respondents had studied above secondary education level. In addition, only 20% of the respondents belong to a farmer group organization. Belonging to Farmer Based Organization (FBO) is expected

to improve farmers' production skills through access to market information, credit and inputs (Asante *et al.,* 2011). Farmers who were not members of any association were either not aware of their existence, or were not aware of benefits of belonging to such groups. In two villages, Awisa and Amponsahkrom, none of the farmers interviewed belonged to a farmer group. The names of the farmer groups mentioned were Asempa, Ebe Ye Yie, Masara Nazaki, Odoneye and Vegetable group. A majority of the farmers (73%) had farms between 2-5 ha, 24.5% had farms greater than 5 ha while a few farmers (2.5%) had farms less than 2 ha.

Table 3. 1: Demographic characteristics of farmers from the six villages

	Frequency	**Percentage**
Age (Years)		
a. 16 – 35	45	37.5
b. 36 – 65	69	57.5
c. > 65	6	5
Sex		
a. Male	83	69.2
b. Female	37	30.8
Level of Education		
a. No formal education	44	36.7
b. Primary	52	43.3
c. Secondary	21	17.5
d. Above secondary	3	2.5
Member of farm group		
a. Yes	24	20
b. No	96	80
Size of farm		
a. <2ha	3	2.5
b. 2-5ha	88	73.3
c. >5ha	29	24.2

3.3.2 Cropping systems and crop production

A majority of the respondents practice mono cropping (93%) with maize as the dominant crop (Table 3.2). The other crops were cowpea, cassava, yam, pepper, okro and groundnut. Asked why they cultivated maize, 100% responded that they sold and consumed it and also made profit. Many farmers (52%) had sufficient land for crop production and allocated much of it to maize. Land preparation involved spraying with herbicides followed by plowing. Most farm capital was from individual savings (68%), while some (30%) was from their turn over. The respondents were not comfortable with loans and explained that loans were difficult to access. Availability of local and improved seeds was not a problem as 71% of the farmers from the various communities had access to seeds during the planting period. The majority of the farmers (70-100%) from the Ejura district cultivated the improved variety Aburohoma, basically because of its high yielding potential and marketability, whiles quite a number cultivated obaatanpa.

At the Wenchi district (Akrobi, Amponsakro and Awisa), the variety grown is a local variety "Kwoappiah". It was interesting to note that the same variety was called Appiah at Akrobi village. Their reasons for the adoption of this variety were that it is high yielding, marketable and easy to transport, because of its very small cob with many grains and long seed depth (Fig. 3.1). Another reason provided was easy disposal of the cobs due to their small sizes as many cobs can be packed and disposed at a time.

Figure 3. 1 Local Variety – Kwoappiah

Table 3. 2 Crop Production Systems by Percentage of Farmers

	Villages						
	Akrobi	Amponsakrom	Awisa	Adiembra	Aframso	Teacherkro	Pooled %
Maize the main crop	100	100	100	100	100	100	100
Land preparation							
a. Plough only	0	0	0	0	0	10	2
b. Spray & Plough	100	100	100	100	100	90	98
c. Hoe	0	0	0	0	0	0	0
Cropping Systems							
a. Mono	95	85	95	95	95	95	93
b. Mixed	5	15	5	5	5	5	7
Farming Capital							
a. Own Saving	80	65	75	60	65	60	68
b. Credit (Loan)	5	0	0	5	0	0	2
c. Turn Over	15	35	25	35	35	40	30
Seed Availability							
a. Good	60	65	60	85	85	70	71
b. Fair	40	35	40	15	15	30	29
c. Poor	0	0	0	0	0	0	0
Adequacy of Land							
a. Yes	70	30	45	55	70	45	52
b. No	30	70	55	45	30	55	48
Varieties grown							
a. Aburohoma	0	0	0	100	70	100	45
b. Kwoappiah	95	60	80	0	0	0	40
c. Dobidi	0	0	5	0	5	0	2
d. Obaatanpa	5	0	5	0	25	40	13
Why do you cultivate maize							
Both sale and comsumption	100	100	100	100	100	100	100
Do you make profit							
a. Yes	100	100	100	100	100	100	100
b. No	0	0	0	0	0	0	0

3.3.3 Constraints to maize production and soil fertility

Low soil fertility was identified as the most important constraint to maize production in all the villages (Fig. 3.2). This was followed by drought, pests and diseases. All the farmers interviewed described the symptoms of low soil fertility as yellowing of leaves, stunted growth and reduced yield, which is related to symptoms of low N or low P or both.

Asked about the factors responsible for low soil fertility, almost 100% of the farmers from all the villages mentioned continuous cropping on the same land as the main cause. Lack of application of fertilizer was perceived by the farmers as the next probable cause, though it was identified as the main cause in Teacherkro (Fig. 3.3). Lack of crop rotation was the third cause. Excessive rainfall was not identified as the cause of low soil fertility by any of the farmers in the locations as they explained that it rarely rained.

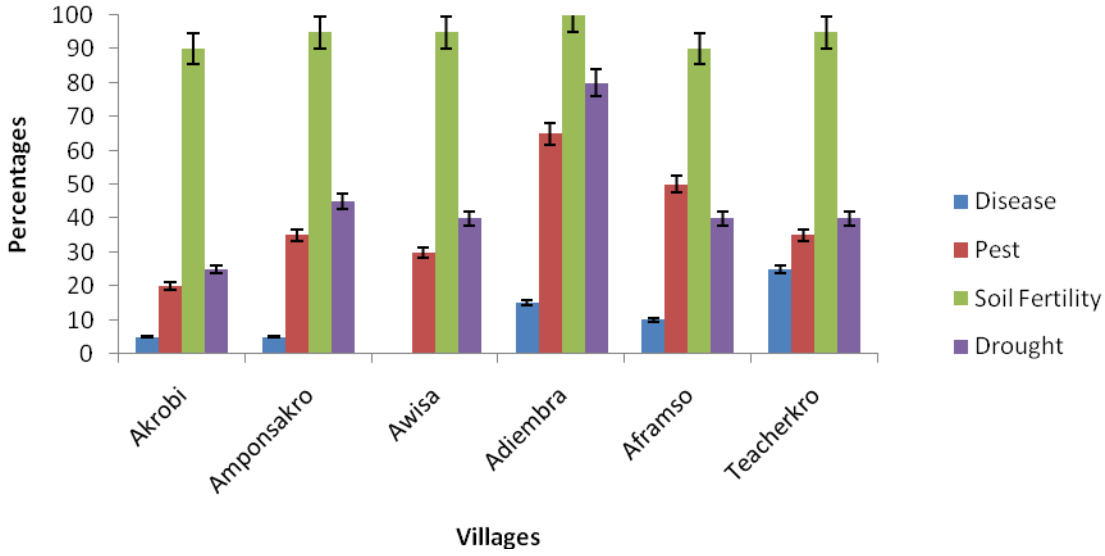


Figure 3. 2 Constraints to maize production in the various villages

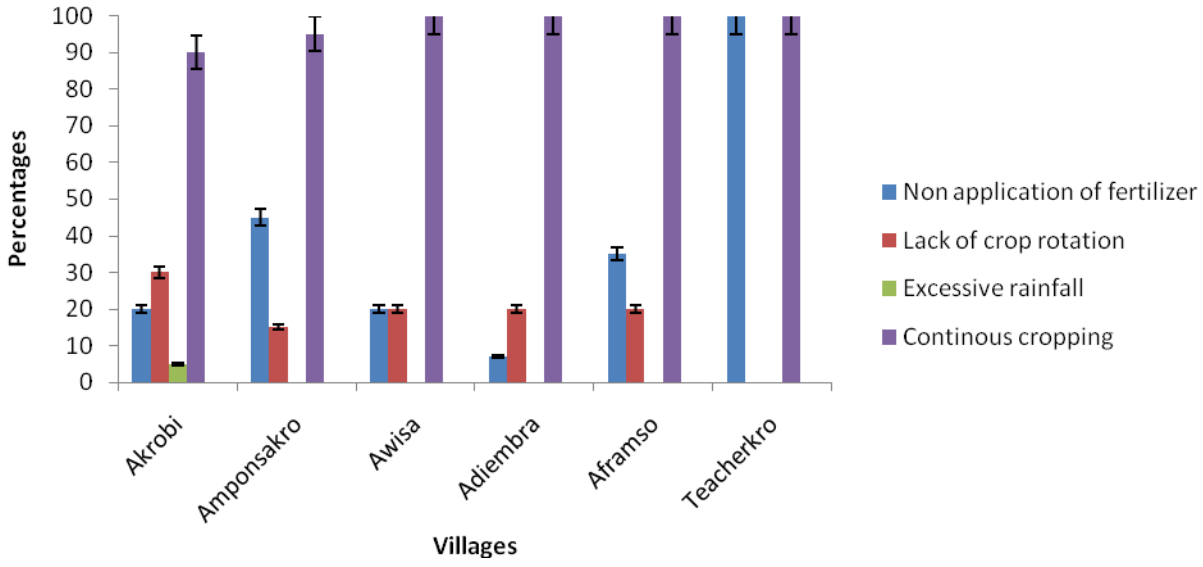


Figure 3. 3 Causes of low soil fertility

3.3.4 Management of low soil fertility (Copping strategies)

The majority of the farmers (94%) mentioned the use of fertilizer as the main way to replenish the soil and reduce low fertility (Fig. 3.4). This was followed by crop rotation (23.3%); only 5% of the farmers identified fallow as a way of managing fertility while none of the farmers mentioned land rotation as land is very scarce.

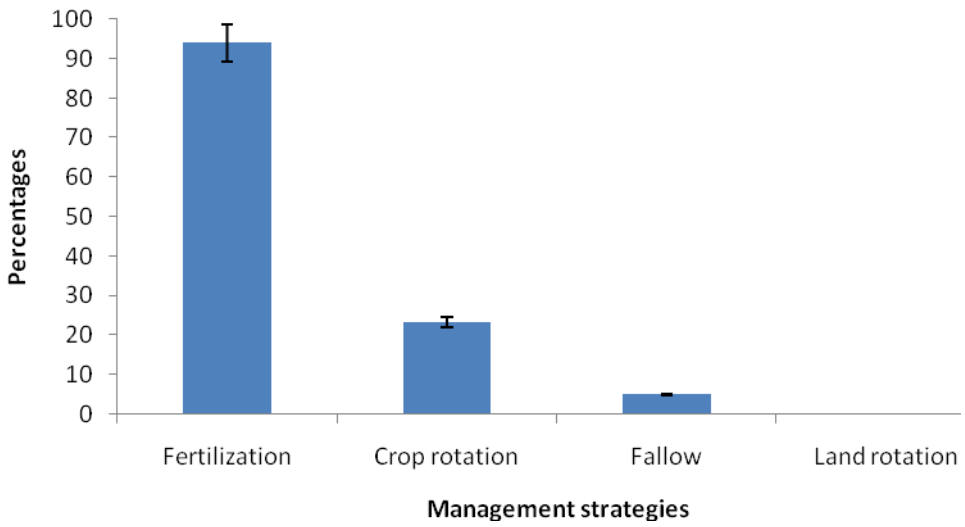


Figure 3. 4 Ways of managing low soil fertility by farmers

3.3.5 Fertilizer use

A good percentage (44.7%) of the farmers use Sulphate of Ammonia, 40.8% use NPK (15:15:15), 14.5% use urea while none of the farmers used manure (Fig. 3.5). It was also revealed that farmers apply fertilizer as top dressing, and do not practice basal dressing. They have subsidized and unsubsidized prices for fertilizers (Table 3.3)

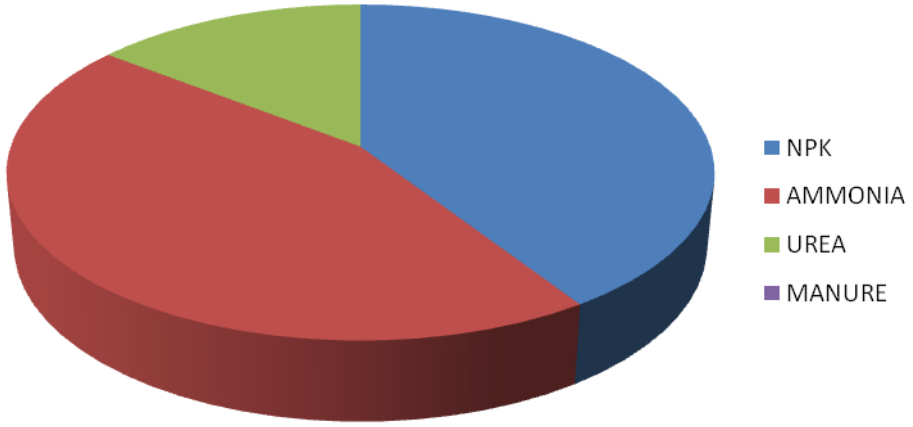


Figure 3. 5 Types of fertilizer use

Table 3. 3 Prices of fertilizers- (Ghana Cedis, 2014)

	Subsidized(GH₵)	Unsubsidized(GH₵)
NPK	38	50
Ammonia	35	45
Urea	None	50

A majority of the farmers (90%) buy unsubsidized fertilizers, which are very expensive. Asked about their reasons for buying fertilizer at such a price, they indicated that although the Government has subsidized fertilizer, sometimes it arrives after planting has already been completed, therefore they have no choice than to buy unsubsidized fertilizer. They felt that some of the agro stores distributed the coupons to friends and relatives, therefore, it was difficult to get coupons for the subsidized fertilizers.

Most of the farmers (78%) have increased the quantity of fertilizer used on their farms over a period of five years because they claimed the soil had degraded over the years.

Consequently, the two bags of fertilizer (100 kg) used five years ago are not enough to replenish nutrients in the soil of their farms thus it has now increased to 4-8 bags depending on the size of farm. Fifty percent of the farmers have decreased the number of bags of fertilizer used as it was too expensive to purchase. The major challenges in using fertilizer were the cost (100% of respondents) and non availability (36%).

3.3.6 Source of information about fertilizer

Majority of the farmers (80.1%) obtained information on fertilizer use and availability from the Ministry of Food and Agriculture, 13.3% obtained information from colleagues while a few (6.6%) had access to information through research scientists in research institutes (Fig. 3 6).

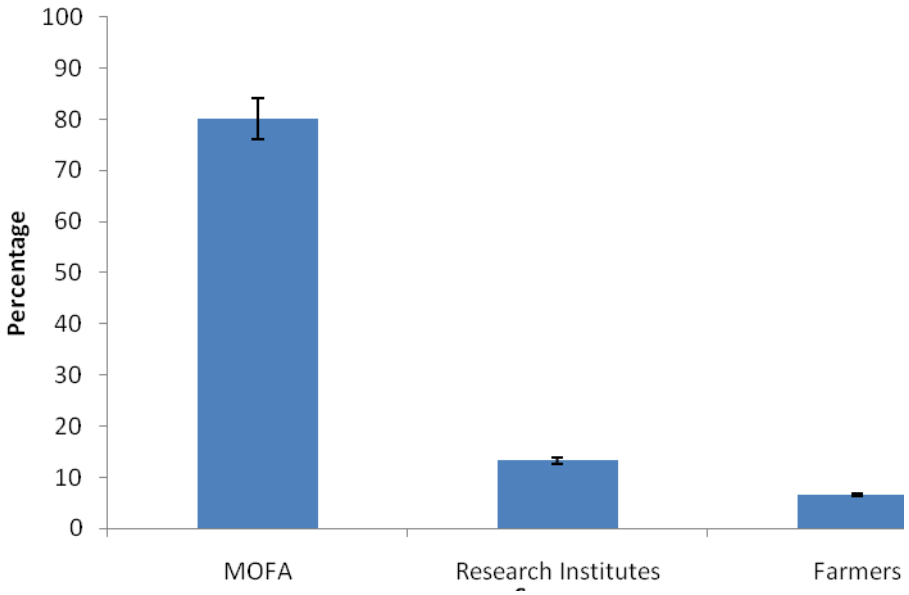


Figure 3. 6 Sources of fertilizer information

3.3.7 Farmers' perception of hybrids

Only (4.1%) of the farmers interviewed had ever planted hybrid seed, although 25.8% had heard about it (Table 3.4). About 95.9% of the farmers prefer to grow the local variety because the seeds can be saved and used over and over again (recycled), which is not so in the case of the hybrids. All (100%) the farmers indicated that the local seeds can be recycled, hence their preference. Their major concern was with the high cost of hybrid seeds. The 25.8% who have heard about hybrid seeds confused them with improved open pollinated (OPV) seeds. Ninety five percent (95%) of the farmers preferred the seeds of local and improved OPV to the hybrid seed because they believed that they were high yielding and responded better to drought than the hybrid seeds. The 4.1% of the farmers that grew hybrids argued that they were high yielding and had some level of tolerance to low soil fertility. From the FGD, it was revealed that hybrid seed will require high levels of fertilizer and the seed may not be available all the time. This showed that farmers need to be trained on the benefits of hybrids.

Table 3. 4 Perception on hybrid seed

Variables	Percentage (%)
Ever planted hybrid seed	
Yes	4.1
No	95.9
Ever heard about hybrid seed	
Yes	25.8
No	74.2
Why don't you plant hybrid seed	
Expensive	100
Cannot be recycled	100

3.3.8 Willingness to use low N tolerant maize varieties

Farmers were asked their opinions on the use of maize hybrids tolerant to low soil N. All of them were interested in such hybrids but expressed reservations about the current seed price. Most of the respondents were not willing to pay anything more than the existing market seed price (4:40 pesewas per kilo, as at 2015). They indicated that they would purchase the new hybrids only after observing their performance in on-farm trials.

3.4 Discussion

Although maize is the most important staple food crop in Ghana, farmers still plant other crops. This is consistent with the findings of Ragasa *et al*. (2013). Other crops planted by the farmers include cassava, cowpea, yam, pepper, okro and groundnut. This indicated that farmers were aware of the uncertainties and risks involved in farming and therefore try to diversify by cultivating crops with lower risk of crop failure as well as practicing crop rotation using nitrogenous crops to help improve soil fertility. It also means that they depend heavily on agriculture for income generation.

Although most small-scale farmers do not produce adequate amount of maize to meet their household needs, they still sell part of the produce to meet other domestic needs. Despite the lack of adequate land, most farmers cultivate their crops on small fields as they lack the necessary financial resources (cash and credit) to purchase farm inputs in order to have large farms.

The production constraints listed by farmers as impeding maize productivity in the smallholder sector are common to the majority of small-scale farmers in sub- Saharan Africa (De Vries and Toenniessen, 2001; Odendo *et al.,* 2002; Wekesa *et al.,* 2003). These are low soil fertility, drought, pests and diseases. Low soil fertility in the study area and its associated symptoms of leaf yellowing, stunted growth and reduced yield was perceived as the major constraint to maize production, as it increases the cost of production. The farmers have employed ways of coping with this problem of low soil fertility. The use of fertilizers is one of the major methods employed to replenish the soil as they consider lack of its application as a major cause of low soil fertility. Farmers

complained of the high price of fertilizer. Furthermore, they indicated that the fertilizer was sometimes not readily available. This meant low levels of application which invariably resulted in low yield. This finding is in disagreement with those of Ragasa *et al*. (2013) who stated that the fertilizer subsidy program may have encouraged farmers to use more fertilizer on their maize plots. They also stressed that the government subsidy on fertilizers is not really benefiting all farmers as some of them barely get access to the coupons for subsidized fertilizers. Again they asserted that occasionally the government subsidy on fertilizers arrives quite late when planting activities were completed hence making it not really useful to them. Efforts at enhancing timely availability of subsidized fertilizers at closer proximities to farmers are highly encouraged.

Application of manure can replenish soil fertility but may also introduce weeds and could be costly to farmers to procure and transport. There is also the problem of its application and availability. As a result, most farmers do not use manure. Manure is promoted by CISR and MOFA and farmers are willing to buy and use it, but a supply is just not available.

Some farmers practice crop rotation. This finding is consistent with those of Morris *et al.* (1999). Farmers in the areas of study use cowpea, cassava and groundnut for this practice. The farmers were aware of the fact that the crops used for the rotation could help fix nitrogen in the soil. Land rotation is scarcely used because land is very scarce and very expensive to acquire.

Appraisal of the economic circumstances of participants and communities where PRAs were conducted revealed that many of the participants could not afford these control measures that required the purchase of fertilizer and land. The cultivation of varieties that

are tolerant to low soil fertility appears to be the most practical and cost-effective means of low soil fertility management.

The majority of farmers planted improved maize varieties. The two most popular improved varieties were Obaatanpa and Aburohoma. Obatanpa, an OPV though released in 1992, has indications that it is becoming more popular even as newer varieties come on the market. Nearly 96 percent of the certified seeds produced in Ghana between 2001 and 2011 were of the Obatanpa variety. The older varieties are still popular. It may be that the new varieties do not perform significantly better than Obatanpa or that they are not being made sufficiently available to farmers via an effective seed system. The rate of varietal turnover for maize in Ghana is 23 years, which is far higher than estimates for other countries and indicates a serious weakness in the research and extension system. Ghana's research system develops and releases seven varieties every 10 years on average, which is very active and high according to African and international standards. However, a very high varietal turnover rate signals a research system that is breeding and producing varieties that do not necessarily address the needs and binding constraints faced by farmers or an ineffective extension system, or a combination of both (Ragasa *et al*.,2013). If varieties take too long to replace, the danger is that variety superiority and performance will collapse before replacement, given average longevity and environmental conditions (Alene and Mwalughali, 2012), which translates into low productivity and economic loss to farmers. There was great interest among the farmers in the new maize varieties and a willingness to adopt them if they incorporated farmers' preferences and were adapted to farming conditions. This finding is consistent with those of Nkonya and Featherstone

(2001), who reported that varieties with farmers' preferred traits were easily adopted. Farmers' personal experience influenced what varieties they grew.

Hybrid varieties have not yet been promoted much in Ghana, and that can be the most likely reason for low uptake by farmers. Ghana has lagged far behind other African countries in hybrid adoption. One sees more than 90 percent adoption of hybrid maize in Zambia, Kenya, and Zimbabwe (Tripp and Mensah-Bonsu, 2013) compared with only 3 percent in Ghana. The seeming lack of interest in hybrid seed among farmers in Ghana needs to be further investigated.

Farmers provided some reasons why they may not invest in hybrid seeds including high hybrid seed prices, non-availability of hybrid seed at local shops, high requirement of fertilizer for cultivation, small or no differences in yield when compared to local or improved varieties, poor storability and poor processing quality. These findings corroborate those of Pixley and Banziger (2001). Increased farmer-breeder interaction would allow the identification of other farmer preferred traits besides low N and the prioritizing of these during the selection process. Low adoption of the new varieties may in part be attributed to lack of interest or knowledge of these varieties by the farmers. Possibly, these varieties do not possess the qualities preferred or farmers may not have had the opportunity to try them as have been reported by (Harris *et al.*, 2001). Farmers in these villages indicated that they preferred low N and drought tolerant varieties with good storability, disease and insect resistance that require low inputs. They also indicated preference for slender cobs, light in weight with lots of grain which can be transported easily from the farm gate to the processing centre without incurring high cost of transportation.

It was surprising that the released hybrids in Ghana were not widely grown by these farmers. The low adoption may, in part, be attributed to lack of interest or knowledge of these varieties by the farmers. These farmers were not educated on hybrids. Since the farmers obtained information on fertilizer used from the extension workers at MOFA, it will therefore be important for the extension and other agencies to educate farmers on the importance and potential impact of hybrids. It will be appropriate for breeders to work with farmers in the process of varietal development as suggested earlier by Sperling *et al.* (2001). This would allow breeders to identify farmer's preferences in order to incorporate them into the improved varieties developed for their specific area. Sibiya *et al.* (2013) also suggested that breeding opportunities exist for improving farmers' local varieties, and maize breeders can take advantage of the preferred traits to incorporate them into existing high yielding varieties.

3.5 Conclusions

The survey revealed that farmers in Ejura and Wenchi districts obtained most of their food from the crops they cultivate, mainly maize, cassava, groundnut, cowpeas and okro. Major constraints to maize production in the districts were low soil fertility, drought, and diseases and insect pests.

Maize farmers in the districts prefer low N tolerant, drought tolerant maize varieties with good storability, disease and insect resistance and little need for inputs. In addition, they prefer slender cobs, light in weight with lots of grains. With such characteristics, the grains can be transported easily from the farm gate to the processing centre at a relatively

lower cost. Farmers will adopt low N tolerant hybrid maize varieties if the price of seeds is not too high. In addition to this trait, priorities of crop improvement should include tolerance to low N, drought, insect pests and diseases. Farmers would purchase new hybrids only after observing their on-farm performance.

It is imperative to breed for maize hybrids tolerant to low soil nitrogen as it will help farmers reduce the problem with lack of fertilizer. There is urgent need to encourage the adoption of hybrid varieties which are more responsive to applications of inputs to raise maize productivity. This will require varieties significantly superior to those now in use, an effective seed system, and mechanisms to overcome some of the market failures that discourage technology adoption.

CHAPTER FOUR

4.0 Combining ability, hybrid performance and heterotic grouping of intermediate maturing maize inbreds under low, high and across soil N environments

4.1 Introduction

Maize production, which has the greatest potential in the Savannas of West Africa due to higher incoming radiation, less diseases and insect pest pressure and lower night temperatures, is severely constrained by several biotic and abiotic stresses (Badu Apraku *et al*., 2013c). The most important abiotic stress factors are drought and low soil nitrogen (Low N) resulting from limited use of nitrogenous fertilizers and declining soil fertility (Sanchez, 2010).

In Ghana, the most limiting factors of maize production in all the agro- ecological zones, especially the savanna agro-ecological zone are erratic rainfall pattern and low soil fertility. The most significant being low soil N (Logah *et al*., 2010). Germplasm improvement for low N tolerance will remain a high priority of the National Maize Breeding Programme of Ghana because significant amount of maize is produced under low-N conditions. Nitrogen absorption by plants is crucial to improved growth. Nitrogen fertilization is therefore a powerful tool for increased maize yield. In Ghana, reduced availability of productive land for agricultural purposes has restricted farmers to cultivation on the same piece of land without fallowing or crop rotation year after year. As a result, most of the cultivated lands are depleted of nutrients. To remain productive and competitive, farmers have resorted to the heavy use of inorganic nitrogen fertilizers to boost yields. While these fertilizers are too expensive for Ghanaian small scale farmers, they also pollute the environment with nitrates. There is therefore an urgent need

for the development and commercialization of low N tolerant maize varieties and hybrids in Ghana.

Crop breeding is often conducted under high-yielding conditions but a considerable amount of maize in the tropics is grown under low nitrogen and drought conditions (Simmonds, 1991; Banziger *et al*., 1997; Banziger and Cooper, 2001). Thus, breeding under optimally managed agronomic conditions is not indicative of the type of conditions under which the majority of African farmers grow their crops (Bolaños *et al*., 1993; Banziger and Diallo, 2004; Muza *et al*., 2004). Success in breeding programs requires evaluation in environments that are representative of the target environments of the farmers (Allen *et al*., 1978).

Utilization of F_1 hybrids in maize has increased rapidly because of the potential of heterosis to tremendously increase yields. Hybrids have become the major driving force behind the success of many crops worldwide, mainly due to the good adaptation and superior yield performance of hybrids. In Ghana, hybrid maize production is still at its infant stage with only about 3% of farmers cultivating hybrids which are mainly imported (Ragasa *et al*, 2013). Breeding maize hybrids that are tolerant to low soil N would increase maize production in Ghana.

Combining ability is the ability of an inbred to contribute superior performance to a F_1 hybrid. Information on combining ability provides a basis for identifying superior heterosis and development of heterotic groups is important for efficient development of high yielding hybrids (Legesse *et al.*, 2009, Romanus *et al.*, 2007). Combining ability estimates give an indication of the type of gene action controlling quantitative characters

or traits, thereby assisting breeders in selecting suitable parent materials (Hallauer and Miranda, 1988).

Hybrids must be high yielding and stable across multiple environments to be successful. GGE biplot is a powerful statistical tool for identifying the best performing cultivar in a given environment and the most suitable environment for each cultivar, comparison of pairs of cultivars in individual environments, mega environment differentiation, average yield, stability of the genotypes and the discriminating ability and representativeness of each environment (Yan *et al*., 2000).

The objectives of the study were to:

(i) Identify high yielding maize hybrids under low and high N growing environments.

(ii) Determine the combining abilities and mode of gene action controlling low N tolerance in intermediate maturing maize inbred lines.

(iii) Classify the inbreds into heterotic groups.

(iv) Assess the performance and stability of the hybrids across low and high N environments.

4.2 Materials and Methods

4.2.1 Genetic materials

The genetic materials included 32 white intermediate maturing inbred lines obtained from International Maize and Wheat Improvement Center (CIMMYT), Institute of Agricultural Research for Development (IRAD) and International Institute of Tropical Agriculture (IITA)) and 3 inbred elite testers (9071, 1368 and 87036) (Table 4.1). Testers 1368 and

9071 are the parents of a commercial single cross hybrid, marketed by several seed companies in Nigeria (Menkir *et al.,* 2003). Tester 87036 is one of the parents of a commercial hybrid in Cameroun (Mafouasson, 2014). The 32 inbred lines were crossed to the three testers in a line by tester mating design to generate 96 hybrids. The testers were used as males and lines as females. Crosses were also made among the testers to generate three hybrids which were used as checks in addition to a released hybrid "Etubi" from Ghana. The crosses were carried out at the research fields of the CSIR-Crops Research Institute at Kwadaso in 2012.

Table 4. 1: List of inbred lines and testers used in the study

Inbred	Pedigree	Source
CLWN 349	HTBAB9 138·5-1.2TL-I-4-2TL-B-ITL-B_	CIMMYT
CML 494	LP~C"F·7-1-2-Z.2.2-8BB	CIMMYT
CLWN 364	SAHCl-5·1·1-5-3-B	CIMMYT
CLWN 341	LP SEQC3-H1-2-2·2-1-1-.a-B	CIMMYT
CLWN 238		CIMMYT
CLRCW 36		CIMMYT
ZM 523B-29-2-1-1-B*6	ZM 523B-29-2-1-1-B*6	CIMMYT
CLWN 359	SA3C4IiC(16X25)-2-4-3-1-B	CIMMYT
CLWN 247	(CL-FAWW11 x CML494)-B-24-2-2-B-B-1-B-8-B-B	CIMMYT
CML 442	CIMMYT M37W/ZM607#bF37sr-2-3sr-6-2-X]-8-2-X-1-BBBB	CIMMYT
CML 444	P43C9-1-1-1-1-1-BBBBB	CIMMYT
CML 198/LPSC	CML198/LPSC3H144-1-2-2-2-2-#-BB]-1-4-1-1-4-B*4-B-B-B	CIMMYT
CML 395/ CML 444	[(CML395/CML444)-B-4-1-3-1-B/CML395//DTPWC8F31-1-1-2-2]-5-1-2-2-BB-B-B-B	CIMMYT
ZM521B-66-4-1-1	ZM521B-66-4-1-1-BB-B-B-B	CIMMYT
CML 444/CML 395/ DTPWC8F31	[CML444/CML395//DTPWC8F31-1-1-2-2-BB]-4-2-2-1-1-BB-B-B-B	CIMMYT
Laposta Seq C7-F71-1-2	La Posta Seq C7-F71-1-2-1-1-B-B-B	CIMMYT
CML 254	TUXSEQ.149-2-BBB"II#'1·BB-f	CIMMYT
Laposta Seq C7-F18-3-2-1	La Posta Seq C7-F18-3-2-1-1-B-B-B-B-B	CIMMYT
J-16-1	Zm 523-16-2-1-1-B*4	CIMMYT
P43SRCq Fs100-1-1-8	P43SRCqFs100-1-1-8#1-B-13-B1	CIMMYT
TZM 501XKU1414XTZM501		CIMMYT
TZL Comp 3	TZL Comp 3-C2-S2-34-4-1-B	CIMMYT
CZL 068	[LZ956441/LZ966205]-B-3-4-4-BB	CIMMYT
CZL 0713	[SYN-USAB2/SYN-ELIB2]-12-1-1-1-BBB	CIMMYT
CLWN 240		CIMMYT

Table 4.1 Continued

Inbred	Pedigree	Source
CZL 00001	INTA-191-2-1-2-B*6	CIMMYT
TZD II 68	TZE-W POP STR 104 S6 40/160-2/3	IITA
TZD II 134	TZE-W POP STR 107 S6 238/254-2/2-3/3-2/4-2/2	IITA
TZD II 140	TZE-W POP STR 105 S6 53/253-1/2-2/3-3/4-2/3	IITA
TZD II 141	TZE-W POP STR 105 S6 53/253-1/2-2/3-2/4-2/3	IITA
CZL 03007	CML445/ZM621B]-2-1-2-3-1-BB	CIMMYT
M131		IRAD
87036*		IRAD
1368*	Across 7721 BC_2 x TZSR	IITA
9071*	N28 x TZSR	IITA

*Inbred testers

4.2.2 Experimental sites and field layout

The study was carried out at three locations; Fumesua, Ejura, and Kwadaso which are experimental fields of the CSIR-Crops Research Institute. Fumesua is in the semi-deciduous forest zone with an altitude of 286 m above sea level and it lies within Latitude 6°41N and Longitude 1°28W. Its mean annual rainfall is 1500 mm with mean minimum and maximum temperatures of 21°C and 31°C, respectively. The soil is Asuansi series, a ferric acrisol. Kwadaso is also located in the forest zone. It has a bimodal rainfall distribution pattern. The major season is from late March to mid July, while the minor season is from mid-September to mid-November. Kwadaso lies within Latitude 6^0 43N and Longitude 1^0 36W. The soil is ferric acrisol. Ejura is in the forest-savanna transition agro-ecological zone. It lies on Latitude 7^0 40N and Longitude 1^0 39W, and 221.9 m above sea level. Temperature at Ejura ranges from 31-34^{0}C with a relative humidity of 55-65%. The soil type at Ejura is Amantin series under the forest and savanna ochrosols and is moderately well drained, permeable and sandy loam (Adu and Asiamah, 1992; MoFA, 2011). The experiments were conducted in 2013 and 2014 in all locations for inbreds and hybrids except 2013 in Ejura for the inbred lines due to poor performance of the inbreds. The 32 inbreds plus the three inbred testers and a check (Ent 70) together with the 96 hybrids plus four checks (9071 x 1368, 9071 x 87036, 1368 x 87036, Etubi) were evaluated separately in two trials planted in adjacent blocks in all environments. The experimental designs were 6 x 6 and 10 x 10 lattice designs with two replications for inbreds and hybrids respectively. Single row plots, each 5 m long, 0.75 m between plants and 0.5 m within plants in each row were used in all the environments. Three seeds of the

lines were planted in each hole and thinned to two plants per hill at two weeks after emergence to give a population density of 53,333 plants per hectare.

4.2.3 Depletion of N in experimental sites

All the study sites were depleted of nitrogen by growing maize at a very high population density without fertilizer application, and removing the biomass after each harvest for a period of 2 years. Soil samples were taken each year before planting for all the test environments and N content was determined at the soil laboratory of the Soil Research Institute in Kumasi. Furthermore, at harvest there was total plant removal from the field.

4.2.4 Determination of the nutrient status of experimental sites

Results of soil properties of experimental fields at Kwadaso, Ejura and Fumesua in 2013 and 2014 are presented in Table 4.2. Nutrient status, in accordance with Landon (1991) interpretation of analyzed soils was generally low in all three locations except for phosphorus level which was very high at Kwadaso. Nitrogen levels were considered low in all locations since amounts less than 0.2% were recorded. Hence, it is expected that results obtained in the study would represent the true response of genotypes to the nitrogen applied externally.

Table 4. 2 Soil chemical properties of experimental sites

	Kwadaso		Ejura		Fumesua		Landon (1991) interpretation	
Soil Properties	0-15cm	15-30cm	0-15cm	15-30cm	0-15cm	15-30cm	High	Low
pH (1:1)	7.2	7.145	4.78	4.472	4.67	4.66	>6.5	<5.8
Organic C (%)	1.05	0.535	0.41	0.26	1.31	1.1	>10.0	<4.0
Total N (%)	0.09	0.032	0.03	0.02	0.12	0.11	>0.5	<0.2
Ex Ca (Cmolc/kg)	7.28	5.32	1.9	1.73	2.73	2.81	>10.0	<4.0
Ex Mg (Cmolc/kg)	1.6	0.76	1.24	1.4	0.53	0.6	>4.0	<0.5
Ex K (Cmolc/kg)	0.37	0.31	0.04	0.02	0.28	0.29	>0.6	<0.2
Ex Na (Cmolc/kg)	0.027	0.022	0.13	0.12	0.52	0.41	>1.0	<1.0
Av P (Mg/kg)	145.7	124	17.41	13.52	27.89	32.12	>50.0	<15.0
2014 N levels	0.09	0.04	0.04	0.04	0.13	0.12		

Ex: Exchangeable, Av: Available

4.2.5 Low soil Nitrogen treatment

The experimental fields were divided into low (30 kg N ha^{-1}) and high (90 kg N ha^{-1}) nitrogen blocks. Nitrogen was applied in the form of sulphate of ammonia. All the experiments received 60 kg ha^{-1} each of triple super phosphate and potassium at planting. In addition, 30 kg N ha^{-1} and 90 kg N/ha were split in to two applications and applied on the low and high N blocks at two and six weeks after planting (WAP), respectively. Weeds were controlled through the use of Atrazine and Gramozone as pre- and post-emergence herbicides and subsequently by hand weeding.

4.2.6 Data collection

Data recorded included days to 50% silking (DTS) as the number of days from planting to when 50% of the plants had emerged silks, and days to anthesis (DTA) when 50% had shed pollen. The anthesis-silking interval (ASI) was calculated as the difference between days to 50% silking and 50% anthesis. Plant height (PHT) was measured as the distance from the base of the plant to the height of the first tassel branch while ear height (EHT) was measured as the distance to the node bearing the upper ear, respectively. Root lodging (RL) percentage of plants leaning more than 30 degrees from the vertical, and stalk lodging (SL) (proportion or percentage of plants with broken stalk below the ear or the stalk bending more than 45 degrees from the upright position), and ear aspect (EASP) (based on a scale of 1 to 9, where 1=clean, uniform, large, and well-filled ears and 9 =ears with undesirable features), were also recorded. Plant aspect (PASP), based on the assessment of the general architecture of plants in a plot as they appeal to sight, was rated on a scale of 1-5 where, 1 = excellent overall phenotypic appeal, 2 = very good overall phenotypic appeal, 3 = good overall phenotypic appeal, 4 = poor overall phenotypic appeal and 5 = very poor overall phenotypic appeal. Ear number per plant (EPP) was obtained by dividing the total number of ears per plot by the number of plants harvested. Chlorophyll concentration of the ear leaf of five plants per plot, randomly selected, was measured at approximately 2 weeks after anthesis (WAA) with a portable SPAD meter (CCM-200 plus-opti sciences). Maize streak and blight diseases were also scored on a scale of 1 to 5, where 1= absence of disease and 5=severe infection. For trials conducted under N stress, harvested ears from each plot were shelled to determine the percentage grain moisture. Grain yield in kg ha^{-1} was computed from the shelled grain weight,

adjusted to 15% moisture. For the high N plot, a shelling percentage of 80% was assumed for all genotypes and grain yield (obtained from ear weight and converted to kg ha^{-1}) was adjusted to 15% moisture.

4.2.7 Statistical Analysis

Analysis of Variance (ANOVA) was performed on plot means for grain yield and other agronomic characters for each environment and across environments using PROC GLM procedure of SAS software, version 9.3 (SAS Institute, 2008). The analysis of variance (ANOVA) was performed separately for inbreds and hybrids. Entry means adjusted for block effects were analyzed according to a lattice design (Cochran and Cox, 1960). Each environment was defined as year x site x nitrogen treatment. Environmental effects were treated as random and genotypes as fixed effects.

The mathematical model underlying the Analysis of Variance for each experiment was as follows:

$$Y_{gjk} = \mu + G_g + BK + R_j + \varepsilon gjK$$

Y_{gjk} is the observation on the gth genotype of the kth block in the jth replication;

μ is the general mean;

G_g is the effect of the gth genotype

BK is the block effect within the jth replication

εgjK is the residual variation contributed by the jth replication for the gth genotype

The mathematical model for Analysis of Variances of the combined data is

$$Y_{igjk} = \mu + E_i + R_{j(i)} + Bk_{(ij)} + G_g + EG_{ig} + \varepsilon gjK$$

Where:

Y_{igjk} is the response of the gth genotype grown in the block k in replicate j of the environment i,

μ is the grand mean;

E_i is the main effect of environment;

$R_{j(i)}$ is the effect of replicate nested within environment effect;

$Bk_{(ij)}$ is the effect of block nested within replicate j by environment i;

G_g is the effect of the genotype;

EG_{ig} is the interaction effect between genotype and environment;

and εgjk is the error term.

i = 1,2,…..10; j = 1,2; k= 1,2….11 and g = 1,2,……121.

Restricted maximum likelihood (REML) estimates of the inbreds and hybrids genetic and phenotypic variances were obtained with SAS PROC Varcomp and were used to compute broad-sense heritability for each trait.

Broad-sense heritability (H^2) was estimated as:

$$h^2 = \sigma_G^2 / (\sigma_E^2/re + \sigma_{GE}^2 /e + \sigma_G^2),$$

Where; σ^2_G is genotypic variance, σ^2_E is error variance, σ^2_{GE} is genotype x environment interaction variance, r is number of replications, and e is number of environments (Fehr, 1991). Correlation coefficients were computed between grain yield and other agronomic traits using the PROC CORR from SAS.

Base Index for selection and Percentage yield reduction

The base index used for identifying low N tolerant genotypes incorporated grain yield (GY), ears per plant (EPP), stay-green characteristic (SG), plant aspect (PASP), ear aspect (EASP) and anthesis silking interval (ASI). N index score was computed as:

$$IN = 2.0\,GY + EPP - SG - ASI - PASP - EASP$$

Where; GY is grain yield under low N, EPP is ears per plant, SG is stay-green characteristic and ASI is anthesis-silking interval, PASP is plant aspect, and EASP ear aspect. Each parameter of the base index score was standardized with a mean of zero and a standard deviation of 1 to minimize the effects of different scales. Positive index value was an indicator of resistance/tolerance to low N while negative value was an indicator of susceptibility to low N.

The percentage grain yield reduction under low and high N environment was computed as the difference between grain yield under high and low, divided by yield under high N. Inbreds with a lower percentage yield reduction were tolerant and efficient under low N. A larger difference means inbreds performed very well under high N but poorly under low N environments. A negative difference means that inbreds performed better under low N than high N environments.

Heterosis

The mid-parent (MPH) and better parent heterosis (BPH) values for a cross were computed for each trait according to the scheme outlined by Matzingar et al. (1962) in the formulae:

MPH = [(F_1-MP)/MP] x 100

BPH = [(F_1-BP)/BP] x 100

Where;

F_1 = Mean of the hybrid,

MP = the mean of the parents that constituted the hybrids and

BP = the mean of the better parent.

MPH and BPH were averaged across low N environments and across high N environments.

Combining abilities

A line x tester analysis (Kempthrone, 1957) was done for crosses excluding the checks for low N environments, high N environments and across environments.

F tests for line, tester and line x tester mean squares were computed using the mean squares for their respective interaction with environment. The mean squares attributable to environment x line x tester was tested using the pooled error mean squares.

The main effects of line and tester represent the general combining ability (GCA) effects while line x tester interaction represents specific combining ability (SCA) effects.

Model 1: model for line x tester analysis at each environment is

$$Y_{ijk} = \mu + R_m + B_k + l_i + t_j + (l \times t)_{ij} + e_{ijk}$$

Model 2: model for line x tester analysis across environments is

$$Y_{eijkm} = \mu + E_e + R_m + B_k + l_i + t_j + (l \times t)_{ij} + (l \times E)_{ie} + (t \times E)_{je} + (l \times t \times E)_{ije} + \varepsilon_{eijkm}$$

Where;

Y_{eijkm} is the response of the of the *i* x *j*th progeny of the *K*th block of the *m*th replication of the *e*th

environment; where;

μ is the experimental mean;

Ee is the main effect of Environment;

R_m is the effect of the *m*th replication;

B*k* is the effect of the *k*th block within the *m*th replication;

l_i is the effect of the *i*th line;

t_j is the effect of the *j*th tester;

$(l \times t)_{ij}$ is the interaction effect of the cross between *i*th line and *j*th tester and;

$(l \times E)_{ie}$ is the interaction effect between line and the Environment;

$(t \times E)_{je}$ is the interaction effect between tester and the Environment;

$(l \times t \times E)_{ije}$ is the interaction effect between line and tester and the Environment;

ε_{eijkm} is the error effect associated with the $eijkm_{th}$ observation;

The source of variation for hybrids was partitioned into variability due to lines, testers and line x testers. Estimates of GCA of a tester (male) were obtained based on its performance in F_1 hybrid combinations with all possible lines (females). Similarly, GCA of a line was determined by its performance in F_1 hybrid combinations with all possible testers. GCA and SCA effects were determined for each agronomic trait under each N

environment and across environments. Estimates of GCA and SCA were calculated and their significance determined by *t* tests. They were computed as follows:

$GCA_l = X_l - \mu$,

$GCA_t = X_t - \mu$ where,

GCA_l and GCA_t= General combining ability of line and tester parents respectively,

X_l and X_t = Mean of line and tester parents respectively and

μ = Overall mean of crosses in the trial

Specific combining ability (SCA) was estimated as:

$SCAX = XX - E(XX) = XX - [GCA_l + GCA_t + \mu]$ where,

SCAx = specific combining ability of the cross x

XX = Observed mean value of the cross

E(XX) = Expected mean value of the cross based on the GCA of both parent

μ = Overall mean of crosses

The relative importance of GCA effects versus SCA effects on progeny performance was calculated as the ratio between sum of square due to GCA and total genotypic sum of squares (GCA and SCA sum of square) (Beck *et al.*, 1990; Pswarayi and Vivek, 2008).

Heterotic grouping of inbreds under contrasting environments

Classification of lines into heterotic groups was based on three methods:

1. Classification of inbred lines into heterotic groups using SCA effects for grain yield and testcross mean grain yield as suggested by (Menkir *et al.*, 2004). Lines with positive SCA with a tester and mean grain yield higher than the yield of the best check were assigned to the group opposite to that tester's heterotic group while lines

with negative SCA when crossed with a tester were classified into the same heterotic group as the tester

2. Heterotic groupings based on specific and general combining ability proposed by Fan *et al*., (2009) and computed as follow:

HSGCA = Cross mean *Xij* – Tester mean (*Xi*) = GCA + SCA,

in which *Xij* is the mean yield of the cross between *i*th tester and *j*th line, X*j* is the mean yield of the *i*th tester, and *Xj* is the mean yield of *j*th line. The computed HSGCA values were subjected to the three classification steps described by Fan *et al*. (2009).

Step 1: Place all inbred lines with negative HSGCA effects into the same heterotic groups as their tester.

Step 2: If an inbred line was assigned to more than one heterotic group in Step 1, keep the line in the heterotic group if its HSGCA had the smallest value (or largest negative value) and remove it from other heterotic groups.

Step 3: If a line had a positive HSGCA effect with all the testers, do not assign that line to any heterotic group because the line might belong to a heterotic group different from the testers used

3. Classification based on GCA effects of multiple traits (HGCAMT) proposed by Badu-Apraku *et al*. (2013, 2015 a, b)

The statistical model used by the HGCAMT method to assign the inbreds into the heterotic groups is as follows:

$$Y = \sum_{i=1}^{n} ((Y_i - \bar{Y}_i)/s) + \varepsilon ij$$

Where:

Y is HGCAMT, which is the genetic value measuring relationship among genotypes based on the GCA of multiple traits *i* to *n*;

Y_i is the individual GCA effect of genotypes for trait $_i$

$\bar{Y}_i$ μ is the mean of GCA effects across genotypes for trait *i*.

s_i is the standard deviation of the GCA effects of trait *i*;

ε_{ij} is the residual of the model associated with the combination of inbred i and trait j.

The grouping by HGCAMT was achieved by standardizing the GCA effects (mean of zero and standard deviation of 1) of the traits that had significant mean squares for genotype under low N and high N growing environments and across test environments to minimize the effects of different scales of the traits. The standardized GCA effects were subsequently subjected to Ward's minimum variance cluster analysis using SAS software version 9.3 (SAS Institute, 2011).

Breeding efficiency was estimated as proposed by Fan *et al*. (2009)

Yield stability

The yield data were further subjected to genotype main effect plus genotype × environment interaction (GGE) biplot analysis to evaluate the G × E interactions of each

experiment using the GGE biplot windows application (Yan *et al.*, 2000; Yan, 2001). The GGE biplot model equation is:

$$Yij - Yj = \lambda 1 \xi i1 \eta j1 + \lambda 2 \xi j2 \eta j2 + \Sigma ij$$

Where, Yij is the average yield of genotype i in environment j,

Yj is the average yield across all genotypes in environment j,

λ1 and λ2 are the singular values for principal component (PC)1 and PC2,

ξi1 and ξj2 are the PC1 and PC2 scores for genotype i,

ηj1and ηj2 are the PC1 and PC2 scores for environment j and

Σij is the residual of the model associated with the genotype i in environment j.

The data were not transformed (Transform=0), not standardized (Scale=0) and were environment-centered (Centering=2).

4.3 Results

4.3.1 Performance of hybrids and inbred parents under low N and high N conditions

4.3.2 Performance of hybrids

The combined Analysis of Variance (ANOVA) of hybrids evaluated under low N environments from 2013 to 2014 across locations showed significant genotype (G), environment (E), and genotype x environment interaction (GEI) mean squares for all traits except GEI for the stay green characteristic (Table 4.3). Similarly, the ANOVA combined across locations under high N revealed significant mean squares due to G, E and GEI for all traits except GEI for ears per plant and ear aspect (Table 4.4). Across environments (low N and high N environments), significant mean squares for G, E, and

GEI were detected for all traits except GEI for the stay green characteristic (Table 4.5). Under low N environments, genotype accounted for (16.29%) of the total sum of squares for grain yield, while GEI accounted for 41.81%, and environment 41.9%. Similarly, under high N environments, genotype accounted for 16.88% of the total sum of squares for grain yield, GEI genotype x environment interactions accounted for 42.55% and environment 40.56%. Across research environments, the test environments contributed about 66.46% of the total sum of squares due to grain yield; while the genotypes accounted for about 6.87% and GEI accounted for 26.67 % (Table 4.6).

Heritability estimates were interpreted based on the classification of Bhateria *et al*. (2006), where > 0.50 values were high, 0.30 - 0.50 values were medium and < 0.30 values were low. Broad sense heritability estimates on plot-mean basis ranged from 8% for plant aspect to 81% for days to anthesis under low N (Table 4.3) and 17% for husk cover to 86% for days to anthesis under high N conditions (Table 4.4). Across environments, broad sense heritability ranged from 35% for ears per plant to 91% for days to anthesis (Table 4.5). Heritability estimates for grain yield were 49%, 50% and 65% for low N, high N and across environments, respectively. The results thus revealed low to high broad sense heritability estimates.

Table 4. 3 Mean squares of intermediate maturing maize hybrids evaluated under low N environments in Fumesua, Ejura and Kwadaso in 2013 and 2014

Source of Variation	DF	GY	DTS	DTA	ASI	PHT	EHT	EPP	SG	PASP	EASP	HC	CC
Envt	5	86049116.5**	4327.05**	3332.37**	153.80**	82997.42**	18467.43**	3.70**	39.90**	85.81**	18.40**	69.04**	7964.80**
Rep(Envt)	6	2004304**	22.72**	23.88**	3.92**	2998.13**	2515.65**	0.23**	5.20**	18.53**	1.86**	1.15**	330.45**
Blk(Rep*Envt)	108	326341.2**	11.80**	7.93**	1.41*	803.88**	324.19**	0.06**	0.88**	0.48**	0.58ns	0.38**	51.10**
Hybrids	99	1689233.1**	23.48**	18.59**	2.28**	931.99**	673.74**	0.07**	0.93**	0.35*	0.93**	0.29**	97.33**
Envt*Hybrids	495	867369.4**	5.49**	3.71**	1.62**	424.06**	155.70**	0.06*	0.456ns	0.32**	0.75**	0.22*	36.43*
Error	486	184530	3.79	2.65	1.11	290.78	106.36	0.04	0.43	0.26	0.5	0.19	30.06
h^2		49	78	81	32	57	77	12	55	8	20	24	66

Table 4. 4 Mean squares of intermediate maturing maize hybrids evaluated under high N environments in Fumesua, Ejura and Kwadaso in 2013 and 2014

Source of Variation	DF	GY	DTS	DTA	ASI	PHT	EHT	EPP	SG	PASP	EASP	HC	CC
Envt	5	159865477.5**	5302.13**	4160.34**	222.21**	2476.77**	23085.31**	22.65**	112.75**	97.35**	14.33**	55.83**	9080.36**
Rep(Envt)	6	8983111.7**	46.06**	39.68**	1.41ns	730.98**	847.72**	0.27**	3.14**	2.83**	5.34**	2.25**	964.91**
Blk(Rep*Envt)	108	693651	5.91**	4.58**	0.85ns	79976.63**	244.05**	0.04ns	0.48**	0.52**	0.69**	0.48**	80.38**
Hybrids	99	3360394.2**	25.88**	19.74**	1.52**	265.67*	591.55**	0.04*	0.58**	0.54**	0.80**	0.54**	187.37**
Envt*hybrids	495	1693989.8**	3.88**	2.89**	1.04**	764.71**	147.47*	0.03ns	0.31*	0.33*	0.56ns	0.42**	49.46*
Error	486	779267	2.89	1.91	0.81	222.3	123.62	0.03	0.26	0.27	0.5	0.25	40.78
h^2		50	86	86	30	67	75	22	53	39	39	17	75

GY: Grain yield; **DTS**: days to silk; **DTA**: days to anthesis; **ASI**: anthesis silking interval; **PHT**: plant height; **EHT**: ear height; **EPP**: number of ears per plant; **SG**: Stay green; **PASP**: plant aspect; **EASP**: ear aspect; **HC**: husk cover; CC: chlorophyll content *, **, Significant at 0.05 and 0.01probability levels, respectively, and ns, not significant, **h^2**: Broad sense heritability

Table 4. 5 Mean squares of intermediate maturing maize hybrids evaluated across environments in Fumesua, Ejura and Kwadaso in 2013 and 2014

Sources of variation	DF	GY	DTS	DTA	ASI	PHT	EHT	EPP	SG	PASP	EASP	HC	CC
Envt	11	314734264**	4583.60**	3522.58**	183.66**	84172.76**	23486.71**	12.16**	108.18**	89.35**	24.22**	60.64**	15371.56**
Rep(Envt)	12	5493708**	34.39**	31.78**	2.67**	2737.45**	1681.68**	0.25**	4.17**	10.68**	3.60**	1.70**	647.68**
Blk(Rep*Envt)	216	509996ns	8.85**	6.25**	1.13ns	767.43**	284.12**	0.05**	0.68**	0.50**	0.63**	0.43**	65.74**
Hybrids	99	3615458**	42.69**	34.04**	2.33**	1387.65**	1126.54**	0.07*	1.09**	0.60**	1.25**	0.54**	230.37**
Envt*hybrids	1089	1275926**	4.72**	3.28**	1.34**	339.21**	146.66**	0.05**	0.37ns	0.32**	0.64**	0.32**	43.31**
Error	972	481898	3.34	2.28	0.96	256.57	114.99	0.04	0.34	0.27	0.5	0.22	35.41
h^2		65	90	91	42	77	88	35	70	48	49	40	83

GY: Grain yield; **DTS**: days to silk; **DTA**: days to anthesis; **ASI**: anthesis silking interval; **PHT**: plant height; **EHT**: ear height; **EPP**: number of ears per plant; **SG**: Stay green; **PASP**: plant aspect; **EASP**: ear aspect; **HC**: husk cover; CC: chlorophyll content *, **, Significant at 0.05 and 0.01probability levels, respectively, and ns, not significant, **$h^{2;}$** Broad sense heritability

Table 4. 6 Proportions of the total variance attributable to the sources of variation for grain yield of maize hybrids evaluated in low, high and across environments in 2013 and 2014

Sources of variation	DF	Sum of squares	% contribution to sum of squares
		High N	
Envt	5	799327387.4	40.56
Blk(Rep* Envt)	108	74914308.4	
Rep(Envt)	6	53898670	
Hybrids	99	332679028.8	16.88
Envt *hybrids	495	838524974.8	42.55
Error	486	378723749	
		Low N	
Envt	5	430245582.3	41.9
Blk(Rep* Envt)	108	35244845.3	
Rep(Envt)	6	12025823.7	
Hybrids	99	167234079.5	16.29
Envt*hybrids	495	429347837.3	41.81
Error	486	89681415	
		Across environments	
Envt	11	3462076899	66.46
Blk(Rep*Envt)	216	110159154	
Rep(Envt)	12	65924494	
Hybrids	99	357930352	6.87
Envt*Hybrids	1089	1389482968	26.67
Error	972	468405164	

Under low N, grain yield ranged from 528 Kg ha^{-1} for TZL Comp3 x 1368 to 2718 Kg ha^{-1} for TZD II 68 x 1368 with a mean of 1784 Kg ha^{-1} (Table 4.7). Grain yield under high N ranged from 2340 Kg ha^{-1} for ZM521B-66-4-1 x 1368 to 5348Kg ha^{-1} for CZL 00001 x 9071 with mean of 3713 Kg ha^{-1} (Table 4.8). Grain yield across environments ranged from 1601 Kg ha^{-1} for TZL Comp3 x1368 to 3852 Kg ha^{-1} for CZL 00001 x 9071 with a mean of 2766 Kg ha^{-1} (Table 4.9). The best check across research environments was 87036 x 9071 which had grain yield of 1876 Kg ha^{-1}, 3558 Kg ha^{-1} and 2717 Kg ha^{-1} under low, high and across N environments, respectively. The high yield of TZD II 68 x 1368 was associated with increased plant height, ears per plant, reduced ASI and good plant aspect. The highest yielding hybrid under low and high N out yielded the best check by more than 10%.

Out of the 100 hybrids evaluated under low N, 50 had positive base indices (an indication of tolerance to low N) with 40 producing grain yield above the mean. Based on grain yield and index for low N tolerance, the best 20 (20%) and worst 10 hybrids were selected and are presented with their respective % yield reduction in Table 4.7. Information on yield reduction as a result of the low N is essential to determine whether the severity of the stress was high enough for the identification of low N tolerant genotypes. Yield reduction was higher in susceptible hybrids than tolerant hybrids. The percentage yield reduction ranged from 17.83% for ZM523B-29-2-1-1-B*6 x 9071 to 80.24% for TZL Comp3 x 1368 (susceptible hybrid) with an average of 51.7%. The top four yielding hybrids based on their yield and base index were CLWN 247 x 9071 , ZM523B-29-2-1-1-B*6 x 9071, TZD II 68 x 1368 and P43SCRq Fs100-1-1-8 x 9071 with a percentage yield reduction of 44%, 17%, 37% and 33%, respectively. They out

yielded the best check by 27%, 30%, 31% and 25%, respectively. Among these four hybrids, TZD II 68 x 1368 was rated the most tolerant since it had the highest grain yield and ranked 3rd based on low N selection index. The poorest hybrids under low N were TZD II 134 x 1368, TZD II 140 x 1368, CLRCW x 1368, CLWN 238 X 1368 and TZL Comp3 x 1368. Seven hybrids (CZL 00001 x 9071, Laposta seqC7-F18-3-2-1 x 9071, CLWN 247 x 9071, CLWN 364 x 9071, TZD II 68 x 1368, CLWN 247 X 87036 and CML 395/CML 444 x 9071) were indentified among the 20 best across environments based on their yields. Only TZL Comp3 x 1368 was identified among the 5 poorest under both low and high N.

Table 4. 7 Grain yield and other agronomic traits of intermediate maturing maize hybrids (best 20 and worst 10) evaluated under low N environments in Fumesua, Ejura and Kwadaso in 2013 and 2014

Hybrids	GY	DTS	DTA	ASI	PHT	EHT	EPP	SG	PASP	EASP	HC	CC	base index	% yield reduction
CLWN 247 X 9071	2596	59.96	58.43	1.53	156.91	93.48	0.92	3.37	2.66	2.01	2.58	16.58	10.53	44.00
ZM523B-29-2-1-1-B*6 X 9071	2680	59.54	57.51	2.03	173.94	85.81	0.83	3.26	2.68	2.29	2.73	20.17	8.12	17.83
TZDII 68 X 1368	2718	56.06	54.48	1.58	168.95	64.30	0.84	3.28	2.84	2.42	2.69	18.89	7.95	37.45
P43SCRq Fs100-1-1-8 X 9071	2523	61.27	59.05	2.22	180.26	86.79	0.76	2.96	2.63	2.43	2.46	17.41	6.83	33.31
CML 395/ CML 444 X 87036	1869	56.54	54.53	2.01	164.05	75.80	0.72	2.59	2.37	2.34	2.23	23.32	6.63	54.56
TZM501 X KU1414 X TZM501 X 9071	2564	60.64	57.98	2.66	152.01	81.27	0.82	3.41	2.54	2.26	2.61	19.72	6.40	33.53
Laposta seq C7-F18-3-2-1 X 87036	1788	55.75	54.53	1.22	175.51	81.46	0.73	2.51	2.55	2.61	2.48	27.14	6.38	43.00
TZDII 68 X 87036	2304	56.25	54.02	2.23	176.59	73.07	0.73	2.71	2.48	2.77	2.48	20.58	5.84	33.27
CLWN 364 X 9071	2329	61.10	58.86	2.25	170.04	80.20	0.79	3.09	2.61	2.45	2.67	13.53	5.75	51.91
CZL 00001 X 9071	2355	61.27	58.50	2.76	170.61	79.25	0.71	3.07	2.29	2.50	2.49	22.77	5.45	55.96
CZL 0713 X 1368	2035	57.69	55.99	1.71	164.43	78.78	0.78	3.09	2.48	2.67	2.26	22.60	5.39	50.40
TZD II 134 X 87036	1941	58.06	56.24	1.82	176.53	87.06	0.82	3.04	2.55	2.59	2.34	24.87	5.27	47.02
CZL 068 X 1368	2127	57.32	55.31	2.01	149.71	63.50	0.69	2.73	2.64	2.43	2.59	23.26	5.25	47.72
Laposta Seq C7-F18-3-2-1 X 9071	2376	59.69	57.50	2.19	172.97	80.02	0.73	3.25	2.49	2.63	2.50	19.98	4.96	52.23
M131 X 1368*	2161	59.59	57.17	2.42	167.33	77.76	0.93	3.10	2.67	2.87	2.68	20.35	4.58	37.46
CLWN 359 X 9071	2463	60.57	58.30	2.27	167.03	78.58	0.66	3.28	2.55	2.44	2.70	15.67	4.50	39.74
CZL 03007 X 9071	1804	60.05	58.50	1.56	161.98	77.14	0.84	3.03	2.66	2.70	2.44	17.92	4.39	46.90
Laposta Seq C7-F71-1-1-2 X 9071	2073	59.28	56.70	2.58	178.42	86.98	0.69	2.98	2.34	2.56	2.39	20.68	4.11	33.21
Laposta Seq C7-F71-1-1-2 X 1368	2057	56.06	54.23	1.83	159.97	67.81	0.78	2.94	2.69	2.90	2.54	24.52	3.82	28.08
CML 395/ CML 444 X 9071	2315	60.36	57.49	2.87	162.69	79.76	0.84	3.02	2.66	2.82	2.51	18.71	3.74	47.72
TZD II 140 X 87036 *	1482	60.46	56.27	4.18	157.54	72.86	0.66	3.31	2.96	3.32	2.87	24.44	-5.45	54.16

Table 4.7 continued

Hybrids	GY	DTS	DTA	ASI	PHT	EHT	EPP	SG	PASP	EASP	HC	CC	Base index	% yield reduction
CLWN 359 X 1368	1329	60.89	56.76	4.14	160.16	65.20	0.61	3.53	2.65	3.29	2.60	19.20	-5.74	69.22
CML 444/CML 395/DTPWC8F31 X1368	1308	61.92	57.93	3.98	152.61	69.87	0.63	3.70	2.85	3.22	2.77	17.77	-6.72	64.27
CML 254 X 1368	1116	62.13	57.50	4.64	158.46	79.13	0.61	3.61	2.75	2.79	2.61	20.20	-7.03	61.61
J-16-1 X 1368	1440	61.19	56.96	4.23	166.24	71.07	0.61	3.68	3.08	2.95	2.80	18.67	-7.11	49.05
TZD II 134 X 1368	1252	60.64	55.74	4.90	163.23	83.55	0.63	3.50	3.01	2.63	2.72	18.39	-7.18	66.54
TZD II 140 X 1368	1560	61.58	56.94	4.64	165.79	73.80	0.67	3.72	2.99	3.44	2.98	18.27	-7.97	53.51
CLRCW 36 X 1368	1074	63.14	58.67	4.47	164.86	76.95	0.57	3.31	2.83	3.23	2.64	16.80	-8.21	69.17
CLWN 238 X 1368	745	63.21	59.37	3.84	141.51	71.07	0.54	3.34	3.07	3.38	3.00	20.39	-10.83	77.51
TZL Comp3 X 1368	528	62.81	58.42	4.38	139.53	63.97	0.54	3.48	3.08	3.81	2.72	16.11	-15.02	80.24
Etubi – (Check)	1124	59.05	56.83	2.22	149.60	69.83	0.57	2.94	2.83	2.95	2.36	21.48	-5.22	64.29
1368 X 87036 (Check)	1462	61.76	57.09	4.67	157.80	73.18	0.57	3.27	2.75	3.04	2.68	18.75	-5.50	50.32
1368 X 9071(Check)	1320	58.86	55.78	3.08	169.33	76.72	0.58	3.14	2.42	2.81	2.39	18.36	-3.93	59.06
87036 X 9071(Check)	1876	58.46	57.01	1.45	162.80	80.73	0.67	3.02	2.37	2.99	2.43	20.41	3.59	47.27
Means	1784	59.55	57.10	2.44	164.23	77.59	0.71	3.19	2.69	2.87	2.61	19.40		
Max	2718	63.21	59.39	4.90	193.85	102.15	0.93	3.82	3.15	3.81	3.00	27.14		
Min	528	55.75	54.02	1.22	139.53	63.50	0.54	2.51	2.29	2.01	2.23	11.53		
SE	137	0.62	0.52	0.34	5.41	3.30	0.06	0.21	0.16	0.24	0.14	1.74		

GY: Grain yield; **DTS**: days to silk; **DTA**: days to anthesis; **ASI**: anthesis silking interval; **PHT**: plant height; **EHT**: ear height; **EPP**: number of ears per plant; **SG**: Stay green; **PASP**: plant aspect; **EASP**: ear aspect; **HC**: husk cover; CC: chlorophyll content

Table 4. 8 Grain yield and other agronomic traits of intermediate maturing maize hybrids (best 20 and worst 10) evaluated under high N environments in Fumesua, Ejura and Kwaadaso in 2013 and 2014

Hybrids	GY	DTS	DTA	ASI	PHT(cm)	EHT(cm)	EPP	SG	PASP	EASP	HC	CC
CZL 00001 X 9071	5348	59.99	57.49	2.50	194.86	95.05	0.76	2.44	2.24	2.20	2.48	27.56
Laposta Seq C7-F18-3-2-1 X 9071	4974	57.85	56.43	1.41	181.37	87.55	0.66	2.16	2.29	2.05	2.32	33.03
CLWN 364 X 9071	4844	57.65	56.05	1.60	186.11	92.06	0.64	1.96	1.99	2.19	2.01	26.24
CML 494 X 1368	4671	57.26	55.82	1.44	173.21	78.09	0.79	2.29	2.27	2.29	2.14	30.38
CLWN 247 X 9071	4636	58.66	56.70	1.96	184.28	98.57	0.75	2.83	2.27	2.17	2.24	27.78
CLWN 247 X 87036	4614	56.54	54.95	1.59	184.28	90.52	0.66	2.26	2.66	2.66	2.19	33.30
CLWN 247 X 1368	4507	57.86	56.37	1.49	179.15	96.80	0.67	2.68	2.28	1.94	2.34	31.05
CML 395/ CML 444 X 9071	4428	56.84	54.97	1.87	177.85	91.59	0.66	2.06	2.23	2.60	2.54	27.66
CLWN 238 X 9071	4427	60.90	57.84	3.06	181.58	97.50	0.59	2.60	2.40	2.41	2.52	25.14
CML 395/ CML 444 X 1368	4422	56.75	54.98	1.77	171.42	84.37	0.72	1.99	2.10	2.26	2.07	30.15
TZDII 68 X 1368	4344	55.16	53.20	1.96	185.17	78.74	0.60	2.38	2.16	2.27	2.21	31.80
CML 494 X 9071	4340	58.69	56.60	2.09	182.30	93.80	0.67	2.38	2.36	2.15	2.39	29.92
TZD II 140 X 9071	4327	57.47	55.61	1.86	181.59	91.25	0.60	2.32	2.34	2.40	2.80	27.32
CLWN 359 X 1368	4316	56.58	55.13	1.46	181.16	81.67	0.70	2.26	2.49	2.42	2.63	33.62
CML 198/ LPSC X 9071	4255	58.87	56.94	1.94	182.78	89.56	0.70	2.54	2.24	2.20	2.11	33.01
CLWN 240 X 9071	4253	58.90	56.84	2.06	185.53	97.05	0.64	2.49	2.14	2.05	2.37	24.89
CML 444/CML 395/DTPWC 8F31X9071	4228	58.86	57.09	1.77	181.79	93.90	0.65	2.28	2.03	2.46	2.05	25.62
P43SRCq Fs100-1-1-8 X87036	4204	56.40	54.98	1.42	182.06	84.74	0.72	1.89	2.03	2.15	1.69	28.79
CML 254 X 9071	4193	59.64	57.47	2.18	177.77	100.44	0.67	2.22	2.45	2.33	2.38	33.61
CLWN 341 X 9071	4172	61.08	58.32	2.76	183.99	90.18	0.71	2.42	2.30	1.81	2.36	28.44
Laposta Seq C7-F71-1-1-2 X 9071	3105	57.96	55.94	2.02	184.53	90.36	0.62	2.57	2.49	2.91	2.77	33.38
CML 198/ LPSC X 1368	3099	56.26	55.09	1.16	168.48	75.82	0.59	2.58	2.58	2.81	2.42	35.19

Table 4.8 continued

Hybrids	GY	DTS	DTA	ASI	PHT(cm)	EHT(cm)	EPP	SG	PASP	EASP	HC	CC
CLWN 359 X 87036	3056	56.76	55.13	1.62	176.53	79.91	0.58	2.23	2.45	2.20	2.72	34.62
ZM 521B-66-4-1-1 X 9071	2990	58.61	56.36	2.25	171.52	86.42	0.60	2.33	2.94	3.12	2.77	28.75
CML 254 X 1368	2907	57.82	56.15	1.67	171.11	85.11	0.53	2.73	2.53	2.15	2.03	33.86
Laposta Seq C7-F71-1-1-2 X 1368	2860	56.47	55.21	1.26	168.31	77.80	0.59	2.48	2.35	2.56	1.96	32.26
CLWN 349 X 1368	2837	57.83	55.99	1.84	182.81	78.95	0.59	2.42	2.30	2.48	2.36	32.74
J-16-1 X 1368	2825	55.60	53.86	1.74	171.07	78.31	0.57	2.91	2.77	2.79	2.90	33.46
TZL Comp3 X 1368	2673	59.26	57.56	1.70	145.54	69.35	0.54	2.91	3.20	3.01	3.25	28.34
ZM 521 B-66-4-1-1 X 1368	2340	57.61	55.65	1.96	157.75	77.86	0.54	2.45	2.84	3.11	2.68	33.12
Checks												
87036 X 9071	3558	57.53	55.32	2.21	177.30	88.54	0.58	2.51	2.31	2.76	2.33	30.86
Etubi – (Check)	3149	56.29	54.87	1.42	164.10	74.63	0.56	2.26	2.74	3.24	2.61	36.69
1368 X 87036	2942	56.94	55.12	1.82	173.64	84.87	0.55	2.51	2.53	2.94	2.34	31.61
1368 X 9071	3225	58.50	56.16	2.34	177.17	84.50	0.59	2.22	2.25	2.35	2.31	32.74
Means	3713	57.42	55.64	1.76	177.86	86.77	0.65	2.35	2.35	2.45	2.34	31.22
Max	5348	61.08	58.32	3.06	196.12	105.06	0.87	2.91	3.20	3.24	3.25	31.22
Min	2340	54.34	52.62	0.85	145.54	69.35	0.53	1.83	1.85	1.81	1.69	22.12
SE	284	0.54	0.44	0.29	4.75	3.55	0.06	0.16	0.16	0.23	0.16	2.05

GY: Grain yield; **DTS**: days to silk; **DTA**: days to anthesis; **ASI**: anthesis silking interval; **PHT**: plant height; **EHT**: ear height; **EPP**: number of ears per plant; **SG**: Stay green; **PASP**: plant aspect; **EASP**: ear aspect; **HC**: husk cover; CC: chlorophyll content

Table 4. 9 Grain yield and other agronomic traits of intermediate maturing maize hybrids (best 20 and worst 10) evaluated across environments in Fumesua, Ejura and Kwadaso in 2013 and 2014

Hybrids	GY	DTS	DTA	ASI	PH	EH	EPP	SG	PASP	EASP	HC	CC
CZL 00001 X 9071	3852.00	60.63	57.99	2.63	182.74	87.15	0.73	2.75	2.27	2.35	2.48	25.17
Laposta Seq C7-F18-3-2-1 X 9071	3675.00	58.77	56.96	1.80	177.17	83.78	0.70	2.71	2.39	2.34	2.41	26.51
CLWN 247 X 9071	3616.00	59.31	57.57	1.74	170.59	96.02	0.83	3.10	2.47	2.09	2.41	22.18
CLWN 364 X 9071	3587.00	59.38	57.45	1.92	178.07	86.13	0.71	2.53	2.30	2.32	2.34	19.89
TZDII 68 X 1368	3531.00	55.61	53.84	1.77	177.06	71.52	0.72	2.83	2.50	2.34	2.45	25.34
CLWN 247 X 87036	3377.00	58.00	56.19	1.82	178.07	87.55	0.68	2.74	2.73	2.74	2.49	26.60
CML 395/ CML 444 X 9071	3371.00	58.60	56.23	2.37	170.27	85.68	0.75	2.54	2.44	2.71	2.52	23.19
CLWN 359 X 9071	3275.00	59.69	57.71	1.97	179.17	86.50	0.64	2.72	2.20	2.34	2.35	21.41
TZD II 134 X 9071	3272.00	59.52	57.23	2.29	187.90	101.62	0.70	2.72	2.59	2.49	2.67	26.30
CML 198/ LPSC X 9071	3260.00	59.64	57.73	1.91	172.04	83.55	0.72	2.97	2.40	2.64	2.34	27.21
CML 494 X 1368	3241.00	58.22	56.49	1.74	162.11	75.26	0.80	2.79	2.53	2.60	2.40	24.36
CLWN 247 X 1368	3240.00	58.64	57.03	1.61	172.47	86.95	0.72	3.11	2.60	2.23	2.62	24.82
CML 395/ CML 444 X 1368	3217.00	57.72	55.81	1.91	164.67	79.40	0.75	2.32	2.52	2.62	2.37	25.70
TZD II 68 X 9071	3212.00	57.87	55.77	2.10	190.72	91.08	0.74	2.73	2.62	2.42	2.46	20.81
CLWN 238 X 9071	3212.00	60.12	57.87	2.25	174.19	93.00	0.65	3.03	2.54	2.67	2.54	20.30
TZM501 X KU1414 X TZM501 X 9071	3211.00	60.40	57.97	2.44	166.48	85.37	0.74	2.98	2.39	2.12	2.47	22.33
CLWN 240 X 9071	3185.00	60.04	57.62	2.42	181.51	97.40	0.72	3.05	2.34	2.57	2.43	18.21
CML 494 X 9071	3180.00	59.94	57.59	2.35	174.06	90.80	0.70	2.80	2.51	2.33	2.45	23.75
TZD II 140 X 9071	3166.00	59.38	57.28	2.09	181.92	87.78	0.63	2.93	2.63	2.66	2.87	21.78
P43SCRq Fs100-1-1-8 X 9071	3153.00	60.48	58.46	2.02	183.81	88.81	0.69	2.77	2.51	2.47	2.43	20.95
TZM501 X KU1414 X TZM501 X 1368	2332.00	59.41	57.79	1.62	161.37	72.25	0.64	3.05	2.62	2.86	2.63	24.35
J-16-1 X 87036	2323.00	57.12	55.18	1.94	177.61	76.49	0.65	2.60	2.35	2.52	2.51	28.61
ZM 521B-66-4-1-1 X 9071	2282.00	59.05	57.25	1.81	165.66	81.41	0.64	2.67	2.80	3.33	2.71	23.99
CLRCW 36 X 1368	2278.00	60.10	58.05	2.05	172.83	78.63	0.58	3.01	2.71	2.79	2.53	21.53
J-16-1 X 1368	2132.00	57.40	55.41	1.98	168.66	74.69	0.59	3.30	2.93	2.87	2.85	26.06

Table 4.9 continued

Hybrids	GY	DTS	DTA	ASI	PH	EH	EPP	SG	PASP	EASP	HC	CC
CLWN 349 X 1368	2105.00	58.55	56.82	1.72	172.23	71.47	0.69	2.85	2.58	2.76	2.53	25.01
CLWN 238 X 1368	2027.00	60.17	58.39	1.78	147.02	76.08	0.60	2.96	2.87	3.01	2.83	22.00
CML 254 X 1368	2011.00	58.98	56.82	2.15	164.79	82.12	0.57	3.17	2.64	2.47	2.32	27.03
ZM 521 B-66-4-1-1 X 1368	1723.00	57.67	55.84	1.83	153.05	74.13	0.58	2.84	2.87	3.11	2.72	27.70
TZL Comp3 X 1368	1601.00	60.03	57.99	2.04	142.53	66.66	0.54	3.19	3.14	3.41	2.98	22.23
Checks												
Etubi – (Check)	2137.00	57.67	55.85	1.82	156.85	72.23	0.56	2.60	2.79	3.10	2.48	29.08
1368 X 9071	2273.00	58.68	55.97	2.71	173.25	80.61	0.59	2.68	2.34	2.58	2.35	25.55
1368 X 87036	2202.00	58.35	56.10	2.25	165.72	79.02	0.56	2.89	2.64	2.99	2.51	25.18
87036 X 9071	2717.00	58.00	56.16	1.83	170.05	84.63	0.63	2.77	2.34	2.88	2.38	25.63
Mean	2766.00	58.37	56.38	1.99	171.24	82.31	0.69	2.77	2.52	2.65	2.47	25.27
Max	3852.00	61.36	58.70	2.85	190.72	101.62	0.83	3.30	3.14	3.41	2.98	33.70
Min	1601.00	55.29	53.37	1.23	142.53	66.66	0.54	2.21	2.16	2.09	2.00	18.21
SE	157.00	0.41	0.34	0.22	3.60	2.43	0.43	0.13	0.12	0.16	0.11	1.34

GY: Grain yield; **DTS**: days to silk; **DTA**: days to anthesis; **ASI**: anthesis silking interval; **PHT**: plant height; **EHT**: ear height; **EPP**: number of ears per plant; **SG**: Stay green; **PASP**: plant aspect; **EASP**: ear aspect; **HC**: husk cover; CC: chlorophyll content

4.3.3 Performance of the inbred lines under contrasting environments

The combined Analysis of Variance of the inbred lines evaluated across low N environments revealed significant mean squares for G, E and GEI for all the traits except G for ASI, EPP and SG and GEI for DTS, ASI, DTA, EPP, PASP and HC (Table 4.10). Across high N environments, (Table 4.11) differences among G were significant ($P<0.05$) for all the traits except ASI, EPP and stay green characteristic. Significant differences were detected for few traits except DTA, DTS ASI, EH PHT, SG, EASP and EPP for GEI. Significant differences were detected in the environments for all the traits. Highly significant ($P<0.01$) differences were observed across environments (Table 4.12) for G, E and GEI for grain yield and all the other traits except G for ASI, EPP and SG, GEI mean square for DTA, DTS, ASI, EHT and EPP.

Table 4. 10 Mean squares of intermediate maturing maize inbred lines evaluated under low N environments in Fumesua, Kwadaso in 2013 and 2014 and Ejura in 2014

Source of Variation	df	GY(Kg/ha)	DTS	DTA	ASI	PHT(cm)	EHT(cm)	EPP	SG(1-9)	PASP	EASP	HC
Envt	4	20235200.6**	3955.47**	2784.29**	497.00**	35472.30**	9450.55**	3.17**	22.42**	6.20**	8.77**	6.21**
Blk(Envt * Rep)	50	264885.5**	29.88ns	42.54**	10.25*	439.92**	178.34	0.14*	0.39*	0.39ns	0.55**	0.4ns
Rep(Envt)	5	634903**	1.77ns	25.91ns	4.54ns	1238.79**	325.01*	0.19ns	0.71*	0.86*	0.69ns	0.73*
Inbreds	35	1358614**	74.65*	33.03**	7.84ns	879.88**	294.17**	0.11ns	0.32ns	0.68**	0.53**	0.48*
Inbreds * Envt	140	964448.5**	37.21ns	21.57ns	7.68ns	617.42**	225.09**	0.1ns	0.40**	0.38ns	0.55**	0.33ns
Error	125	161814.1	45.45	19.1	7.11	256.86	131.20	0.09	0.25	0.38	0.31	0.3
h^2		32	43	31	49	52	38	21	48	42	34	32

Table 4. 11 Mean squares of intermediate maturing maize inbred lines evaluated under high N environments in Fumesua, Kwadaso in 2013 and 2014 and Ejura in 2014

Source of Variation		GY(Kg/ha)	DTS	DTA	ASI	PHT(cm)	EHT(cm)	EPP	SG(1-9)	PASP	EASP	HC
Envt	4	42587852.2**	2178.34**	1556.98**	233.81**	30987.32**	6682.24*	3.12**	40.91**	21.23**	3.11**	13.29**
Blk (Envt * Rep)	50	816850.6ns	23.02ns	17.43ns	2.62ns	507.6ns	156.81ns	0.11ns	0.32ns	0.38ns	0.41ns	0.48**
Rep(Envt)	5	485656.3ns	18.17ns	17.66ns	1.49ns	1167.1ns	444.68**	0.21ns	0.31ns	1.17**	1.87**	1.13**
Inbreds	35	3587457**	40.93*	35.15**	1.64ns	1090.05**	349.88*	0.09ns	0.39ns	0.73**	0.57*	0.62**
Inbreds * Envt	140	1402927.4**	22.03ns	16.29ns	2.4ns	598.53ns	164.12ns	0.10ns	0.36ns	0.47*	0.43ns	0.37*
Error	125	7880652	26.6	18.93	2.12	589.78	194.83	0.12	0.31	0.34	0.34	0.27
h^2		56	45	42	51	56	645	34	55	66	56	43

GY: Grain yield; **DTS**: days to silk; **DTA**: days to anthesis; **ASI**: anthesis silking interval; **PHT**: plant height; EHT: ear height; **EPP**: number of ears per plant; **SG**: Stay green characteristic; **PASP**: plant aspect; **EASP**: ear aspect; **HC**: husk cover; *, **, Significant at 0.05 and 0.01probability levels, respectively, and ns, not significant

Table 4. 12 Mean squares of 36 intermediate maturing maize inbred lines across environments in Fumesua, Kwadaso in 2013 and 2014 and Ejura in 2014

Sources of variation	Df	GY	DTA	DTS	ASI	PHT	EHT	EPP	SG	PASP	EASP
Envt	9	31303317.9**	1945.96**	2736.26**	325.48**	30007.19**	7387.53**	2.85**	30.15**	17.34**	5.53**
Blk(Rep*Envt)	100	540868ns	29.99**	26.45	6.44**	473.76ns	167.58ns	0.13ns	0.36ns	0.43ns	0.48*
Rep(Envt)	10	560279.6ns	21.79ns	9.97ns	3.01ns	1202.95**	387.34**	0.20*	0.51ns	0.96**	1.26**
Inbreds	35	3806013.8**	48.63**	78.42**	4.95ns	1216.35**	386.98**	0.14ns	0.4ns	0.64**	0.53*
Envt*Inbreds	315	1153537.9**	18.28	29.81	4.98ns	624.29**	196.15	0.09	0.37**	0.48**	0.49**
Error	250	474940	19.01	36.03	4.61	423.32	163.02	0.1	0.28	0.37	0.33
h^2		53	48	54	61	66	67	42	53	62	60

GY: Grain yield; **DTS**: days to silk; **DTA**: days to anthesis; **ASI**: anthesis silking interval; **PHT**: plant height; **EHT**: ear height; **EPP**: number of ears per plant; **SG**: Stay green characteristic; **PASP**: plant aspect; **EASP**: ear aspect; *, **, Significant at 0.05 and 0.01probability levels, respectively, and ns, not significant h^2: Broad sense heritability

Grain yield of inbreds ranged from 208 Kg ha^{-1} for CML 494 to 2022 Kg ha^{-1} for ZM 523B-29-2-1-1B*6 with a mean of 908 Kg ha^{-1} across low N environments (Table 4.13). Mean grain yield ranged from 483 Kg ha^{-1} for CLWN 341 to 3446 Kg ha^{-1} for CML 198/LPSC for high N environments (Table 4.14). There was a trend of increased plant height, reduced ASI, more EPP and increased plant and ear aspects among the higher yielding inbred lines. For example, TZL Comp3 had the highest EPP of 0.81 while CLWN 341 had the lowest EPP of 0.41 under low N. Out of the 36 inbred lines evaluated under low N, 16 had positive base indices (an indication of tolerance to low N) with 9 producing grain yield above the mean (Table 4.13). The percentage yield reduction ranged from -44.85% for TZD 11 134 to 88.63% for CML 494 with an average of 27%. The negative sign indicated that the inbreds had higher yield under the low N than high N condition thus, there was reduction in yield under optimal conditions. The absence of the negative sign indicated that the inbreds had higher yield under high N environments than under low N. Among the inbred lines that had positive base indices, TZLComp3, CZL 068 and CML 444 had inherent ability for good performance under both low N and high N environments with a percentage yield reduction of 0.03, 1.04 and 17% respectively. Inbred lines TZD II 134, CML 442 and CLWN 364 performed better under low N than high N with percentage yield reduction of -44.85, -44.41 and -14.19 respectively, hence these lines were the best based on yield reduction. The inbreds CLWN 247, ENT 70, CML 198/LPSC, and Laposta SeqC7F18-3-2-1 performed better under high N than low N with percentage yield reduction of 40.33, 54.11, 52.76 and 37.15, respectively. Four of the top ten high yielding inbreds under low N (CLWN 247, TZL Comp 3, ENT 70, ZM523B-29-2-1-1-B*6) also ranked among the top ten high

yielding inbreds under high N. The best inbred lines based on percentage yield reduction under low N and grain yield above the mean were TZLComp3, CZL 068, CML 444, and ZM523B-29-2-2-1B*6. In contrast, the lowest yielding inbred lines based on their yield, selection indices and percentage yield reduction under low N were CML 494, CLWN 349, CZL 0713 and CZL 00001.

Table 4. 13 Grain yield and other agronomic traits of intermediate maturing maize inbreds (best 20 and worst 10) evaluated across low N environments in Fumesua, Kwadaso in 2013 and 2014 and Ejura in 2014

Inbred line	GY(Kg/ha)	ASI	PHT(cm)	EHT(cm)	PASP	SG	EPP	EASP	Selection index	Rank index	% Yield reduction
TZL Comp 3	1761	3.2	139.83	67.65	2.69	2.83	0.81	2.63	9.91	1	0.03
ZM 523B-29-2-1-1B*6	2022	3.33	129.86	63.92	2.28	3.01	0.82	2.64	9.06	2	26.86
87036	1114	3.54	130.67	59.62	3.12	2.63	0.61	3.08	5.66	3	-4.9
CZL 068	861	3.27	119.51	51.52	3.09	2.55	0.63	2.93	5.23	4	1.04
CLWN 247	1401	3.17	130.88	63.17	2.98	2.65	0.69	2.99	5.18	5	40.33
CML 444	1693	4.47	111.2	57.05	2.81	3.11	0.56	2.99	5.07	6	17.18
TZd II 134	1059	4.26	112.64	50.15	3.16	2.59	0.63	3.3	4.92	7	-44.85
Ent 70 (Chceck)	890	4.7	125.52	58.83	3.4	3.05	0.51	3.29	3.21	8	54.11
CML 442	1177	3.8	120.46	53.4	3.06	2.69	0.75	2.79	3.03	9	-44.41
CML444/CML395/DTPWC8F31	814	3.81	111.48	53.62	2.99	2.66	0.79	2.92	2.35	10	17.55
CML 198/LPSC	1628	4.45	115.35	52.22	3.13	3.02	0.51	3.32	1.37	11	52.76
J-16-1	772	4.11	120.82	57.01	2.91	2.86	0.8	3.38	1.36	12	37.87
CLWN 364	1450	4.3	124.37	54.89	2.59	3.03	0.69	2.93	1.25	13	-14.19
CLWN 359	884	4.21	132.41	59.52	3.21	2.6	0.6	2.82	0.94	14	37.15
Laposta Seq C7-F18-3-2-1	635	4.64	132.66	67	2.8	2.61	0.73	3.13	0.77	15	61.4
CML 395/ CML 444	650	4.36	102.54	52.39	2.96	2.59	0.65	3.33	0.71	16	41.64
ZM521B-66-4-1-1 CML 444	748	3.44	110.48	54.1	3.12	2.77	0.56	3.22	-0.29	17	51.26
CLWN 240	914	4.86	99.72	52.21	3.09	2.82	0.52	3.36	-0.52	18	-26.97
CML 254	620	4.97	117.57	61.5	2.99	2.85	0.46	2.83	-0.76	19	20.65
P43SRCq Fs100-1-1-8	623	3.44	111.7	49.96	2.78	2.91	0.51	3.54	-1	20	59.8
CLWN 238	880	4.13	129.93	62.46	3.18	3.03	0.65	3.59	-3.21	27	38.92
CZL 00001	435	4.29	113.83	51.54	2.82	3.09	0.59	3.26	-3.26	28	43.78
CZL 0713	557	4.04	113.65	58.54	2.92	2.83	0.62	3.38	-3.5	29	57.06
CZL 03007	748	4.47	118.26	56.89	3.22	2.79	0.38	3.27	-4.63	30	1.89
CML 494	208	4.73	97.33	46.34	3.82	3.04	0.39	3.49	-4.66	31	88.63

Table 4.13 continued

Inbred line	GY(Kg/ha	ASI	PHT(cm)	EHT(cm)	PASP	SG	EPP	EASP	Selection index	Rank index	% Yield reduction
CLWN 349	729	4.47	129.18	57.59	3.62	2.79	0.51	3.27	-4.8	32	41.35
TZdII 141	813	4.9	122.05	55.69	3.58	2.83	0.64	3.31	-4.83	33	16.09
TZM 501XKU1414XTZM501	428	5.03	101.2	41.81	3.62	3.24	0.52	3.18	-4.92	34	18.77
TZd II 68	817	4.99	120	56.41	3.32	3.13	0.6	2.9	-5.54	35	-23.45
CLWN 341	408	5.04	105.34	46	3.18	2.81	0.41	3.35	-7.3	36	15.34
Mean	908	4.47	118.19	55.4	3.09	2.87	0.61	3.17			
SE	150.0	0.97	5.8	4.2	0.22	0.18	0.11	0.21			

GY: Grain yield; **ASI**: anthesis silking interval; **PHT**: plant height; **EHT**: ear height; **EPP**: number of ears per plant; **SG**: Stay green; **PASP**: plant aspect; **EASP**: ear aspect;

Table 4. 14 Grain yield and other agronomic traits of intermediate maturing maize inbreds (best 20 and worst 10) evaluated under high N environments in Fumesua, Kwadaso in 2013 and 2014 and Ejura in 2014

Inbred	GY	ASI	PH	EH	EPP	SG	PASP	EASP
CML 198/LPSC	3446	3.74	132.45	51.46	0.78	2.07	2.18	2.86
ZM 523B-29-2-1-1-B*6	2764	3.76	118.39	59.15	0.75	2.49	2.9	2.66
CLWN 247	2347	4.14	118.88	56.43	0.68	2.31	3.18	2.96
9071	2070	3.85	132.8	60.2	0.65	2.36	2.5	2.62
ZM521B-66-4-1-1	2044	4.41	118.79	59.75	0.71	2.76	2.82	2.97
Ent 70	1940	4.7	15.38	57.64	0.77	2.51	2.92	2.49
CML 494	1826	4.58	120.69	61.94	0.63	2.22	2.95	2.65
TZL Comp 3	1762	5.17	129.62	57.14	0.73	2.87	3.44	3
Laposta Seq C7-F18-3-2-1	1646	4.07	113.88	51.08	0.74	2.37	3.14	2.9
M131	1637	4.7	18.62	55.6	0.59	2.41	2.92	2.59
P43SRCq Fs100-1-1-8	1550	3.71	99.97	41.07	0.65	2.76	3.32	3.17
CML 444	1534	3.66	100.82	51.08	0.86	2.66	3.4	3.12
CLWN 238	1441	4.21	114.93	55.55	0.69	2.46	3.22	3.07
CLWN 359	1407	3.46	104.1	42.2	0.82	2.76	3.1	2.92
CZL 0713	1298	3.94	119.79	55.57	0.74	2.17	3.08	2.94
CLWN 364	1270	3.99	104.67	50.56	0.53	2.71	3.22	2.99
J-16-1	1243	4.51	114.36	49.54	0.78	2.6	3.26	3.27
CLWN 349	1243	4.46	119.7	49.34	0.68	2.76	3	3.22
CML 395/ CML 444	1114	4.54	111.01	47.51	0.44	2.59	3.27	3.57
87036	1062	4.03	30.16	58.28	0.55	2.53	3.24	3.11
CLRCW 36	1051	4.74	126.81	54.65	0.67	2.49	3.37	3.07
1368	1020	4.9	103.47	56.76	0.59	2.82	3.67	3.02
CML444/CML395/DTPWC8F31	987	4.03	110.01	45.57	0.86	2.43	3.55	3.14
TZD II 141	969	4.03	125.22	64.54	0.57	2.53	3.14	2.91

Table 4.14 continued

Inbred	GY	ASI	PH	EH	EPP	SG	PASP	EASP
TZd II 140	963	3.91	121.58	59.44	0.71	2.8	3.12	2.78
CZL 068	870	5.07	79.1	37.32	0.56	2.25	3.86	3.07
CML 442	815	4.07	93.8	44.2	0.53	2.75	3.66	3.07
CML 254	782	4.13	117.03	55.95	0.42	2.33	3.05	3.34
CZL 00001	773	3.95	118.46	50.08	0.73	2.56	3.1	3.22
CZL 03007	763	4.91	114.68	55.98	0.68	2.8	3.32	2.78
TZd II 134	731	4.14	110.39	48.25	0.76	2.37	2.98	3.26
CLWN 240	720	4.42	122.67	56.39	0.56	2.68	3.33	3.28
TZd II 68	662	3.46	120.37	52.6	0.63	2.49	3.3	3.46
Laposta Seq C7-F71-1-2	661	5.1	91.03	40.86	0.7	2.44	3.79	3.53
TZM 501XKU1414XTZM501	527	4.94	90.04	41.96	0.66	2.73	3.24	3.14
CLWN 341	483	4.67	97	45.44	0.44	3.05	3.96	3.27
Mean	1317	4.28	113.35	52.25	0.66	2.55	3.21	3.04
SE	355	0.58	9.7	5.3	0.13	0.22	0.25	0.22

GY: Grain yield; **ASI**: anthesis silking interval; **PHT**: plant height; **EHT**: ear height; **EPP**: number of ears per plant; **SG**: Stay green; **PASP**: plant aspect; **EASP**: ear aspect;

4.3.4 Relationship between performance of parental inbred lines and their hybrids

The mid-parent heterosis under low N ranged from -56.50% for TZLComp3 x 1368 to 313.89% for CML 494 x 1368 with an average of 97.04% (Table 4.15) The top three hybrids with high mid-parent heterosis were CML 494 x 1368, TZD II 68 x 1368 and CZL 0713 x 1368. TZD 11 68 x 1368 was among the best five hybrids under low N. Better parent heterosis ranged from -70.00% for TZL Comp3 x 1368 to 232.61% for TZD II68 x 1368 with an average of 63.27%.

Under high N environmets mid-parent heterosis ranged from 34.92% for ZM523B-29-2-1-1-B*6 X 9071 to 421.28% for CLWN 341 X 1368, while better parent heterosis ranged from -10.08% for CML 198/ LPSC x 1368 to 325.98% for TZD II 68 x 1368 (Table 4.16). The average was 199.37% and 150.29% for mid- and better- parent heterosis respectively. Hybrids CML 198/ LPSC x 1368, ZM 521 B-66-4-1-1 x 1368, and ZM523B-29-2-1-1-B*6 x 1368 had lower values for both mid- and better- parent heterosis. ZM 521 B-66-4-1-1 x 1368 was among the lowest yielding hybrids (2340.25kg/ha) under high N. The relationship between inbred line and hybrid performance was investigated using simple linear correlation between inbred line *per se* and hybrid performance in the same environment. Grain yield of inbreds was significantly correlated with hybrid grain yield at low N ($r=0.26$, $P< 0.01$) and ($r=0.19$, $P <0.05$) under high N conditions, but the correlations were weak.

Table 4. 15 Mid-parent and better parent heterosis for grain yield under low N environments in Fumesua and Kwadaso in 2013 and 2014

Hybrids	Mid parent heterosis %	Hybrids	Better parent heterosis %
CML 494 X 1368	313.89	TZDII 68 X 1368	232.61
TZDII 68 X 1368	265.98	Laposta Seq C7-F71-1-1-2 X 1368	207.88
CZL 0713 X 1368	232.11	CZL 0713 X 1368	204.58
CLWN 341 X 1368	212.42	CML 395/ CML 444 X 1368	201.26
Laposta Seq C7-F71-1-1-2 X 1368	208.74	Laposta Seq C7-F18-3-2-1 X 1368	172.05
CZL 00001 X 1368	207.93	CML 494 X 1368	171.28
TZM501 X KU1414 X TZM501 X 9071	205.40	CZL 00001 X 1368	154.19
CML 395/ CML 444 X 1368	205.38	CLWN 341 X 1368	151.73
CZL 00001 X 9071	179.38	CZL 068 X 1368	146.99
Laposta Seq C7-F18-3-2-1 X 1368	178.86	TZD II 140 X 1368	133.50
CZL 068 X 1368	178.17	CZL 03007 X 1368	133.13
CML 494 X 9071	176.86	P43SCRq Fs100-1-1-8 X 1368	126.49
P43SCRq Fs100-1-1-8 X 9071	169.19	CLWN 240 X 1368	116.49
TZD II 140 X 1368	161.03	M131 X 1368*	107.49
M131 X 1368*	152.82	TZDII 68 X 87036	106.74
ZM 521B-66-4-1-1 X 9071	6.92	ZM523B-29-2-1-1-B*6 X 87036	-10.70
CML 198/LPsc X 87036	5.98	CML 198/LPsc X 87036	-10.74
CLWN 238 X 1368	-3.81	CLWN 238 X 1368	-15.40
ZM 521 B-66-4-1-1 X 1368	-6.38	ZM 521 B-66-4-1-1 X 1368	-34.72
TZL Comp3 X 1368	-56.50	TZL Comp3 X 1368	-70.00
Mean	97.04		63.27
Max	313.89		232.61
Min	-56.50		-70.00

Table 4. 16 Mid-parent and better parent heterosis for grain yield under high N environments in Fumesua and Kwadaso in 2013 and 2014

Hybrids	Mid parent heterosis %	Hybrids	Better parent heterosis %
CLWN 341 X 1368	421.28	TZDII 68 X 1368	325.98
TZDII 68 X 1368	416.67	CZL 0713 X 1368	302.24
TZM501 X KU1414 X TZM501 X 1368	369.05	CZL 068 X 1368	298.88
Laposta Seq C7-F71-1-1-2 X 87036	342.88	CML 395/ CML 444 X 1368	297.00
TZM 501 X KU 1414 X43 TZM 501 X 87036	338.59	CLWN 341 X 1368	283.95
CLWN 240 X 87036	337.80	CLRCW 36 X 87036	281.65
CZL 068 X 1368	330.50	TZD II 141 X 87036	277.33
TZD II 134 X 1368	327.24	CML 395/ CML 444 X 87036	269.27
CZL 03007 X 87036	322.92	CLWN 240 X 87036	267.32
CLWN 341 X 87036	315.30	TZD II 134 X 1368	266.82
CML 395/ CML 444 X 1368	314.48	CZL 03007 X 87036	263.30
TZD II 134 X 87036	308.58	Laposta Seq C7-F71-1-1-2 X 87036	259.19
TZDII 68 X 87036	300.44	CML 444/CML 395/DTPWC8F31 X 1368	258.88
CZL 00001 X 1368	297.92	TZM501 X KU1414 X TZM501 X 1368	255.65
TZD II 141 X 87036	294.69	CZL 068 X 87036	253.47
CML 198/LPsc X 87036	54.26	ZM523B-29-2-1-1-B*6 X 9071	17.99
ZM 521 B-66-4-1-1 X 1368	52.76	ZM523B-29-2-1-1-B*6 X 1368	15.70
ZM 521B-66-4-1-1 X 9071	45.33	ZM 521 B-66-4-1-1 X 1368	14.49
CML 198/ LPSC X 1368	38.77	CML 198/LPsc X 87036	0.90
ZM523B-29-2-1-1-B*6 X 9071	34.92	CML 198/ LPSC X 1368	-10.08
Mean	199.37		150.29
Max	421.28		325.98
Min	34.92		-10.08

4.3.5 Correlation between grain yield and secondary traits

Phenotypic correlations (r) of GY with secondary traits under low and high N environments are shown in Table 4.17. The results revealed that GY is positively correlated ($p \leq 0.01$) with plant height (r= 0.54), ear height (r= -0.44), ears per plant (r= 0.46) and chlorophyll content (r-0.39), but negatively correlated with days to anthesis, days to silking, anthesis silking interval, plant aspect, stay green characteristic, and husk cover (r=-0.36, -0.38, -0.23, -0.49, -0.43, -0.08, and -0.46 respectively) under low N. Grain yield and ASI are negatively correlated, showing the importance of shorter ASI for increased grain yield. Similar results were observed under high N environments and across environments.

Table 4. 17 Pearson correlation coefficient between grain yield and other agronomic traits under low, high and across N environments

	Grain yield		
Trait	**Low**	**High**	**Across**
DTA	-0.36**	-0.36**	-0.37**
DTS	-0.38**	-0.35**	-0.39**
ASI	-0.23**	-0.12**	-0.23**
PH	0.54**	0.43**	0.5**
EH	0.44**	0.3**	0.42**
EPP	0.46**	0.4**	0.28**
EASP	-0.49**	-0.29**	-0.43**
PASP	-0.43**	-0.48**	-0.46**
SG	-0.08**	-0.37**	-0.44**
HC	-0.46**	-0.36**	-0.41**
CC	0.39**	0.39**	0.58**

*, **, Significant at 0.05 and 0.01probability levels, respectively

4.3.6 Genetic analysis of intermediate maturing maize inbred lines under low and high N environments

Across low N environments, the ANOVA of the single crosses revealed significant to highly significant mean squares for G, E and GEI for all the traits except the stay green characteristic for GEI (Table 4.18). Significant differences were observed among hybrids, environments and GEI for all traits except G for ears per plant and GEI for ears per plant and plant aspect under high N environments. (Table 4.19). There were significant differences among G, E, and GEI for all traits across environments (Table 4.20).

Partitioning of the entries into components revealed that GCA of line (GCA_l) and GCA of tester (GCA_t) and SCA mean squares were significant for all measured traits under low N except line GCA for plant aspect, SCA for plant height, ears per plant, stay green characteristic, plant aspect, husk cover and chlorophyll content (Table 4.18). GCA_l x environment interactions were significant for all traits except stay green characteristic, plant aspect, husk cover and chlorophyll content while GCA_t x environment interactions were significant for all traits except days to silking and days to anthesis. SCA x environment interactions were not significant for most traits. The GCA of line and tester variances were larger than those of SCA for all traits under low N environments.

Under high N environments, GCA_l and GCA_t were significant for all traits except ears per plant (Table 4.19). SCA effects were significant for grain yield, days to silking and anthesis, anthesis silking interval, plant aspect and husk cover. GCA x environment interaction for line and tester were significant for all traits except ears per plant and chlorophyll content for GCA_l x environment and ear height and plant height for GCA_t x

environment. Similar to low N environments, SCA by environment interactions were not significant for most measured traits and GCA of line and tester variances were larger than those of SCA for all traits.

The combined analysis across environments also revealed significant GCA_l, GCA_t, GCA_l x environment interaction and GCA_t x environment interaction for all traits (Table 4.20). SCA effect was significant for all traits except ears per plant, stay green characteristic, plant aspect and chlorophyll content. GCA_l and GCA_t effects were considerably larger than SCA effects for all measured traits.

Table 4. 18 Line x tester analysis of grain yield and other agronomic traits under low N environments at Fumesua, Ejura and Kwadaso in 2013 and 2014

GY: Grain yield; **DTS**: days to silk; **DTA**: days to anthesis; **ASI**: anthesis silking interval; **PHT**: plant height; **EHT**: ear height; **EPP**: number of ears per plant; **SG**: Stay green; **PASP**: plant aspect; **EASP**: ear aspect; **HC**: husk cover; *, **, Significant at 0.05 and 0.01probability levels, respectively, and ns: not significant.

Source of Variation	DF	GY(Kg/ha)	DTS	DTA	ASI	EHT(cm)	PHT(cm)	EPP	SG(1-9)	PASP	EASP	HC	CC
Envt	5	83797560.7**	4181.42**	3236.49**	146.61**	17887.60**	80400.10**	3.69**	39.03**	81.52**	18.82**	65.58**	7601.39**
Hybrid	95	1676145.7**	24.14**	19.17**	2.22**	687.27**	942.12**	0.06**	0.94**	0.35*	0.96**	0.29**	97.88**
Envt*Hybrid	475	853336.5**	5.40**	3.63**	1.64**	155.69**	425.13**	0.06**	0.45ns	0.32*	0.73**	0.23*	36.67**
Line(GCA)	31	2048032.7**	38.17**	31.46**	3.02**	891.24**	1874.65**	0.08**	1.67**	0.44ns	1.27**	0.44**	183.55**
Tester(GCA)	2	18190061**	574.93**	427.30**	15.13**	12710.50**	8322.58**	0.24**	16.20**	1.80**	1.76*	0.90*	1402.61**
Envt*Line(GCA)	155	937557.3**	6.95**	4.962**	1.90**	219.26**	609.27**	0.07**	0.57ns	0.37ns	1.03**	0.26ns	42.43ns
Envt*Tester(GCA)	10	5405532.2**	6.82ns	3.08ns	4.20**	740.99**	1192.72**	0.18**	1.43**	0.85*	2.05**	0.95**	100.81**
Line*Tester(SCA)	62	1192775.9**	7.72**	5.19*	2.02**	285.92**	546.32ns	0.06ns	0.60ns	0.36ns	0.89**	0.27ns	45.39ns
Envt*Line*Tester(SCA)	310	851950.5**	6.95**	4.68**	1.68**	166.57ns	460.79ns	0.06**	0.50ns	0.34ns	0.61ns	0.25ns	36.36ns
Error	575	226378	5.25	3.59	1.21	167.03	415.662	0.04	0.57	0.42	0.53	0.23	36.09

Table 4. 19 Line x tester analysis of grain yield and other agronomic traits under high N environments at Fumesua, Ejura and Kwadaso in 2013 and 2014

Source of variation	DF	GY	DTS	DTA	ASI	EHT(cm)	PHT(cm)	EPP	SG(1-9)	PASP(1-5)	EASP(1-5)	HC	CC
Envt	5	154917972**	5161.99**	4066.14**	215.47**	21941.92**	77045.40**	21.76**	107.82**	94.15**	15.39**	53.80**	8989.30**
Hybrid	95	3315746.9**	26.51**	20.44**	1.59**	598.29**	787.41**	0.04ns	0.61**	0.55**	0.87**	0.56**	192.09**
Envt*Hybrid	475	1723694.2**	3.86**	2.83**	1.06**	146.79*	262.64*	0.03ns	0.31**	0.33*	0.54ns	0.43**	49.19**
Line(GCA)	31	4788592.3**	38.82**	31.92**	1.46**	817.63**	1288.91**	0.04ns	0.86**	0.66**	1.43**	0.45*	329.84**
Tester(GCA)	2	31287146.8**	669.70**	505.37**	15.71**	11011.63**	8498.81**	0.11ns	12.15**	4.11**	2.27*	3.22**	2821.78**
Envt*Line(GCA)	155	2072081.4**	5.61**	4.25**	1.18**	284.11**	523.82**	0.04ns	0.42*	0.45*	0.72*	0.56**	63.34ns
Envt*Tester(GCA)	10	4894530.4**	19.10**	14.23**	2.09**	161.44ns	305.1ns	0.09**	0.71*	1.22**	1.46**	1.61**	177.20**
Line*Tester(SCA)	62	2076478.3**	6.31**	4.34**	1.39**	197.48ns	469.1ns	0.04ns	0.36ns	0.47*	0.58ns	0.53**	57.79ns
Envt*Line*Tester(SCA)	310	1648246.2**	4.62*	3.32*	1.25**	146.94ns	287.94ns	0.03ns	0.29ns	0.38	0.492	0.43**	43.93ns
Error	575	820467	3.93	2.83	0.78	152.09	338.48	0.04	0.32	0.35	0.57	0.31	56.29

GY: Grain yield; **DTS**: days to silk; **DTA**: days to anthesis; **ASI**: anthesis silking interval; **PHT**: plant height; **EHT**: ear height; **EPP**: number of ears per plant; **SG**: Stay green; **PASP**: plant aspect; **EASP**: ear aspect; **HC**: husk cover; *, **, Significant at 0.05 and 0.01probability levels, respectively, and ns: not significant.

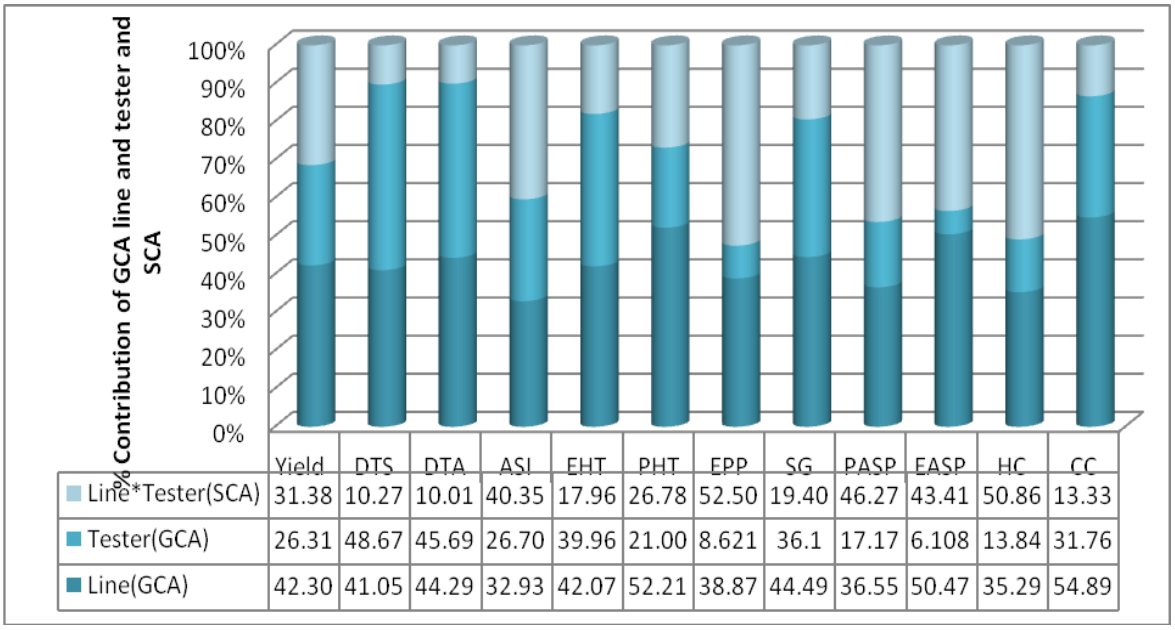
Table 4. 20 Line x tester analysis of grain yield and other agronomic traits across N environments at Fumesua, Ejura and Kwadaso in 2013 and 2014

Sources of variation	DF	GY(Kg/ha)	DTS	DTA	ASI	EHT(cm)	PHT(cm)	EPP	SG(1-9)	PASP(1-5)	EASP(1-5)	HC	CC
Envt	11	305193537**	4449.88**	3432.49**	176.36**	22496.40**	81219.37**	11.81**	104.98**	85.92**	24.89**	58.35**	14783.06**
Hybrid	95	3582219**	44.15**	35.07**	2.33**	1147.96**	1418.75**	0.06**	1.12**	0.60**	1.25**	0.55**	233.60**
Envt*Hybrid	1045	1285303**	4.65012**	3.22**	1.34**	146.6459**	339.7874**	0.05**	0.38*	0.32**	0.62**	0.32**	43.33**
Line(GCA)	31	5050710**	67.71**	57.82**	2.50**	1608.08**	2694.55**	0.08**	2.08**	0.77**	2.02**	0.62**	452.86**
Tester(GCA)	2	48688191**	1244.26**	924.61**	31.46**	23677.89**	16799.07**	0.27**	26.12**	5.60**	3.79**	3.78*	4061.10**
Envt*Line(GCA)	341	1527327**	6.54**	4.67**	1.57**	238.51**	557.89**	0.05**	0.49ns	0.4ns	0.86**	0.39**	53.51*
Envt*Tester(GCA)	22	4738603**	12.17**	8.05**	2.82**	416.62**	684.41**	0.13**	1.16**	0.97**	1.61**	1.23**	140.66**
Line*Tester(SCA)	62	1873596**	8.47**	6.54**	1.53**	343.30**	691.17**	0.05ns	0.45ns	0.49ns	0.87**	0.45**	55.01ns
Envt*Line*Tester(SCA)	682	1263499**	5.75**	3.91**	1.46**	155.17ns	370.52ns	0.05*	0.41ns	0.36ns	0.55ns	0.34**	40.84ns
Error	1151	529842	4.59	3.21	1.02	161.85	378.76	0.04	0.44	0.39	0.55	0.27	46.22

GY: Grain yield; **DTS**: days to silk; **DTA**: days to anthesis; **ASI**: anthesis silking interval; **PHT**: plant height; **EHT**: ear height; **EPP**: number of ears per plant; **SG**: Stay green; **PASP**: plant aspect; **EASP**: ear aspect; **HC**: husk cover; *, **, Significant at 0.05 and 0.01probability levels, respectively, and ns, not significant

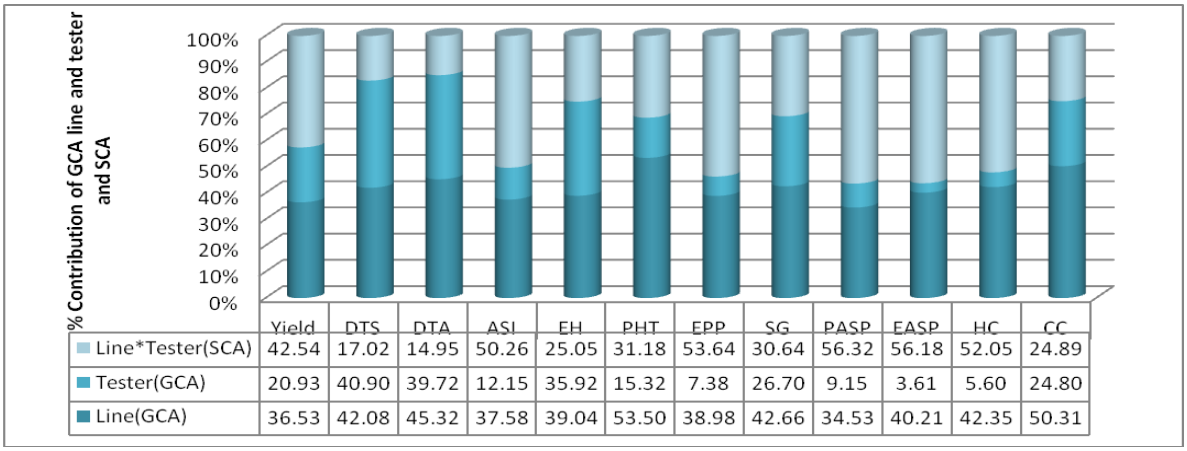
4.3.7 Relative contributions of combining ability effects (Mode of gene action)

The relative importance of GCA and SCA effects was determined as the ratio of GCA effects to the total genetic effects using the sum of squares. The closer the ratio is to unity, the greater the predictability based on GCA (Baker, 1978). GCA sums of squares (GCA of line plus GCA of tester) varied from 47.49% for ears per plant to 89.98% for days to anthesis. The SCA sum of squares varied from 10.01% for days to anthesis to 52.5% for ears per plant. The SCA sum of squares were larger than GCA sum of squares for ears per plant (52.50%) and husk cover (50.86%) whereas GCA sum of squares were larger for all other traits across environments (Fig. 4.1). Under low N environments, GCA effects varied from 43.68% for plant aspect to 85.04% for days to anthesis (Fig. 4.2). GCA accounted for 57.46% of the total genetic effects for grain yield while 31.38% was the contribution from SCA. SCA also varied from 14.95% for days to anthesis to 56.32% for plant aspect. SCA sum of squares were larger than GCA sum of squares for ears per plant (53.64%), anthesis silking interval (50.26%), plant aspect (56.32%), ear aspect (56.18) and husk cover (52.05%) whereas GCA sum of squares were larger for the other traits. Across high N environments, the contribution of GCA to genotypic sum of squares ranged from 35.3% for ears per plant to 88.15% for days to anthesis (Fig. 4.3), while SCA varied from 11.85% for days to anthesis to 64.69% for ears per plant. GCA effects accounted for 62.11% of the total genetic effects for grain yield. Similarly, under low N environments, SCA sum of squares were larger than GCA sum of squares for ears per plant (64.69%), anthesis silking interval (52.94%), plant aspect (50.48%), and husk cover (61.51%) whereas GCA sums of squares were larger for the other traits.



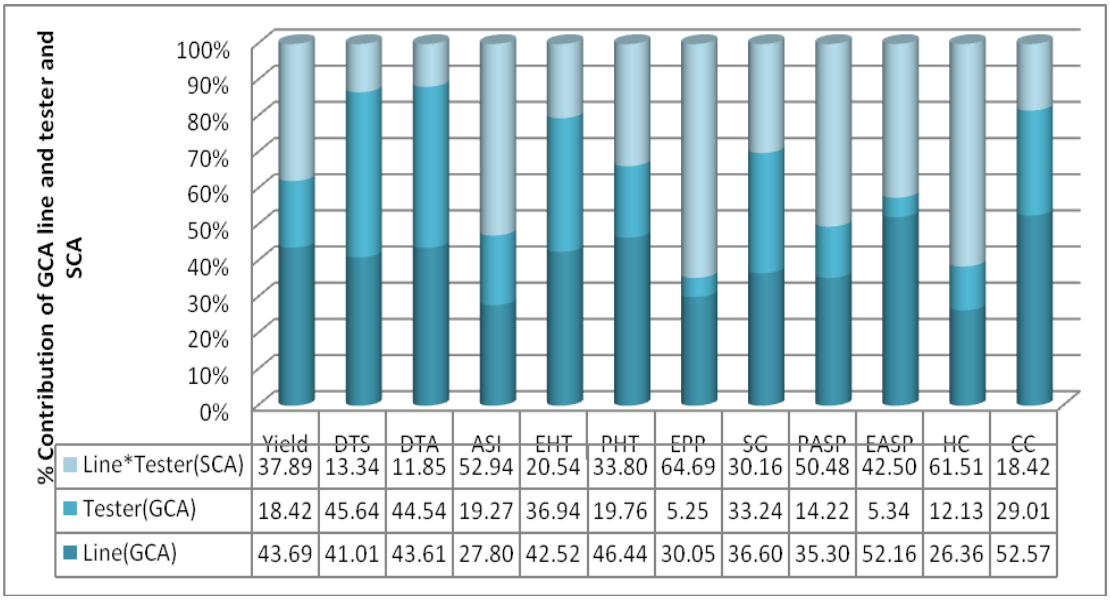
	Yield	DTS	DTA	ASI	EHT	PHT	EPP	SG	PASP	EASP	HC	CC
Line*Tester(SCA)	31.38	10.27	10.01	40.35	17.96	26.78	52.50	19.40	46.27	43.41	50.86	13.33
Tester(GCA)	26.31	48.67	45.69	26.70	39.96	21.00	8.621	36.1	17.17	6.108	13.84	31.76
Line(GCA)	42.30	41.05	44.29	32.93	42.07	52.21	38.87	44.49	36.55	50.47	35.29	54.89

Figure 4. 1 Proportion of total genotypic sum of squares of grain yield and other agronomic traits of intermediate maturing inbred lines attributable to GCA_l, GCAt and SCA across environments.



	Yield	DTS	DTA	ASI	EH	PHT	EPP	SG	PASP	EASP	HC	CC
Line*Tester(SCA)	42.54	17.02	14.95	50.26	25.05	31.18	53.64	30.64	56.32	56.18	52.05	24.89
Tester(GCA)	20.93	40.90	39.72	12.15	35.92	15.32	7.38	26.70	9.15	3.61	5.60	24.80
Line(GCA)	36.53	42.08	45.32	37.58	39.04	53.50	38.98	42.66	34.53	40.21	42.35	50.31

Figure 4. 2 Proportion of total genotypic sum of squares of grain yield and other agronomic traits of intermediate maturing inbred lines attributable to GCA_l, GCAt and SCA under low N environments.



	Yield	DTS	DTA	ASI	EHT	PHT	EPP	SG	PASP	EASP	HC	CC
Line*Tester(SCA)	37.89	13.34	11.85	52.94	20.54	33.80	64.69	30.16	50.48	42.50	61.51	18.42
Tester(GCA)	18.42	45.64	44.54	19.27	36.94	19.76	5.25	33.24	14.22	5.34	12.13	29.01
Line(GCA)	43.69	41.01	43.61	27.80	42.52	46.44	30.05	36.60	35.30	52.16	26.36	52.57

Figure 4. 3 Proportion of total genotypic sum of squares of grain yield and other agronomic traits of intermediate maturing inbred lines attributable to GCA_l, GCAt and SCA under high N environments.

4.3.8 General combining ability effects (GCA effects)

Under low N, GCA effects for grain yield ranged from -399.98 for TZL Comp3 to 239.97 for CML395/CML444 (Table 4.21). Out of the 32 inbred lines, only CLWN 247, TZD II 68 and ZM523B-29-2-1-1-B*6 showed significant positive GCA for grain yield. Only one of the testers, 9071 showed significant positive GCA. Negative significant GCA effects for days to silking and anthesis silking interval were observed for inbred lines CLWN 349, CML 442, Laposta Seq C7-F18-3-2-1, Laposta Seq C7-F71-1-1-2, TZD II 68, ZM 521B-66-4-1-1, ZM523B-29-2-1-1-B*6. Two testers 1368 and 87036 also showed significant negative GCA for days to silking. Inbred lines Laposta Seq C7-F18-3-2-1 and TZD II 68, as well as the tester 9071 displayed significant positive GCA for plant height. Significant negative GCA effects for stay green characteristic were observed for

inbred lines CML 395/ CML 444 , CML 442, CZL 068, Laposta Seq C7-F71-1-1-2 and the tester 87036. Only CZL 00001 showed significant negative GCA for plant aspect. A positive significant GCA for chlorophyll content was obtained for CML 198/ LPSC, CML 254, CML 442, CZL 0713, Laposta Seq C7-F18-3-2-1, Laposta Seq C7-F71-1-1-2 and the tester 9071.

Under high N environments, significant positive GCA effects for grain yield was observed for lines CLWN 247, CLWN 364, CML 395/ CML 444 CZL 00001 and tester 9071 (Table 4.22). Inbred lines CML 395/ CML 444, CML 442, CZL 068, CZL 0713, J-16-1, Laposta Seq C7-F18-3-2-1, Laposta Seq C7-F71-1-1-2, TZD II 140, TZD II 68 and tester 1368 exhibited significant negative GCA for days to silking. Inbred lines CML 395/ CML 444, CZL 068 and CZL 0713 showed significant negative GCA for stay green characteristic. Line CZL 0713 had significant negative GCA for both plant and ear aspects, while CLWN 240 and CML 494 had significant negative GCA for ear aspect. The two laposta lines, Laposta Seq C7-F18-3-2-1 and Laposta Seq C7-F71-1-1-2 had significant positive GCA for chlorophyll content.

GCA effects across environments are presented in (Table 4.23). Significant positive GCA effect for grain yield were obtained for CLWN 247, CLWN 364, CML 395/ CML 444, TZD II 68 and the tester 9071. Inbred lines CML 395/ CML 444 , CML 442, CZL 068, Laposta Seq C7-F18-3-2-1, Laposta Seq C7-F71-1-1-2, TZD II 68, ZM 521B-66-4-1-1 and ZM523B-29-2-1-1-B*6 showed significant negative GCA for days to silking. Significant and negative stay green characteristic was observed for CML 395/ CML 444, CZL 068, CZL 0713, Laposta Seq C7-F18-3-2-1, Laposta Seq C7-F71-1-1-2 and tester 1368. Similarly, significant negative GCA for ear aspect was observed for lines CLWN

247, CZL 0713 and TZD II 134 while significant negative GCA for plant aspect was observed for lines CZL 00001, CZL 068 and CZL 0713. Best combiners for chlorophyll content was observed for lines CML 198/ LPSC , CML 442, CZL 068, CZL 0713, Laposta Seq C7-F18-3-2-1, Laposta Seq C7-F71-1-1-2, TZD II 134, ZM523B-29-2-1-1-B*6 and tester 1368.

Table 4. 21 General combining ability effects of lines and testers for grain yield and other agronomic traits under low N environments

Line	GY	DTS	DTA	ASI	EHT(cm)	PHT(cm)	EPP	SG	PASP	EASP	HC	CC
CLRCW 36	-301.76	2.44**	2.00**	0.44	-0.20	2.03	-0.04	0.11	0.04	0.17	0.11	-2.90*
CLWN 238	-213.50	0.41	1.00**	-0.59*	0.52	-9.21*	-0.03	0.11	0.09	-0.03	0.06	-1.26
CLWN 240	106.49	0.94*	0.64	0.30	5.72*	3.56	-0.02	0.36**	-0.02	0.00	0.03	-3.94**
CLWN 247	425.20**	0.36	0.72	-0.37	7.90**	-0.72	0.07	0.14	0.12	-0.28	0.14	-1.65
CLWN 341	-142.58	1.66	1.39**	0.27	0.62	-0.95	0.01	0.11	0.06	0.03	0.00	-3.13**
CLWN 349	-316.64	-0.09*	0.03	-0.12	-6.90**	3.34	0.01	0.05	0.06	0.19	0.09	0.08
CLWN 359	48.69	-0.26	-0.11	-0.14	-4.86*	1.11	-0.07	-0.14	0.01	-0.03	0.06	0.57
CLWN 364	197.14	0.38	0.25	0.13	-1.98	3.92	-0.02	-0.11	-0.13	-0.17	-0.03	-1.81
CML 444/CML 395/DTPWC8F31	-319.19*	0.66	0.72	-0.06	-3.99	-12.28	-0.05	0.08	0.06	0.33*	0.03	-1.77
CML 198/ LPSC	124.29	-0.70	-0.47	-0.23	-4.45	-1.01	0.01	0.17	-0.10	0.11	-0.08	2.83**
CML 254	-262.59	0.66	0.47	0.19	5.10*	5.14	-0.04	0.28	0.06	-0.06	0.09	2.58*
CML 395/ CML 444	239.97	-0.70	-0.81*	0.11	-1.14	-4.11	0.05	-0.50**	-0.05	-0.08	-0.14	1.02
CML 442	-57.35	-1.28**	-1.50**	0.22	-4.85*	-6.05	0.01	-0.25*	-0.10	0.22	0.00	5.06**
CML 444	-56.24	1.44**	1.28**	0.16	7.73	1.71	-0.04	0.28*	0.01	-0.11	-0.03	-3.29**
CML 494	46.64	0.11	0.00	0.11	1.19	-6.87	0.03	0.00	-0.13	-0.03	-0.11	-0.43
CZL 00001	42.13	0.24	0.03	0.22	-4.79*	-1.99	0.06	-0.08	-0.27**	-0.31	-0.14	1.94
CZL 03007	-95.51	0.36	0.42	-0.06	-3.05	1.11	0.05	0.30*	0.12	-0.17	0.06	-2.32*
CZL 068	114.28	-0.64	-0.97**	0.33	-9.57**	-9.89*	0.05	-0.39**	-0.10	0.05	-0.08	1.53
CZL 0713	-106.27	-0.62	-0.50	-0.12	5.32*	4.57	-0.03	-0.22	-0.08	-0.17	-0.11	3.06**
J-16-1	-228.90	0.80	0.42	0.38	1.58	6.37	-0.10*	0.17	0.06	0.05	0.03	-2.01
Laposta Seq C7-F18-3-2-1	239.33	-1.73**	-1.22**	-0.50*	3.53	11.99**	0.02	-0.17	-0.10	0.11	-0.11	2.40*
Laposta Seq C7-F71-1-1-2	49.22	-1.20**	-1.22**	0.02	-2.35	0.89	0.02	-0.39**	-0.02	0.17	-0.03	3.35**
M131	201.86	-0.34	-0.56	0.22	4.19	5.76	0.09	0.08	-0.02	0.05	0.00	0.76
P43SRCq Fs100-1-1-8	100.20	-0.09	0.33	-0.42	1.53	5.05	0.00	-0.11	-0.16	-0.11	-0.25	0.13
TZL comp 3	-399.98*	1.13	1.28**	-0.14	-7.01**	-19.48**	-0.05	-0.06	0.17	0.22	0.09	-1.96

Table 4.21 continued

Line	GY	DTS	DTA	ASI	EHT(cm)	PHT(cm)	EPP	SG	PASP	EASP	HC	CC
TZM 501 X KU 1414 X43												
TZM 501	-97.86	0.80	0.55	0.25	-4.24	-9.31*	0.03	0.22	-0.05	-0.08	0.00	0.11
TZD II 134	48.09	-0.01	-0.33	0.33	11.53**	7.89	0.01	0.03	0.09	-0.28	-0.03	1.72
TZD II 140	-90.11	0.16	0.03	0.13	0.06	5.22	-0.07	0.19	0.29**	0.42*	0.34**	0.21
TZD II 141	30.64	0.24	0.36	-0.12	4.82*	0.68	-0.04	0.17	0.09	-0.14	0.14	-2.23*
TZD II 68	580.35**	-1.98**	-2.00**	0.02	-2.98	12.17**	0.08	-0.11	-0.05	-0.14	-0.16*	-1.54
ZM 521B-66-4-1-1	-340.44*	-1.45**	-0.86	-0.59*	-2.93	-8.37*	-0.03	-0.22	0.04	0.33*	-0.03	1.23
ZM523B-29-2-1-1-B*6	434.42**	-1.73**	-1.33**	-0.39	3.95	7.73	0.05	-0.08	0.01	-0.25	0.03	1.67
SE	158.84	0.43	0.37	0.23	2.43	4.05	0.04	0.12	0.10	0.17	0.08	1.07
Tester												
1368	-174.86	-0.23*	-0.09	-0.14	-4.73**	-4.80**	-0.02	0.15**	0.08*	0.05	0.05	0.07
9071	243.758*	1.32**	1.10	0.23	6.41**	4.49**	0.03	0.09	-0.04	-0.07	0.01	-1.95**
87036	-68.90	-1.09**	-1.01	-0.08	-1.68	0.31	0.00	-0.23**	-0.04	0.02	-0.05	1.87**
SE	96.87	0.11	0.07	0.86	1.13	1.44	0.02	0.05	0.04	0.06	0.04	0.42

GY: Grain yield; **DTS**: days to silk; **DTA**: days to anthesis; **ASI**: anthesis silking interval; **PHT**: plant height; **EHT**: ear height; **EPP**: number of ears per plant; **SG**: Stay green; **PASP**: plant aspect; **EASP**: ear aspect; **HC**: husk cover; *, **, Significant at 0.05 and 0.01probability levels, respectively, and ns: not significant.

Table 4. 22 General combining ability effects of lines and testers for grain yield and other agronomic traits under high N environments

Line	GY	DTS	DTA	ASI	EHT(cm)	PHT(cm)	EPP	SG	PASP	EASP	CC
CLRCW 36	-3.18	1.94**	1.60**	0.34	2.80	7.89*	-0.01	0.07	0.04	-0.13	-6.21**
CLWN 238	2.91	1.47**	1.05**	0.42*	3.63	-6.93	0.02	0.13	-0.02	-0.05	-5.64**
CLWN 240	110.06	0.72	0.71*	0.01	4.20	1.78	-0.03	-0.04	-0.07	-0.30*	-3.31*
CLWN 247	850.63**	0.30	0.32	-0.02	8.76**	5.38	0.04	0.29*	0.04	-0.19	-1.30
CLWN 341	20.45	2.44**	2.21	0.23	-2.00	-4.05	0.01	0.13	0.21	-0.19	-5.46**
CLWN 349	-121.26	0.78*	0.55	0.23	-5.45*	2.56	-0.03	-0.04	0.01	0.12	-1.65
CLWN 359	56.71	-0.14	-0.01	-0.13	-0.09	6.30	-0.02	-0.09	-0.07	-0.13	1.86
CLWN 364	574.61*	-0.45	-0.40	-0.05	1.36	5.47	0.05	-0.04	-0.07	0.12	-2.87*
CML 198/ LPSC	-118.89	-0.09	-0.23	0.15	-5.00	-3.30	-0.02	0.18	0.04	0.37	3.69**
CML 254	-384.91	1.03**	0.74	0.29	5.26	-5.22	-0.08**	0.13	0.10	0.01	1.77
CML 395/ CML 444	623.24**	-1.31**	-1.15**	-0.16	-0.19	-1.32	0.04	-0.37**	-0.21	-0.05	0.30
CML 442	-270.94	-0.89*	-0.82*	-0.08	-4.96	-7.39	0.02	-0.04	0.07	0.31*	3.70**
CML 444	-227.34	1.28**	1.35**	-0.08	9.07**	4.57	-0.02	0.04	-0.07	0.09	-2.59*
CML444/CML395/DTPWC8F31	-63.15	0.33	0.46	-0.13	-0.81	-5.24	-0.05	0.10	-0.10	0.12	-1.36
CML 494	460.67	0.00	-0.07	0.06	0.69	-0.25	0.07*	-0.04	-0.13	-0.30*	0.70
CZL 00001	464.90*	0.30	0.35	-0.05	-2.92	3.58	0.00	-0.07	-0.21	-0.10	-0.13
CZL 03007	-265.60	-0.39	-0.32	-0.08	-3.47	-3.53	0.00	0.04	0.04	-0.13	-1.20
CZL 068	258.83	-1.78**	-2.04**	0.26	10.62**	-9.70**	0.04	-0.26*	-0.21	0.01	1.93
CZL 0713	181.21	-0.97*	-0.84*	-0.13	8.59	2.14	0.04	-0.29**	-0.29**	-0.38**	4.00**
J-16-1	-129.98	-0.84*	-0.82*	-0.02	-2.31	3.90	-0.02	0.04	-0.02	0.11	1.94
Laposta Seq C7-F18-3-2-1	104.00	-1.45**	-1.07**	-0.38*	0.86	10.50**	0.01	-0.21	-0.04	0.04	5.78**
Laposta Seq C7-F71-1-1-2	-426.49	-1.00*	-0.84*	-0.16	-3.77	-1.56	-0.01	-0.07	0.04	0.20	4.51**

Table 4.22 continued

Line	GY	DTS	DTA	ASI	EHT(cm)	PHT(cm)	EPP	SG	PASP	EASP	CC
M131	-154.23	0.83*	0.55	0.29	2.90	0.52	0.00	0.24*	0.04	0.26	0.87
P43SCRq Fs100-1-1-8	278.24	-0.03	0.21	-0.24	-0.55	5.62	0.01	-0.01	-0.07	-0.08	-2.68*
TZL Comp3	-652.06**	1.14**	1.18**	-0.05	-3.82	-12.50**	-0.04	0.18	0.18	0.17	-0.89
TZM501 X KU1414 X TZM501	-168.85	0.47	0.43	0.04	-4.87	-1.83	-0.03	0.27*	0.07	-0.13	-0.86
TZD II 134	117.69	-0.45	-0.32	-0.13	6.98*	5.91	-0.02	-0.09	0.07	-0.24	4.41**
TZD II 140	-108.01	-0.78*	-0.59	-0.19	-2.44	-0.79	0.04	-0.09	-0.07	-0.02	-0.11
TZD II 141	143.66	-0.20	0.05	-0.24	5.80*	8.32*	-0.02	0.07	0.07	-0.02	-1.63
TZD II 68	149.95	-1.86**	-1.73**	-0.13	-2.14	4.26	-0.02	-0.09	0.10	-0.16	-0.66
ZM 521B-66-4-1-1	-859.66**	-0.09	-0.29	0.20	-5.54*	-13.17**	-0.02	0.02	0.37**	0.42	1.05
ZM523B-29-2-1-1-B*6	-443.22	-0.34	-0.26	-0.08	0.03	-1.97	0.04	-0.09	0.18	0.26	2.08
SE	236.13	0.39	0.34	0.18	2.77	3.75	0.03		0.11	0.14	1.31
Tester											
1368	-214.88*	-0.30	-0.16	-0.14*	-4.74**	-5.00**	-0.02	0.19	0.12	0.03	-0.46
9071	325.00*	1.45**	1.21	0.24**	5.82**	4.36**	0.00	-0.03	-0.04	-0.09	-2.47**
87036	-110.12	-1.15**	-1.05	-0.09	-1.08*	0.63	0.01	-0.16	-0.08	0.06	2.94**
SE	92.18	0.18	0.16	0.06	0.53	0.73	0.01	0.04	0.05	0.05	0.55

GY: Grain yield; **DTS**: days to silk; **DTA**: days to anthesis; **ASI**: anthesis silking interval; **PHT**: plant height; **EHT**: ear height; **EPP**: number of ears per plant; **SG**: Stay green; **PASP**: plant aspect; **EASP**: ear aspect; CC: chlorophyll content *, **, Significant at 0.05 and 0.01probability levels, respectively, and ns: not significant.

Table 4. 23 General combining ability effects of lines and testers for grain yield and other agronomic traits across environments

Line	GY	DTS	DTA	ASI	EHT	PHT	EPP	SG	PASP	EASP	HC	CC
CLRCW 36	-152.47	2.19**	1.80**	0.39**	1.30	4.96	-0.03	0.09	0.04	0.02	0.04	-4.56**
CLWN 238	-105.29	0.94**	1.02**	-0.08	2.07	-8.06**	-0.01	0.12	0.04	-0.04	-0.03	-3.45**
CLWN 240	108.28	0.83**	0.68**	0.15	4.96**	2.67	-0.02	0.16*	-0.05	-0.15	0.01	-3.62**
CLWN 247	637.92**	0.33	0.52*	-0.19	8.33**	2.33	0.06	0.22**	0.08	-0.23*	0.00	-1.47
CLWN 341	-61.06	2.05**	1.80**	0.25	-0.69	-2.50	0.01	0.12	0.13	-0.08	0.00	-4.29**
CLWN 349	-218.95	0.34	0.29	0.06	-6.18**	2.96	-0.01	0.01	0.04	0.16	0.10	-0.78
CLWN 359	52.70	-0.20	-0.06	-0.14	-2.48	3.70	-0.05	-0.12	-0.03	-0.08	0.09	1.22
CLWN 364	385.87**	-0.03	-0.07	0.04	-0.31	4.70	0.01	-0.08	-0.10	-0.02	-0.04	-2.34**
CML 198/ LPSC	2.70	-0.39	-0.35	-0.04	-4.72**	-2.15	0.00	0.17*	-0.03	0.24	-0.04	3.26**
CML 254	-323.75*	0.84**	0.61	0.24	5.18**	-0.04	-0.06	0.20*	0.08	-0.02	0.04	2.18
CML 395/ CML 444	431.60**	-1.00**	-0.98**	-0.03	-0.67	-2.71	0.04	-0.44**	-0.13	-0.07	-0.13	0.66
CML 442	-164.15	-1.09**	-1.16**	0.07	-4.90**	-6.72*	0.01	-0.14	-0.02	0.27*	-0.01	4.38**
CML 444	-141.79	1.36**	1.31**	0.04	8.40**	3.14	-0.03	0.16*	-0.03	-0.01	0.00	-2.94
CML444/CML395/DTPWC8F31	-191.17	0.50	0.59*	-0.10	-2.40	-8.76**	-0.05	0.09	-0.02	0.23*	0.06	-1.56
CML 494	253.66	0.05	-0.03	0.09	0.94	-3.56	0.05	-0.02	-0.13	-0.16	-0.10	0.05
CZL 00001	253.51	0.27	0.19	0.09	-3.85*	0.80	0.03	-0.08	-0.24**	-0.21	-0.11	0.91
CZL 03007	-180.55	-0.02	0.05	-0.07	-3.26	-1.21	0.03	0.17*	0.08	-0.15	0.01	-1.76*
CZL 068	186.55	-1.21**	-1.51**	0.29	-10.10**	-9.79**	0.04	-0.33**	-0.16*	0.03	-0.15*	1.74*
CZL 0713	37.47	-0.80	-0.67**	-0.12	6.95**	3.36	0.00	-0.26**	-0.18**	-0.27*	-0.10	3.53**
J-16-1	-179.44	-0.02	-0.20	0.18	-0.37	5.14	-0.06	0.11	0.02	0.08	0.04	-0.03
Laposta Seq C7-F18-3-2-1	171.66	-1.59**	-1.14**	-0.44**	2.20	11.25**	0.01	-0.19*	-0.07	0.07	-0.11	4.09**
Laposta Seq C7-F71-1-1-2	-188.64	-1.10**	-1.03**	-0.07	-3.06	-0.33	0.01	-0.23**	0.01	0.18	0.00	3.93**
M131	23.81	0.25	-0.01	0.25	3.55*	3.14	0.04	0.16*	0.01	0.16	-0.03	0.82

Table 4.23 continued

Line	GY	DTS	DTA	ASI	EHT(cm)	PHT(cm)	EPP	SG	PASP	EASP	HC	CC
P43SCRq Fs100-1-1-8	189.22	-0.06	0.27	-0.33	0.49	5.34	0.01	-0.06	-0.12	-0.09	-0.18**	-1.27
TZL Comp3	-526.02**	1.14**	1.23**	-0.10	-5.42**	-15.99**	-0.05	0.06	0.18*	0.20	0.14	-1.42
TZM 501X KU 1414 X43TZM501	-133.35	0.64*	0.49*	0.14	-4.56*	-5.71*	0.00	0.24**	0.01	-0.11	-0.06	-0.37
TZD II 134	82.89	-0.23	-0.32	0.10	9.26**	6.90*	-0.01	-0.03	0.08	-0.26*	0.11	3.07**
TZD II 140	-99.06	-0.31	-0.28	-0.03	-1.19	2.22	-0.02	0.05	0.11	0.20	0.24**	0.05
TZD II 141	87.15	0.02	0.20	-0.18	5.31**	4.50	-0.03	0.12	0.08	-0.08	0.11	-1.93*
TZD II 68	365.15*	-1.92**	-1.87**	-0.05	-2.56	8.22**	0.03	-0.10	0.02	-0.15	-0.07	-1.10
ZM 521B-66-4-1-1	-600.05**	-0.77*	-0.57*	-0.19	-4.23*	-10.77**	-0.02	-0.10	0.20**	0.38**	0.10	1.14
ZM523B-29-2-1-1-B*6	-4.40	-1.03**	-0.80**	-0.23	1.99	2.88	0.04	-0.09	0.09	0.00	0.08	1.88*
SE	143.35	0.30	0.25	0.15	1.79	2.74	0.03	0.08	0.07	0.11	0.07	0.85
Tester												
1368	-194.87**	-0.27	-0.12	-0.14**	-4.73**	-4.91**	-0.02*	0.17**	0.10**	0.04	0.06	-0.19**
9071	284.38**	1.39	1.15	0.23**	6.11**	4.43**	0.02	0.03	-0.04	-0.08	0.02	-2.20**
87036	-89.51	-1.12	-1.03	-0.09	-1.38*	0.47	0.01	-0.20**	-0.06	0.04	-0.08	2.40**
SE	64.14	0.10	0.08	0.05	0.60	0.77	0.01	0.03	0.03	0.04	0.03	0.35

GY: Grain yield; **DTS**: days to silk; **DTA**: days to anthesis; **ASI**: anthesis silking interval; **PHT**: plant height; **EHT**: ear height; **EPP**: number of ears per plant; **SG**: Stay green; **PASP**: plant aspect; **EASP**: ear aspect; **HC**: husk cover; CC: chlorophyll content *, **, Significant at 0.05 and 0.01probability levels, respectively, and ns: not significant.

4.3.9 Specific combining ability

Both negative and positive estimates of SCA effects (line by tester) for grain yield were observed among crosses under low N, high N and across environments (Tables 4.24, 4.25 and 4.26, respectively). Desirable SCA effects (significant and positive) were obtained for crosses TZD II 68 x 1368, CZL 0713 x 1368, MI31 x 1368, CLWN 359 x 9071, TZM 501 x KU 1414 x 43 TZM501 x 9071 and TZL comp 3 x 87036 under low N environment, with the highest SCA detected for TZD II 68 x 1368. All these crosses with the exception of TZL comp 3 x 87036 were among the best 20 hybrids selected under low N environments. Under high N, significant positive SCA were observed for crosses CLWN 359 x 1368, CML 494 x 1368, CZL 00001 x 9071, Laposta Seq C7-F18-3-2-1 x 9071, CLWN 349 x 87036 and Laposta Seq C7-F71-1-1-2 x 87036. CZL 00001 x 9071 had the highest SCA effect. All these hybrids also produced high yields and were among the best 20 with the exception of Laposta Seq C7-F71-1-1-2 x 87036. The crosses CZL 068 x 1368, CZL 0713 x 1368, TZD II 68 x 1368, CZL 00001 x 9071, Laposta Seq C7-F18-3-2-1 x 9071, CLWN 349 x 87036 and ZM 521B-66-4-1-1 x 87036 exhibited significant SCA effects across environments. In contrast, significant negative SCA were observed for crosses CLWN 238 x 1368, TZL comp 3 x 1368, TZM 501 x KU 1414 x 43, TZM501 x 1368, TZD II 68 x 9071 under low N, Laposta Seq C7-F18-3-2-1 x 87036 under high N and CLWN 238 x 1368, TZL comp 3 x 1368, CZL 0713 x 9071, CZL 00001 x 87036, and Laposta Seq C7-F18-3-2-1 x 87036 across environments.

Table 4. 24 Specific combining ability effects for grain yield under low N environments

	Testers		
Line	1368	9071	87036
CLRCW 36	-117.585	-12.068	129.653
CLWN 238	-564.686**	262.86	301.825
CLWN 240	89.3	-18.642	-70.657
CLWN 247	-110.216	105.063	5.153
CLWN 341	89.251	-382.466	293.215
CLWN 349	68.656	-233.335	164.679
CLWN 359	-403.486	497.053*	-93.566
CLWN 364	-305.534	113.98	191.554
CML 444/CML395/DTPWC8F31	20.573	-203.678	183.105
CML 198/ LPSC	294.811	37.096	-331.907
CML 254	-183.9	-221.86	405.759
CML 395/ CML 444	123.6	-103.034	-20.566
CML 442	202.631	-169.719	-32.913
CML 444	-17.862	51.597	-33.735
CML 494	155.775	-41.537	-114.237
CZL 00001	126.995	273.339	-400.334
CZL 03007	335.762	-81.91	-253.852
CZL 068	419.436	-370.001	-49.436
CZL 0713	483.604*	-360.317	-123.286
J-16-1	34.14	117.361	-151.501
Laposta Seq C7-F18-3-2-1	19.625	100.734	-120.358
Laposta Seq C7-F71-1-1-2	349.008	-141.344	-207.665
M131	426.499*	-169.475	-257.024
P43SRCq Fs100-1-1-8	-208.056	320.697	-112.641
TZL comp 3	-564.846**	100.211	464.635*
TZM 501 X KU 1414 X43 TZM501	-565.313**	522.52*	42.793
TZD II 134	-406.066	202.708	203.358
TZD II 140	77.859	68.762	-146.621
TZD II 141	-283.797	101.731	182.066
TZD II 68	537.606**	-432.119*	-105.488
ZM 521B-66-4-1-1	-238.822	-50.607	289.43
ZM523B-29-2-1-1-B*6	115.039	116.399	-231.438
SE	214	214	214

*, **, Significant at 0.05 and 0.01 probability levels, respectively

Table 4. 25 Specific combining ability effects for grain yield under high N environments

	Testers		
Line	1368	9071	87036
CLRCW 36	58.449	-502.388	443.939
CLWN 238	-265.197	300.303	-35.106
CLWN 240	-339.864	181.673	158.191
CLWN 247	0.786	-16.741	15.954
CLWN 341	205.775	260.781	-466.555
CLWN 349	-392.277	-233.888	626.164*
CLWN 359	596.705*	-88.117	-508.588
CLWN 364	-37.941	230.503	-192.561
CML 444/CML395/DTPWC8F31	170.749	55.946	-226.695
CML 198/ LPSC	-374.548	144.856	229.692
CML 254	-430.328	520.803	-90.475
CML 395/ CML 444	367.267	-281.958	-85.309
CML 442	-89.267	-48.692	137.959
CML 444	-190.488	37.589	152.899
CML 494	657.519*	-209.327	-448.192
CZL 00001	-250.905	918.015**	-667.11*
CZL 03007	4.363	-302.461	298.098
CZL 068	384.591	-208.894	-175.697
CZL 0713	420.481	-567.462	146.98
J-16-1	-377.741	382.102	-4.361
Laposta Seq C7-F18-3-2-1	-176.831	821.15**	-644.319*
Laposta Seq C7-F71-1-1-2	-292.909	-462.029	754.938*
M131	-45.394	-174.545	219.939
P43SRCq Fs100-1-1-8	375.159	-474.243	99.085
TZL comp 3	-174.99	-60.502	235.493
TZM 501 X KU 1414 X43 TZM501	133.179	-87.036	-46.142
TZD II 134	148.237	8.169	-156.406
TZD II 140	-89.399	419.609	-330.21
TZD II 141	-239.269	11.083	228.186
TZD II 68	536.957	-107.059	-429.898
ZM 521B-66-4-1-1	-303.576	-160.581	464.157
ZM523B-29-2-1-1-B*6	10.707	-306.658	295.951
SE	297.84		

*, **, Significant at 0.05 and 0.01 probability levels, respectively

Table 4. 26 Specific combining ability effects for grain yield across environments

	Testers		
Line	1368	9071	87036
CLRCW 36	-29.57	-257.23	286.80
CLWN 238	-414.94**	281.58	133.36
CLWN 240	-125.28	81.52	43.77
CLWN 247	-54.72	44.16	10.55
CLWN 341	147.51	-60.84	-86.67
CLWN 349	-161.81	-233.61	395.42**
CLWN 359	96.61	204.47	-301.08
CLWN 364	-171.74	172.24	-0.50
CML 444/CML395/DTPWC8F31	95.66	-73.87	-21.80
CML 198/ LPSC	-39.87	90.98	-51.11
CML 254	-307.11	149.47	157.64
CML 395/ CML 444	245.43	-192.50	-52.94
CML 442	56.68	-109.21	52.52
CML 444	-104.18	44.59	59.58
CML 494	406.65	-125.43	-281.22
CZL 00001	-61.96	595.68**	-533.72**
CZL 03007	170.06	-192.19	22.12
CZL 068	402.01*	-289.45	-112.57
CZL 0713	452.04*	-463.89*	11.85
J-16-1	-171.80	249.73	-77.93
Laposta Seq C7-F18-3-2-1	-78.60	460.94*	-382.34*
Laposta Seq C7-F71-1-1-2	28.05	-301.69	273.64
M131	190.55	-172.01	-18.54
P43SRCq Fs100-1-1-8	83.55	-76.77	-6.78
TZL comp 3	-369.92*	19.85	350.06
TZM 501 X KU 1414 X43 TZM501	-216.07	217.74	-1.68
TZD II 134	-128.91	105.44	23.48
TZD II 140	-5.77	244.19	-238.42
TZD II 141	-261.53	56.41	205.13
TZD II 68	537.28**	-269.59	-267.69
ZM 521B-66-4-1-1	-271.20	-105.59	376.79*
ZM523B-29-2-1-1-B*6	62.87	-95.13	32.26
SE	184.40		

*, **, Significant at 0.05 and 0.01 probability levels, respectively

4.3.10 Heterotic groupings, relationship among different grouping methods and grouping efficiencies

Three testers (1368, 9071, 87036) were adopted to group the lines into three different heterotic groups using three methods:

Groupings based on SCA of grain yield

The specific combining ability (SCA) effects and mean grain yields of testcrosses of the lines with the three testers were used to classify the inbred lines into heterotic groups for each of the two growing conditions and across environments using the procedure described by Menkir *et al*. (2003).

Lines with positive SCA with one tester and negative SCA with other testers with a mean grain yield higher than or equal to the yield of the best tester cross (hybrid) were assigned to the group opposite to the testers heterotic group. These opposite tester heterotic groups will be designated as anti groups. For example, since there were three testers used in the study there are anti (anti1368) group A, (anti 9071) group B and (anti 87036) group C heterotic groups. Under high N, the mean of the best tester hybrid was 87036 x 9071(4252.90 kg/ha), therefore, CLWN 247 was placed under anti 1368 because it had a positive SCA with the tester 1368 and a mean grain yield of 4506.51kg/ha. This meant that it belongs to a heterotic group other than 1368. Again, lines with positive SCA and higher yields than the yield of the best tester cross (hybrid) with two testers were classified into heterotic groups opposite to both of these testers. Grouping of inbred lines based on the SCA method under low N, high N and across environments are presented in Table 4.26. Under low N, 9 inbreds were assigned to the anti 1368 (group A) heterotic

group while 15 and 3 inbreds were assigned to anti 9071 and anti 87036 heterotic groups, respectively. Similarly, 10, 15 and 3 inbreds were assigned to anti 1368, anti 9071 and anti 87036 heterotic groups, respectively under high N. Groupings across environments revealed 9 inbreds assigned to anti 1368, 14 to anti 9071 and 5 to anti 87036. Out of the 32 inbred lines, 5 could not be classified under low; while four each could not be classified under high N and across environments respectively. There was correspondence among the growing environments in terms of placement of inbred lines into the same heterotic group. Examples of such inbreds were TZD II 68 and CML 395/CML444 were assigned to anti 1368 under all the growing environments while CLWN 238, CLWN 364, CML 198/ LPSC, CZL 00001, and Laposta Seq C7-F18-3-2-1 were all placed in the anti 9071 group. It is interesting to note that inbred TZD II 68 had the highest SCA effect with 1368 under low N. CLWN 247 was the only inbred classified into the anti 87036 group across growing environments.

Table 4. 27 Classification of the 15 inbreds into heterotic groups based on SCA effects of grain yield under low, high and across N environments

Group A(Anti 1368)	Group B (Anti 9071)	Group C (Anti 87036)
	Low	
CLWN 240	CLWN 238	CLWN 247
CML 198/ LPSC	CLWN 359	CLWN 364
CML 395/ CML 444	CLWN 364	TZD II 134
CZL 068	CML 198/ LPSC	
CZL 0713	CLWN 247	
Laposta Seq C7-F71-1-1-2	CML 444	
M131	CZL 00001	
TZDII 68	J -16-1	
ZM523B-29-2-1-1-B*6	Laposta Seq C7-F18-3-2-1	
	P43SCRq Fs100-1-1-8	
	TZD II 134	
	TZD II 140	
	TZD II 141	
	TZM501 X KU1414 X TZM501	
	ZM523B-29-2-1-1-B*6	
	High	
CLWN 247	CLWN 238	CLRCW 36
CLWN 341	CLWN 240	CLWN 240
CML 444/CML 395/DTPWC8F31	CLWN 341	CLWN 247
CML 395/ CML 444	CLWN 364	CLWN 349
CML 494	CML 198/ LPSC	CML 442
CZL 068	CLWN 247	CZL 0713
CZL 0713	CML 254	CZL 03007
P43SCRq Fs100-1-1-8	CML 444	Laposta Seq C7-F71-1-1-2
TZD II 134	CML 444/ CML 395/DTPWC 8F31	M131
TZD II 68	CZL 00001	P43SRCq Fs100-1-1-8
	J -16-1	TZD II 141
	Laposta Seq C7-F18-3-2-1	
	TZD II 134	
	TZD II 140	
	TZD II 141	
	Across	
CLWN 341	CLWN 238	CLRCW 36
CLWN 359	CLWN 240	CLWN 240
CML 395/ CML 444	CLWN 247	CLWN 247
CML 494	CLWN 359	CLWN 349
CZL 068	CLWN 364	TZD II 134
CZL 0713	CML 198/ LPSC	
M131	CML 254	
P43SCRq Fs100-1-1-8	CML 444	
TZD II 68	CZL 00001	
	J -16-1	
	Laposta Seq C7-F18-3-2-1	
	TZD II 134	
	TZD II 140	
	TZM501 X KU1414 X TZM501	

Groupings based on Heterotic group's specific and general combining ability (HSGCA)

The results of the heterotic grouping based on HSGCA are shown in Table 4.28. Under low N, 15 inbreds were grouped in heterotic group (group A), 3 in 9071 heterotic group (group B), and 14 in 87036 heterotic group (group C), whilst under high N 16 inbreds were grouped in heterotic group 1368 (group A), 3 in 9071 heterotic group (group B) and 12 in heterotic group 87036 (group C). Eighteen inbreds were grouped in 1368, 1 in 9071 and 10 in 87036 heterotic group across environments. CLWN 238, CLWN 247, CLWN 349, CML 444/CML395, CML 254, CML 444 and TZL Comp 3 were classified in heterotic group 1368 (group A) under all the growing conditions, whilst CML 395/CML 444, CML 494, Laposta Seq C7-F18-3-2-1 and TZD II 140 were grouped in heterotic group 87036 (group C).

Table 4. 28 Classification of intermediate maturing maize inbreds into heterotic groups based on HSGCA effects of grain yield under low N, high N and across N environments

1368	9071	87036
	Low	
CLRCW 36	CLWN 341	CLWN 240
CLWN 238	CZL 068	CML 198/LPsc
CLWN 247	TZD II 68	CML 395/ CML 444
CLWN 349		CML 442
CLWN 359		CML 494
CLWN 364		CZL 00001
CML 444/CML 395/DTPWC8F31		CZL 03007
CML 254		CZL 0713
CML 444		J-16-1
P43SRCq Fs100-1-1-8		Laposta Seq C7-F18-3-2-1
TZL comp 3		Laposta Seq C7-F71-1-1-2
TZM 501 X KU 1414 X43 TZM 501		M131
TZD II 134		TZD II 140
TZD II 141		ZM523B-29-2-1-1-B*6
ZM 521B-66-4-1-1		
	High	
CLWN 238	CLRCW 36	CLWN 341
CLWN 240	CZL 0713	CLWN 359
CLWN 247	P43SRCq Fs100-1-1-8	CLWN 364
CLWN 349		CML 395/ CML 444
CML 444/CML 395/DTPWC8F31		CML 494
CML 198/LPsc		CZL 00001
CML 254		CZL 068
CML 442		J-16-1
CML 444		Laposta Seq C7-F18-3-2-1
CZL 03007		TZD II 68
Laposta Seq C7-F71-1-1-2		TZM 501 X KU 1414 X43 TZM 501
M131		TZD II 134
TZL comp 3		TZD II 140
TZD II 141		
ZM 521B-66-4-1-1		
ZM523B-29-2-1-1-B*6		
	Across	
CLRCW 36	CZL 0713	CLWN 341
CLWN 238		CLWN 359
CLWN 240		CML 395/ CML 444
CLWN 247		CML 494
CLWN 349		CZL 03007
CLWN 364		CZL 068
CML 444/CML 395/DTPWC8F31		Laposta Seq C7-F18-3-2-1
CML 198/LPsc		M131
CML 254		TZD II 68
CML 442		TZD II 140
CML 444		
CZL 00001		
J-16-1		
Laposta Seq C7-F71-1-1-2		
P43SRCq Fs100-1-1-8		
TZL comp 3		
TZM 501 X KU 1414 X43 TZM 501		
TZD II 134		
TZD II 141		
ZM 521B-66-4-1-1		

Heterotic grouping based on GCA of multiple traits (HGCAMT)

Dendograms constructed for groupings based on HGCAMT for low, high and across N environments are shown in Figs 4.4, 4.5 and 4.6. The HGCAMT identified three groups each under low N, high N, and across both environments. Tester 1 which is 1368 (group A) identified 16 inbreds, tester 2 which is 9071 (group B) identified 4 inbreds while 12 inbreds were grouped by tester 3 which is 87036 (group C) under low N environments. Under high N, 18 inbreds were grouped by tester 1 (1368), 5 inbreds by tester 2 (9071) and 9 by tester 3 (87036). Across environments, 13 inbreds were grouped by tester 1 (1368), 5 by tester 2 (9071) and 14 by tester 3 (87036), respectively. There was correspondence among the growing environments in terms of placement of inbred lines into the same heterotic group.

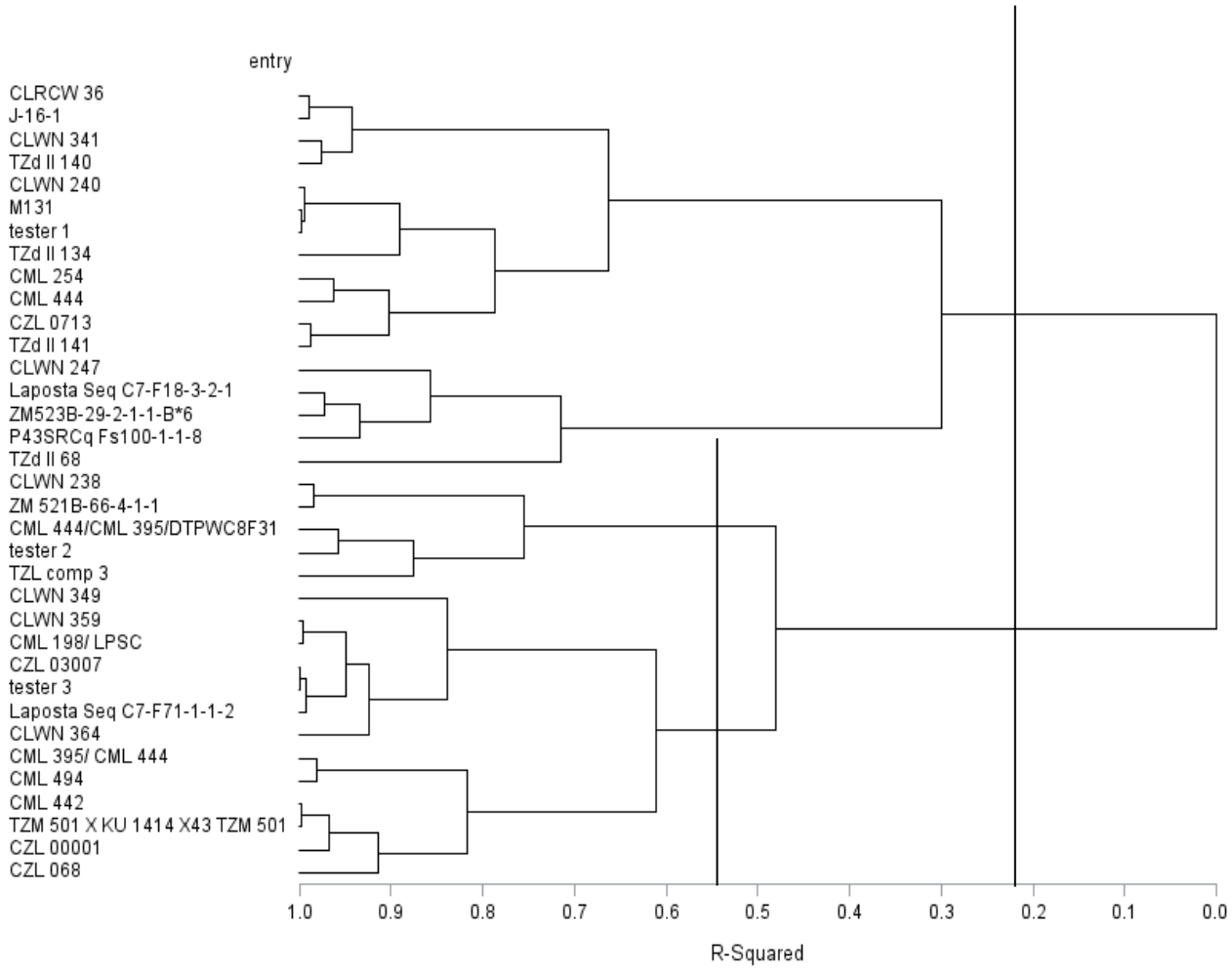


Figure 4. 4 Classification of intermediate maturing maize inbreds into heterotic groups based on HGCAMT method under low N environment. Note: tester 1= 1368, tester 2= 9071, tester 3=87036

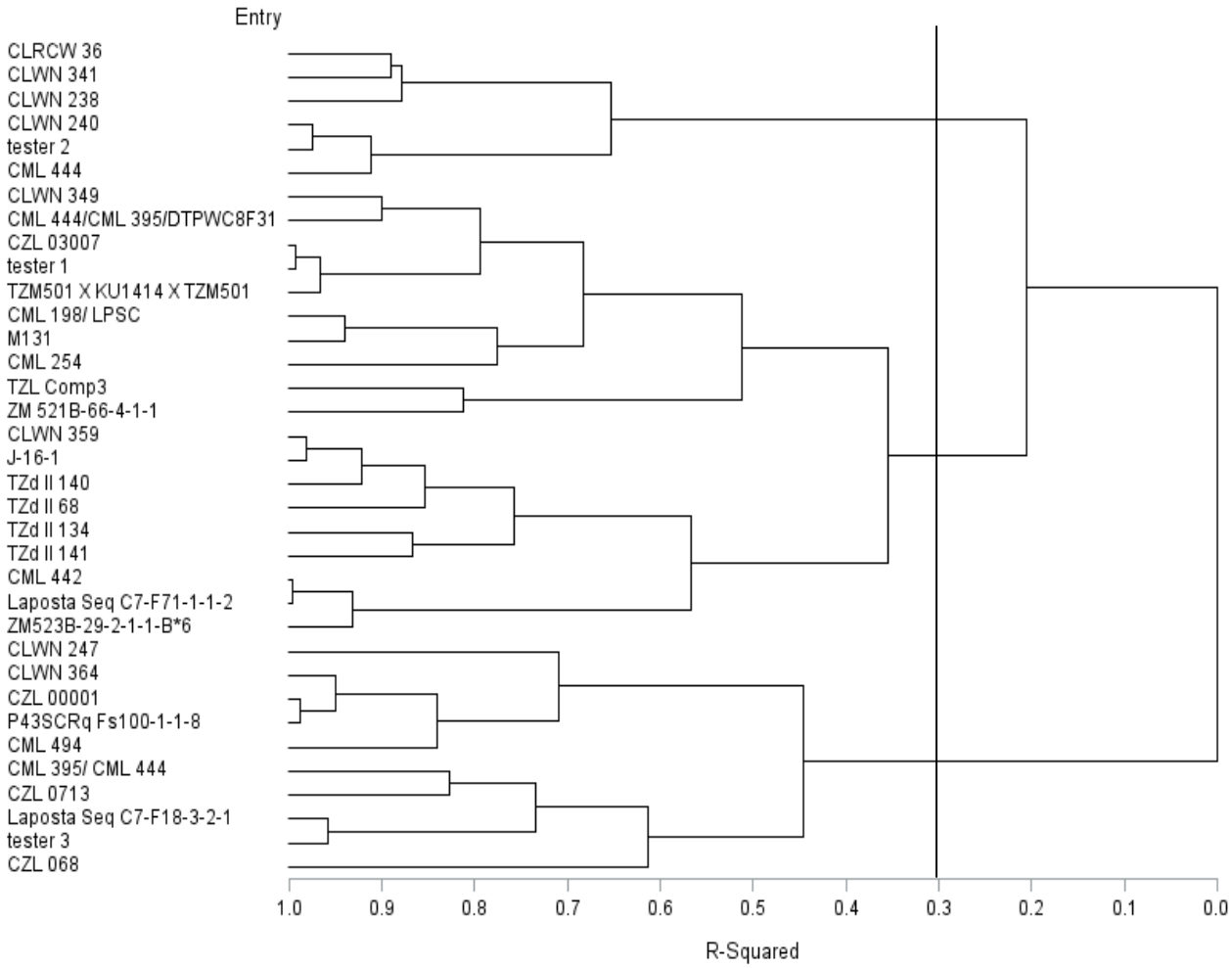


Figure 4. 5 Classification of intermediate maturing maize inbreds into heterotic groups based on HGCAMT method under high N environments. Note: tester 1= 1368, tester 2= 9071, tester 3=87036

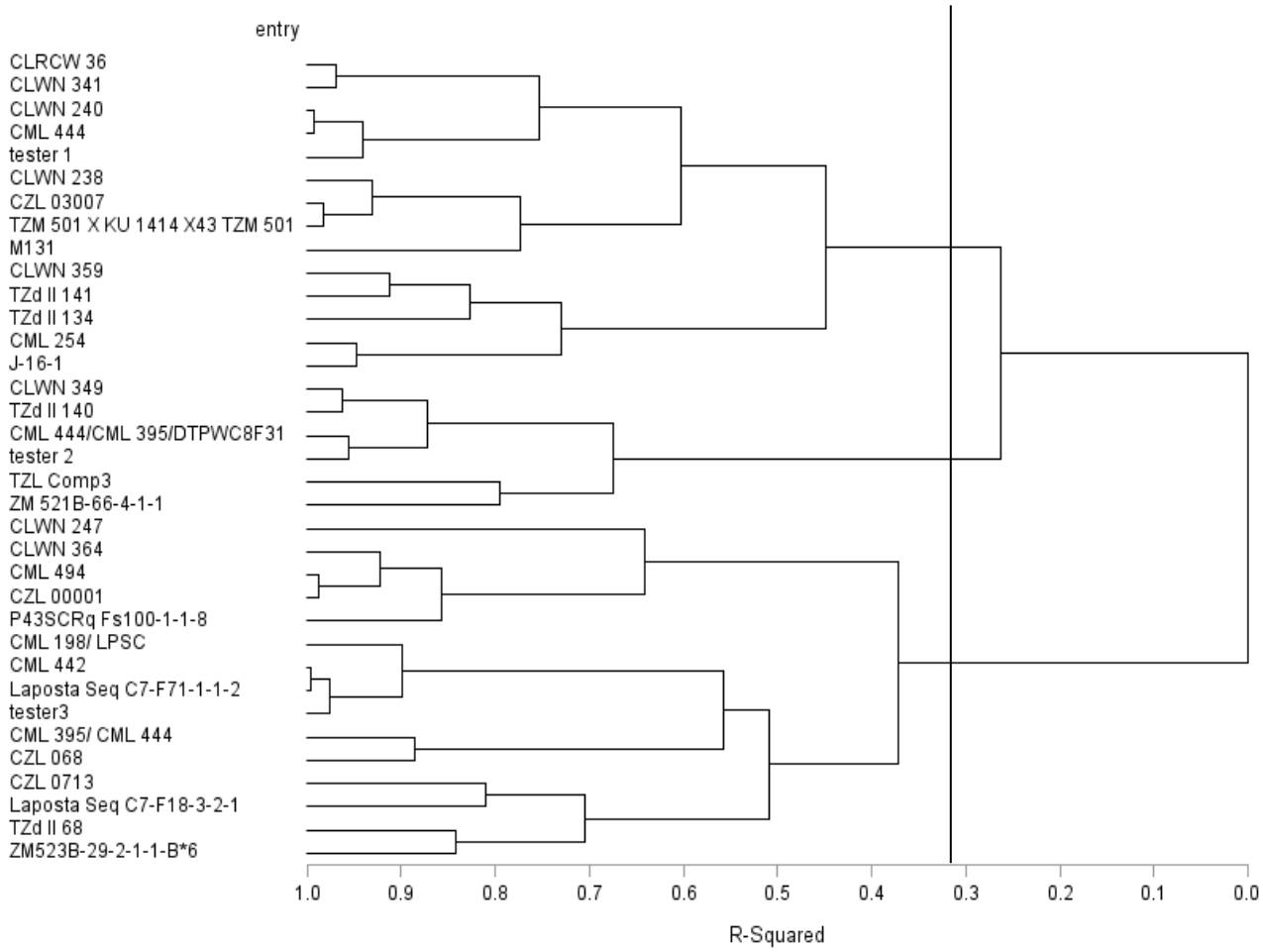


Figure 4. 6 Classification of intermediate maturing maize inbreds into heterotic groups based on HGCAMT method across low and high N environments. Note: tester 1= 1368, tester 2= 9071, tester 3=87036

Breeding (Grouping efficiency)

Grouping efficiency as defined by Fan *et al*. (2009) is the percentage of superior high-yielding hybrids obtained across the total number of inter-heterotic group crosses.

To compare the efficiency of the three heterotic grouping methods, the 99 hybrids were arranged from the highest to the lowest based on grain yield under low N, high N and across research environments. The procedure consisted of dividing the total number of hybrids for each method into two major groups i.e. inter-group and intra-group crosses. These two groups were subsequently divided into high yielding hybrids (yield group 1 with a mean grain yield ranking among the first thirty three); intermediate hybrids (yield group 2 with a mean grain yield between 34th and 66th) and low yielding hybrids (yield group 3 with a mean grain yield between 67th and 99th) (Table 4. 29). The best classification method is the one whose heterotic groups allowed inter-heterotic group crosses to produce more superior hybrids than the within-group crosses. Based on this, the SCA method identified 24, HGCAMT 20, HSGCA 29, high yielding hybrids out of the total intergroup crosses under low N. Under high N, SCA method revealed 18, HGCAMT 23, and HSGCA 30, high-yielding hybrids of the total number of intergroup crosses identified by the grouping methods (Table 4.29). Across research environments, the SCA revealed 17, HGCAMT 20 and HSGCA 31, high yielding intergroup crosses. The breeding efficiency of the SCA method was the highest under low N (57%), high N (56%) and across environments (53%) (Table 4.30). The next highest breeding efficiency was observed for HSGCA for all the growing environmets. Grouping method HGCAMT had the least breeding efficiency for all the growing environments.

Overall, the SCA method was identified as the most efficient method for the classification of the inbreds into heterotic groups because it had the highest breeding efficiency for all the test environments.

Table 4. 29 Number of hybrids within the best 33 arranged in descending order of their yield (group 1), from 34th to 66th (group 2) and from 67th to 99th (group 3)

Yield Group	Cross type	HSGCA	HGCAMT	SCA
Low-N environments				
1	Inter	29	20	24
1	Intra	3	7	2
2	Inter	25	23	9
2	Intra	7	7	5
3	Inter	9	15	9
3	Intra	22	14	13
High N environments				
1	Inter	30	23	18
1	Intra	3	7	11
2	Inter	22	22	9
2	Intra	11	10	10
3	Inter	15	19	5
3	Intra	18	13	10
Across low and high N environments				
1	Inter	31	20	17
1	Intra	2	5	9
2	Inter	22	16	7
2	Intra	11	8	12
3	Inter	14	19	8
3	Intra	19	13	13

Table 4. 30 Breeding efficiency (%) of HSGCA, HGCAMT and the SCA heterotic grouping methods under low-N, high-N and across research environments.

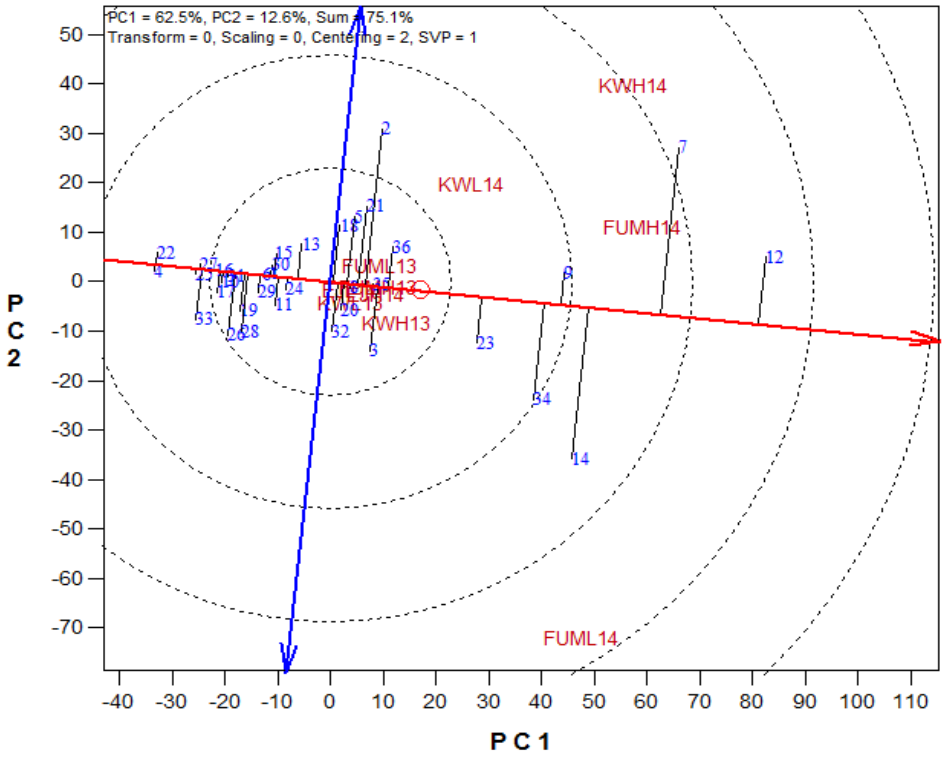
Environment	HSGCA	HGCAMT	SCA
Low-N	46.03	34.48	57.14
High-N	44.77	35.94	56.25
Across	46.27	36.36	53.13

4.3.11 Stability Analysis of Grain Yield of Intermediate Maturing Inbreds and Hybrids

4.3.11.1 Stability Analysis of Inbreds

The GGE biplot of grain yield of thirty two intermediate- maturing inbreds, three testers and a check revealed that PC1 explained 62.5% of the total variance while PC2 explained 12.6 %, thus both PCs accounted for 75.1 % of the total variation for grain yield of the inbreds across test environments. In the GGE biplot display, the double-arrow line (ATC ordinate) separates entries with below average means from those with above average means (Fig.4.7). The average yield of a genotype is approximated by the projections of their markers on the average-tester axis while stability of the genotypes is measured by their projection onto the average-tester coordinate y axis single-arrow line (ATC abscissa). The greater the absolute length of the projection of a genotype, the less stable it is. The entry/tester GGE biplot revealed inbreds CML 198/LPSC (12), ZM 523B-29-2-1-1-B*6 (7), ZM521B-66-4-1-1 (14), CLWN 247 (9), 9071 (34), TZL Comp 3 (23) and Ent 70 (36) were the highest yielding while TZM 501XKU1414XTZM501 (22) and CLWN 341 (4) were the lowest yielding inbreds. The check ENT 70 (36) was high yielding and was among the best 20 lines across the contrasting environments. The inbreds ZM 523B-

29-2-1-1-B*6 (7), ZM521B-66-4-1-1 (14) were high yielding but highly unstable while TZL Comp 3 (23) and CLWN 247 (9) were high yielding and moderately stable, and both were among the best 20 under both growing conditions. CLWN 341 (4), CLRCW 36 (6), CZL 0713 (25), M131 (35), CLWN 359 (8), Laposta Seq C7-F71-1-1-2 (16), CZL 00001 (27), CZL 03007 (31) and CML 442 (10) were the most stable inbreds. Among the stable inbreds, only CLWN 359 and CML 442 were among the best 20 inbreds under low N, while M131, CLWN 359 and CZL 0713 were among the best under high N, and across environments. An important feature of a GGE biplot is its ability to display top performing cultivars in a specific environment as well as the low yielding cultivars across environments (Figure 4.8). There are eight sectors in the biplot created by the perpendicular line that starts from the origin of the biplot and runs perpendicular to the side of the polygon. Out of the eight sectors, three have environments within them and five sectors have no environments within them. Hence inbred(s) that fall in sectors where environment(s) are included indicate the association of the inbred(s) with that specific environment(s). The inbreds at the different vertices of the polygon are expected to be responsive as they are the furthest from the origin. However, the responsive vertex inbreds can be either the best performing or the poorest at one or additional environments (Yan and Rajcan, 2002). Based on this, ZM523B-29-2-1-1-B*6 (7) was the vertex inbred (highest yielding) in KWH14, whereas CML 198/LPSC (12) was the vertex inbred at FUMH14. The highest yielding inbred at FUML14 was ZM521B-66-4-1-1 (14). The other inbreds, though were the vertex inbreds in some sectors, did not fall into any environment and thus are the lowest in most or all the test environments.



Entry	Inbred	Entry	Inbred
1	CLWN 349	19	TZD II 68
2	CML 494	20	J-16-1
3	CLWN 364	21	P43SRCq Fs100-1-1-8 TZM
4	CLWN 341	22	501XKU1414XTZM501
5	CLWN 238	23	TZL Comp 3
6	CLRCW 36	24	CZL 068
7	ZM 523B-29-2-1-1-B*6	25	CZL 0713
8	CLWN 359	26	CLWN 240
9	CLWN 247	27	CZL 00001
10	CML 442	28	TZD II 134
11	CML 444	29	TZD II 140
12	CML 198/LPSC	30	TZD II 141
13	CML 395/ CML 444	31	CZL 03007
14	ZM521B-66-4-1-1	32	87036
15	CML 444/CML 395/ DTPWC8F31	33	1368
16	Laposta Seq C7-F71-1-2	34	9071
17	CML 254	35	M131
18	Laposta Seq C7-F18-3-2-1	36	Ent 70

Figure 4. 7 An entry/tester genotype main effect plus genotype x environment biplot of grain yield of 36 intermediate maturing maize inbreds across low and high N environments in 2013 and 2014

Code	EJH14	EJL14	EJH13	EJL13	FUML13	FUMH13	FUML14	FUMH14	KWL13	KWH13	KWH14	KWL14
Environment	Ejura high 2014	Ejura low 2014	Ejura high 2013	Ejura low 2013	Fumesua low2013	Fumesua high 2013	Fumesua low 2014	Fumesua high 2014	Kwadaso low 2013	Kwadaso high 2013	Kwadaso high 2014	Kwadaso low 2014

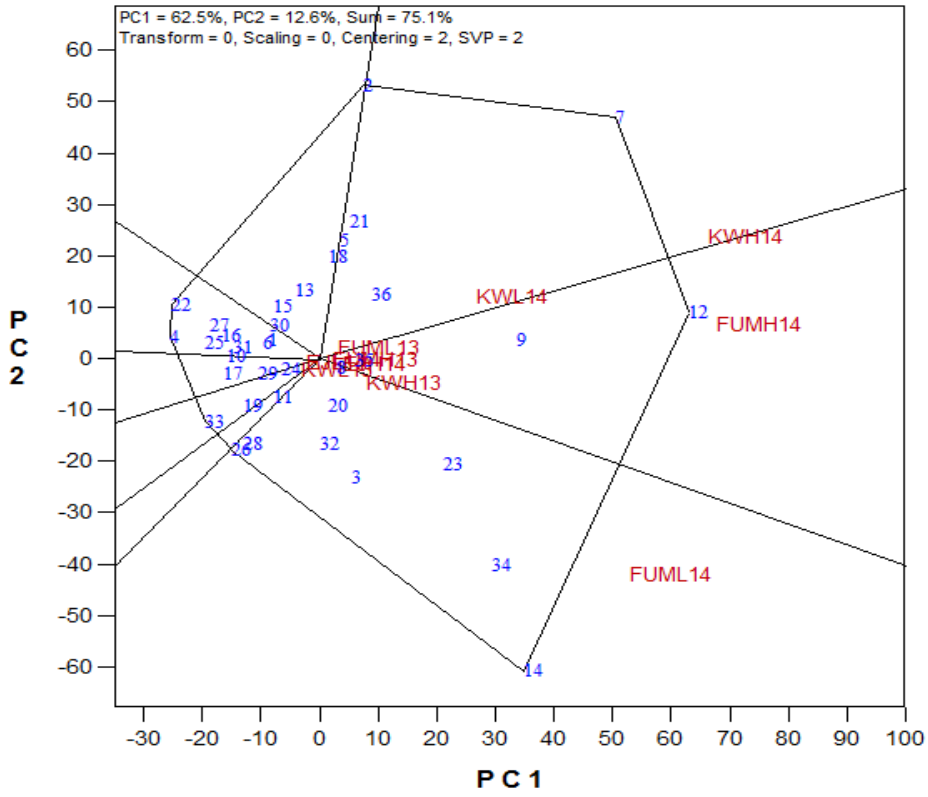


Figure 4. 8 A 'which won where' GGE biplot of grain yield of 36 intermediate maturing maize inbreds evaluated across low N and high N environments in 2013 and 2014

4.3.11.2 Stability Analysis of Hybrids

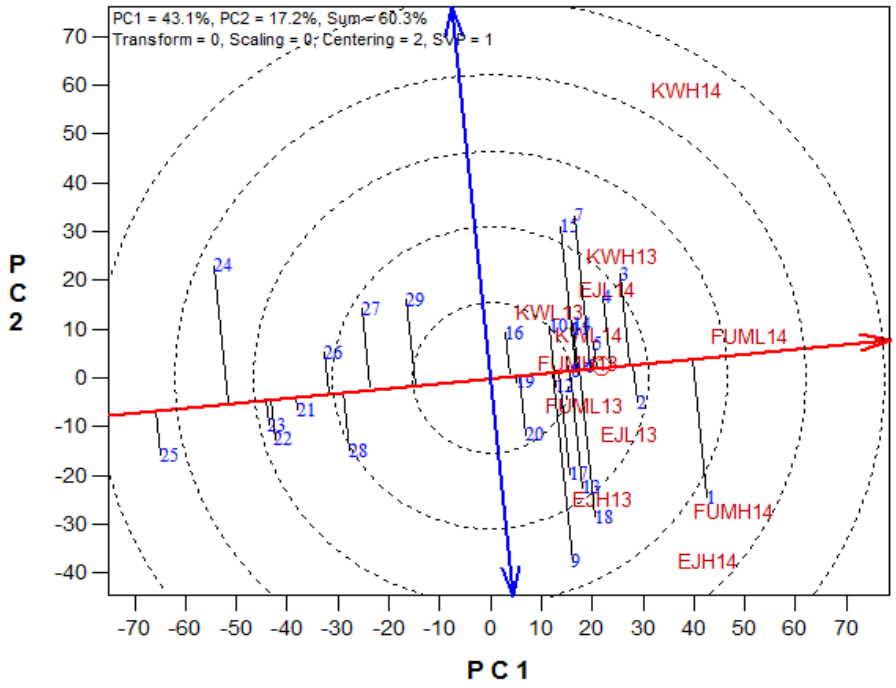
The GGE biplot analysis of grain yield of the best 20 and worst five hybrids and the four checks across 12 environments revealed that the principal component axis 1 (PC1) explained 43.1% of total variation while PC2 explained 17.2% of the total variation in grain yield across the environments with both axes explaining 60.3% of the total variation in grain yield. In figure 4.9, the entry/tester GGE biplot revealed CZL 00001 x 9071 (1), Laposta Seq C7-F18-3-2-1 x 9071 (2), CLWN 247 x 9071 (3), CLWN 364 x 9071 (4) and TZD II 68 x 1368 (5) as the five top yielding hybrids. The lowest yielding hybrids were TZL Comp3 x 1368 (25), ZM 521 B-66-4-1-1 x 1368 (24), CML 254 x 1368 (23) and CLWN 238 x 1368 (22). The hybrids CZL 00001 x 9071 (1), and CLWN 247 x 9071 (3), were high yielding but highly unstable while CML 395/ CML 444 x 9071 (6), TZD II 134 x 9071 (8), CML 494 x 1368 (12), CML 494 x 9071 (19) and CLWN 349 x 1368 (21) were the most stable hybrids. Among the hybrids that were most stable, CML 395/ CML 444 x 9071 (6), and CML 494 x 1368 (12) were among the best 20 under high N, while CML 395/ CML 444 x 9071 (6) was among the best 20 under low N. Across environments, hybrids CML 494 x 1368 (12), CML 494 x 9071 (19) and CML 395/ CML 444 x 9071 (6), were among the best 20. Hybrids CML 494 x 1368(12), TZD II 134 x 9071(8), CML 395/ CML 444 x 9071(6) and TZDII 68 x 1368 (5) were the best hybrids in terms of stability and yield performance across test environments.

In Figure 4.10, the vertex cultivar in each sector of the polygon view represents the highest yielding cultivar in the location that falls within that particular sector. Hybrids 7, 15 and 3 were the highest yielding at KWH14 and KWH13 (High N environments at

Kwadaso, 2013, 2014), while hybrid 1 had the highest performance at FUML14, FUMH14 and EJH14. The vertex hybrids, 9, 25 and 24 were the lowest yielding at all or some locations. Furthermore, no environment fell into the sectors with 29, 27, 26, 21, 23, 22, 28, 20, 17, 13, and 18, indicating that these hybrids were not the best in any of the environments. Hybrids within the polygon, particularly those located close to the biplot origin were less responsive than the vertex hybrids.

The representativeness and discriminating ability of the environments is represented in Figure 4.11. The straight line from the origin to the coordinates where an environment falls is called the research environment vector while the straight line with a single arrow which passes through the origin and the average environment represents the average environment axis (AEA). The vector length measures its discriminating power to assess cultivars under the test environments, that is, the longer the vector length the more discriminating the environment. The angle between an environment and AEA measures its representativeness, therefore, the shorter the projection is from the marker of an environment, the more representative the environment. According to Yan *et al.* (2010b), the shorter environmental vectors indicate that the specific environments were not strongly correlated with environments having longer vectors and were probably not strongly correlated with one another. Based on these requirements, KWH14, FUML14 FUMH14 and EJH14 with longer vectors and far away from the origin were more powerful in discriminating among the hybrids while FUML14 environment was the most representative. An ideal test environment should effectively discriminate genotypes and represent their mega-environment (Yan & Rajcan, 2002). The biplot identified FUML14 as the ideal test environment. Also, EJH14 and KWH14 with long vectors and large

angles cannot be used in selecting superior hybrids, but can be used effectively in culling unstable genotypes.



Entry	Hybrid	Entry	Hybrid
1	CZL 00001 X 9071	**15**	TZD II 68 X 9071
2	Laposta Seq C7-F18-3-2-1 X 9071	**16**	TZM501 X KU1414 X TZM501 X 9071
3	CLWN 247 X 9071	**17**	CLWN 240 X 9071
4	CLWN 364 X 9071	**18**	TZD II 140 X 9071
5	TZD II 68 X 1368	**19**	CML 494 X 9071
6	CML 395/ CML 444 X 9071	**20**	TZD II 141 X 9071
7	CLWN 247 X 87036	**21**	CLWN 349 X 1368
8	TZD II 134 X 9071	**22**	CLWN 238 X 1368
9	CLWN 359 X 9071	**23**	CML 254 X 1368
10	CLWN 247 X 1368	**24**	ZM 521 B-66-4-1-1 X 1368
11	CML 198/ LPSC X 9071	**25**	TZL Comp3 X 1368
12	CML 494 X 1368	**26**	1368 X 9071
13	CLWN 238 X 9071	**27**	1368 X 87036
14	CML 395/ CML 444 X 1368	**28**	Etubi – (Check)
		29	87036 X 9071

Figure 4. 9 An entry/tester genotype main effect plus genotype x environment biplot of grain yield of 29 intermediate maturing maize hybrids across low and high N environments in 2013 and 2014

Code	EJH14	EJL14	EJH13	EJL13	FUML13	FUMH13	FUML14	FUMH14	KWL13	KWH13	KWH14	KWL14
Environment	Ejura high 2014	Ejura low 2014	Ejura high 2013	Ejura low 2013	Fumesua low2013	Fumesua high 2013	Fumesua low 2014	Fumesua high 2014	Kwadaso low 2013	Kwadaso high 2013	Kwadaso high 2014	Kwadaso low 2014

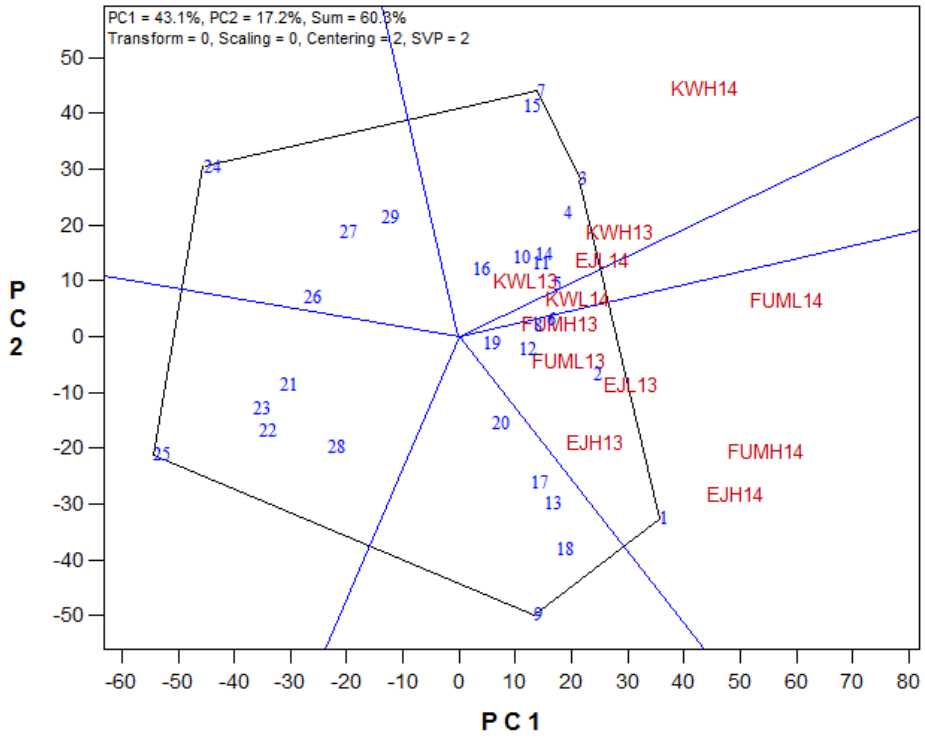
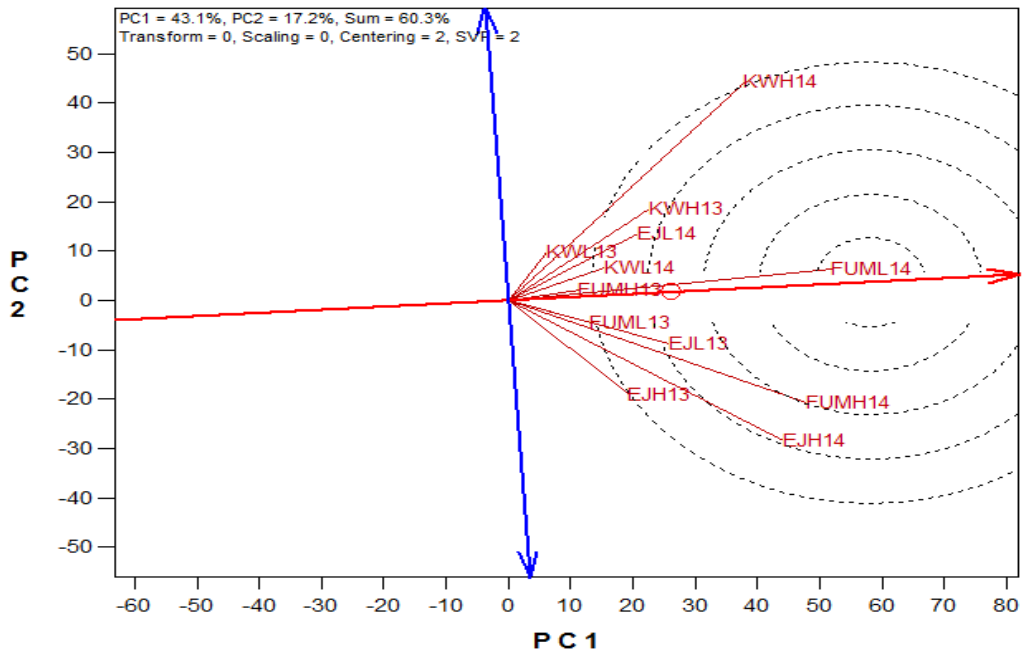


Figure 4. 10 A 'which won where' GGE biplot of grain yield of 29 intermediate maturing maize hybrids evaluated across low N and high N environments in 2013 and 2014.



Code	Environment
EJH14	Ejura high 2014
EJL14	Ejura low 2014
EJH13	Ejura high 2013
EJL13	Ejura low 2013
FUML13	Fumesua low2013
FUMH13	Fumesua high 2013
FUML14	Fumesua low 2014
FUMH14	Fumesua high 2014
KWL13	Kwadaso low 2013
KWH13	Kwadaso high 2013
KWH14	Kwadaso high 2014
KWL14	Kwadaso low 2014

Figure 4. 11 The 'discriminating power and representativeness' view of GGE biplot on a genotype X environment yield data of 29 intermediate maturing maize hybrids evaluated in 12 environment

4.4 Discussion

The effects of N on grain yield and other traits confirmed that N stress was a major limiting factor in maize productivity. The use of fields that had been previously depleted of N resulted in severe N stress as indicated by soil nutrient levels and crop responses. Increased N fertilization was accompanied by increase in grain yield of the maize genotypes. This is consistent with findings of other studies (Akintoye *et al*., 1998, Banzinger *et al*., 1999, Badu Apraku, 2011d; 2013c, Ifie, 2013, Mafouasson, 2014). Significant differences observed among genotypes for most traits under low N and high N indicated that adequate genetic variation existed among the hybrids and inbreds which could allow good progress from selection under contrasting environments. It also indicated good probability for the improvement of these genotypes under stress and optimal conditions across a range of environments. Results showed significant environmental variation for grain yield and all other traits for both inbreds and hybrids under low and high N environments implying that each environment was unique and different. This observation is in agreement with findings of Badu-Apraku *et al.* (2007a). Variations in response of maize hybrids to environmental stress have previously been reported by several authors (Betran *et al*., 2003d; e; Mosisa *et al*., 2007; Derera *et al*., 2008).

Genotype x environment effect was significant for most traits of the hybrids. This suggested that the performance of hybrids for most traits was not consistent across environments. These results are in agreement with the findings of Ifie (2013), and Badu-Apraku *et al.* (2012, 2013b) who also reported significant genotype x environment interactions for maize grain yield and other agronomic traits under low N. GEI effect,

however, was significant for only few traits of the inbreds suggesting that the inbreds responded similarly for most of the traits studied at the different locations. Similar results were reported by Makumbi (2011) who found significant GEI in only two traits under low N. The environment and genotype x environment interaction effects across environments were significant for most traits indicating that the individual environments were unique and that hybrid and inbred selection would not be consistent across the environments. Except for high N conditions, the analysis of variance showed that the environment accounted for the largest sum of squares for grain yield followed by the GEI and the genotypes. The large environmental effects indicated that the test environments were highly variable and substantiated the need for the testing of genotypes in a wide range of sites over years as reported by Badu-Apraku *et al* (2007a) and Badu-Apraku and Lum (2010). Moderate heritability estimates for grain yield, days to silking and anthesis, anthesis-silking interval, plant height, ear height, stay-green characteristic, and chlorophyll content suggested that early generation testing for these traits to improve low N tolerance would be effective. Heritability for grain yield was 49% under low N, and was comparable to the 41% reported by Sibale and Smith (1997). Heritability estimates for grain yield were higher than that for ASI, ears per plant, plant aspect and ear aspect but lower than that of days to anthesis and silking, plant height, ear height, stay green characteristic and chlorophyll content. This means that much of the grain yield differences under low N was not determined by genotypic effects alone suggesting that selection based on grain yield alone under low N environments will not be effective as its heritability usually decreases under stressed condition, while the heritability of some secondary traits remains high and their genetic correlation with grain yield increases

sharply under stress. Banziger *et al*. (2000) recommended that information on GY, EPP, ASI and stay green characteristic should be used in selecting genotypes that tolerate low N. Badu-Apraku *et al.* (2011a) expanded the parameters for selection to include plant height, days to silking, days to anthesis, ears per plant, anthesis–silking interval, stay green characteristic, ear aspect, and plant aspect. In the present study, grain yield and ASI were negatively correlated, indicating the importance of shorter ASI for increased grain yield. Other studies using different germplasm under stress conditions reported similar results (Bolaños and Edmeades, 1993b; Lafitte and Edmeades, 1995; Bänziger and Lafitte, 1997; Bänziger *et al*., 2002; Betrán *et al*., 2003c). Grain yield showed a positive phenotypic correlation with ears per plant (0.43**). Bänziger and Lafitte (1997) indicated that ears per plant reflect the ability of a plant to produce a grain-bearing ear under N stress. Grain yield showed a negative correlation with stay green characteristic and this is in agreement with results reported by Betrán *et al*. (2003c) and Makumbi *et al*. (2011). Delayed leaf senescence should allow for improved grain filling in the genotypes that maintain more green leaves. Highly significant correlation of grain yield and traits used in computation of the base index for selection of low N tolerant genotypes were recorded in this study. This justified the use of a base index that integrates high grain yield, number of ears per plant, short ASI, improved plant aspect, better ear aspect and good stay-green characteristic.

The intensity of the stress observed under low soil N, resulting in grain yield reduction of 52% for hybrids in this study, fell within the range of yield reduction observed under stress levels applied during selection of hybrids for tolerance to low N by other workers (Meseka *et al.*, 2006; Makumbi *et al.*, 2011; Badu-Apraku *et al.*, 2010). These authors

reported 52%, 51% and 40% reduction in grain yield, respectively for hybrids under low N. The 52% yield reduction of hybrids in this study fell outside the limits of the 20 to 30% set by Bolanos and Edmeades (1996) and it was high enough to facilitate the identification of hybrids that possess genes for low N tolerance. A yield reduction of 27% under low N environments compared to high N environments was observed for the inbred lines. This reduction in grain yield is lower than that reported by Betran *et al*. (2003a), who observed 68% reduction in grain yield of inbreds under low N, but fell within the limits of the 20-30% set by Bolanos and Edmeades (1996). Ifie (2013) also reported a yield reduction of 27% among hybrids under low and high nitrogen environments. Differences in the results of this study and those of other authors could be due to the level of N tolerance in the set of inbred lines used in the present study.

In the present study, a higher yield, a positive base index and a low percentage yield reduction indicated tolerance to low N. Tolerant genotypes suffered less yield reduction under low N stress. Thus CLWN 247 x 9071, ZM523B-29-2-1-1-B*6 x 9071, TZD II 68 x 1368 and P43SCRq Fs100-1-1-8 x 9071 hybrids were identified as the best low N tolerant hybrids. They were among the best five inbreds identified under low N using the base index and had low yield reduction under low N. Again they out yielded the best check by 27%, 30%, 31% and 25%, respectively. These hybrids should be tested across more locations and released for cultivation by resource poor farmers. Six hybrids: CZL 00001 x 9071, LapostaSEQC7-F18-3-2-1 x 9071, CLWN 364 x 9071, CLWN 247 x 9071, CML 395/CML 444 x 9071 and TZD II 68 x 1368 were identified among the 20 best hybrids under low N, high N and across environments. These hybrids had inherent ability for outstanding performance under both low N and high N environments. Because

farmers cultivate maize under varying soil fertility levels, high yield under low N and high N conditions is desirable and these hybrids are appropriate as they posses genes for general adaptability. TZD II 68 x 1368 was identified as an outstanding low N tolerant hybrid as well as good hybrid under high N. The inbred TZD II 68, an IITA line developed from crosses between the extra-early *Striga* resistant population, TZEE-W Pop STR C4 and *Zea diploperennis* confirms the report by Badu-Apraku *et al.* (2009; 2015), that recurrent selection for *Striga* resistance under artificial infestation results in selection gains in grain yield under low and high N environments. TZL Comp 3 x 1368 was among the 5 poorest yielding hybrids under both low N and high N environments. This hybrid can be used as a susceptible check in other studies. The inbred lines, TZLComp3, CZL 068, CML 444, and ZM523B-29-2-2-1B*6 were low N tolerant while CML 494, CLWN 349, CZL 0713 and CZL 00001 were sensitive inbreds. Based on these results, CML 444 and CML 494 were selected as low N tolerant and low N susceptible inbreds respectively for developing a mapping population for identification of QTL's for low N tolerance in subsequent studies.

Superior heterosis for grain yield is critical in any hybrid breeding programme. The average degree of mid parent heterosis and better parent heterosis in the present study was 97.04% and 63.27% under low N environments while, under high N, it was 199.37% and 150.29%. These values are higher than those reported by Betran *et al.* (2003a), who reported mid parent heterosis and better parent heterosis of 157% and 126% under high N and 34% and 8% under low N. However, the values reported in this study were lower than those reported by Mafouasson (2014) who showed mid parent heterosis and better parent heterosis of 265.64% and 189.72% under low N. The expression of heterosis was

greater under high N than low N stress conditions. This could be explained by the fact that inbred lines used were bred for tolerance to low N stress conditions so they tended to perform better under low N environments, thereby resulting in an increase in parental and better parent means. These results are contrary to findings of Makumbi *et al.* (2011) and Mafouasson (2014) who reported higher mid parent heterosis and higher parent heterosis values under low N stress than under high N environments. However, the findings from this study are in agreement with those of Betan *et al.* (2003.a) who found higher heterosis in high N than low N environments. The hybrid TZD II 68 x 1368 had the highest value for better parent heterosis under both low and high N environments and the second highest value for mid parent heterosis. It was also rated as one of the best hybrids under all research environments. The use of inbred line *per se* information to predict hybrid performance under stress could reduce the need for hybrid evaluation. Correlation between inbred line *per se* and hybrid performance was weak but positive and significant under both low and high N environments. The correlation was higher at low N ($r= 0.26$, $P < 0.01$) than high N ($r= 0.19$, $P <0.05$). Similar results were reported by Betran *et al* (2003a), who obtained correlations between line and hybrid performance higher (0.33) for low N than for high N (0.08). The weak correlations observed in the present study are consistent with the findings of Meseka *et al. (*2006) and Betran *et al.* (2003a). This suggests that crosses between high yielding inbred lines under low and high N environments may not result in high yielding hybrids. The results are however, contrary to those of Edmeades (1995) and Ifie (2013) who reported no significant correlations between grain yield of parental and hybrids under low N. Despite the positive correlations between inbreds and hybrids in all environments in this study, comparative

yield trials of the hybrids are still needed, especially in the light of the weak correlations observed.

An important objective of the present study was to examine the combining abilities of the thirty- two intermediate maturing inbreds and three inbred testers under both low and high N environments. Under low N, the presence of significant GCA_l, GCA_t and SCA mean squares for all measured traits except the GCA_l for plant aspect and SCA for plant height, ears per plant, stay green characteristic, plant aspect, husk cover and chlorophyll content indicated that both additive and non-additive gene actions were important for most of the traits and that there was scope for the improvement of these traits through selection under low N. The non-significant SCA mean squares for plant height, ears per plant, stay green characteristic, plant aspect, husk cover and chlorophyll content in the present study indicates that non-additive gene effects were not important in the inheritance of these traits. Badu-Apraku *et al.* (2013b) and Ifie (2013) also reported non-significant SCA for stay green characteristic under low N. The preponderance of GCA mean squares over SCA mean squares implies that additive gene action was more important than non-additive gene action for most traits and that GCA was the major component accounting for the differences among the hybrids under low N environments. This is consistent with other reported results (Below *et al.*, 1997; Kling *et al.*, 1997; Tamilarasi *et al.* (2010), Badu-Apraku *et al.*(2013) and Ifie (2013). However, this is contradictory to the results of Betràn *et al.* (2003a), Meseka *et al.* (2006), Makumbi *et al.* (2011), Meseka *et al.* (2013), Ndhlela (2012), and Mafouasson (2014) who reported preponderance of non-additive gene effects over additive gene effects for grain yield

under low N. The differences in these results may be attributed to the differences in the germplasm used.

Under high N environments, $GCA_{l,}$ and GCA_t mean squares were significant for all the measured traits except ears per plant. SCA effects were significant for grain yield, days to silking and anthesis, anthesis-silking interval, plant aspect and husk cover. This indicates that both additive and non-additive gene action were important in the inheritance of grain yield and other traits under high N environments. The significant SCA effects observed for ears per plant and the non significant GCA for ears per plant is an indication that non-additive gene action was important in the inheritance of ears per plant under high N environments. This is consistent with the findings of Ifie (2013). The preponderance of GCA mean squares over SCA mean squares implied that additive gene action was more important than non-additive gene action for most traits and that GCA was the major component accounting for the differences among the single cross hybrids. This suggested a greater contribution of inbred parents with high GCA effects to the performance of the hybrids across environments (Baker, 1978). However the prediction of hybrid performance based on GCA effects of the parents alone might not be accurate as has been observed in many studies, since SCA effects are also significant. The results of this study corroborate that of Makumbi *et al.* (2011) and Mafouasson (2014) who reported that additive genetic effects control grain yield under high N. With preponderance of GCA over SCA variance, early generation testing may be more effective and promising hybrids can be identified and selected mainly based on the prediction from GCA effects. This has an implication in breeding in that good parents can be identified using the GCA effects and then crossed to produce high yielding hybrids.

The significant GCA_l x environment and GCA_t x environment interactions for most traits under low and high N, indicated that GCA effects associated with the lines and testers were not consistent over environments, and that there was significant variation in the combining ability of the lines under different environments. This agrees with findings of other authors (Derera *et al*., 2008; Gissa, 2008). SCA x environment interactions were not-significant for most traits, indicating that the response of the hybrids for those traits did not differ in the research environments. This result is consistent with Meseka *et al.* (2006), who reported non-significant SCA x environment interactions for grain and most secondary traits under low N.

Inbred lines with high GCA effects for grain yield and other traits are likely to transmit their characteristics to the progeny and could be useful in a breeding program. Such inbreds could be used as parents to form a synthetic population that could be improved for stress environments (Makumbi *et al*. 2011). The significant positive GCA effects observed for grain yield of inbreds; CLWN 247 , TZD II 68 and ZM523B-29-2-1-1-B*6 suggests that these lines could be useful for contributing favourable alleles for breeding for improved grain yield under low N environments. CLWN 247 showed consistency in performance exhibiting good GCA effects for grain yield under low N, high N and across environments. This line is, therefore, a good general combiner for grain yield across all environments. This observation was further confirmed by the hybrids it produced that were among the 10 best performing hybrids across all environments. This line was also a good general combiner for other traits such as ear height in all environments, stay green characteristic under high N and across environments, days to anthesis, and ear aspect across environments. CLWN 247, CLWN 364, CML 395/ CML 444 and CZL 00001 had

significant positive GCA effects for grain yield under high N environments. These inbred lines are expected to contribute to higher grain yield in their hybrids under high N environments. The negative and significant GCA effects observed for the stay-green characteristic for CML 395/ CML 444, CML 442, CZL 068, Laposta Seq C7-F71-1-1-2 under low N and CML 395/ CML 444, CZL 068 and CZL 0713 under high N implied that these inbreds will transmit the trait to their progenies and thus slow down their rates of leaf senescence under the contrasting environments. In much the same way, inbreds with negative and significant GCA effects for days to silk, days to anthesis, ear aspect and ASI and those with positive and significant GCA effects for plant and ear heights and chlorophyll content would contribute positively to the improvement of these traits across the research environments. The tester 9071 was a good general combiner for grain yield, plant height and chlorophyll content under low N and grain yield under high N. This was confirmed by the high yielding hybrids it produced in combination with other inbreds under all environments. The inbred, 87036 was a good general combiner tester for stay green characteristic while 1368 was a good combiner for days to silking under low N. This indicated that these testers were capable of contributing to the hybrids favorable alleles for improvement of these traits.

The occurrence of significant SCA is a consequence of fluctuations in dominance relationships among parents (Wassimi *et al*., 1986). Based on the significant SCA effects observed under low N environments in this study, seven promising hybrids , TZD II 68 x 1368, CZL 0713 x 1368, MI31 x 1368, CLWN 359 x 9071, TZM 501 x KU 1414 x 43 TZM501 x 9071 and TZL comp 3 x 87036 were identified. All the crosses with the exception of TZL comp 3 x 87036 were among the best 20 hybrids selected under low N

environments. This indicated that selection of parents for hybrid production on the basis of only SCA may not be useful as positive estimates of SCA are not indicative of high performance in hybrids. Therefore, selection of crosses on the basis of high SCA in addition to the mean grain yield of test crosses as suggested by Menkir *et al.* (2004) is more practical.

In the present study, heterotic grouping was based on three different methods; (SCA of grain yield, HGSCA, and HGCAMT). Classification by the three methods showed similar but not identical trends. For example, under low N, CML 444, TZD II 134, TZD II 141 were placed into the same group which is group A (1368) by the three methods, while others, such as CLRCW 36, CML 254, CLWN 247 and J-16-1 were placed into the same group by two of the three methods. Under high N, five inbreds (CML198/LPSC, CML 444/CML395, CZL 03007 and M131 were placed in the same heterotic group A (1368). In heterotic group C (87036), under low N, CML 395/CML444, CML 494, CZL 0001 and CML198/LPSC were found. In terms of placement of inbred lines into the same group, the HSGCA and the HGCAMT methods appear to be more similar. For example, under low N seven inbreds were classified into heterotic group C (87036) with HSGCA and HGCAMT methods. Similar findings were reported by (Badu-Apraku and Oyekunle 2012, Badu Apraku *et al*., 2015), who showed close correspondence with the classification by the HGCAMT and HSGCA method. On the contrary, Badu Apraku *et al.* (2013) found the HSGCA and the molecular marker methods to be more similar than the other comparisons. But there was close correspondence among the three groups. Similar results were reported by Badu-Apraku *et al. (*2013) who found close correspondence in the classification of seven extra-early yellow inbreds using the SCA,

HGCAMT and HSGCA methods. The breeding efficiency was highest for SCA method under all growing conditions, which indicated that the SCA method was more efficient in classifying the inbreds. These results are in disagreement with the findings of Fan *et al*. (2009), Badu-Apraku *et al*. (2013c, 2015) and Akinwale *et al*. (2014) who found the HSGCA method to be more efficient in classifying inbreds than the SCA method. However, it is in agreement with Laouali (2014) who reported that the SCA method was efficient in classifying inbreds and found that the HSGCA method offered no advantage over the SCA method. But then, in a situation where we have just few traits with significant and positive GCA effects, the efficiency of the HGCAMT method may be compromised (Badu-Apraku *et al.*, 2013). However, it is important to note that no heterotic grouping method is perfect due to unlimited genetic combinations between any two inbred lines which may result in the development of superior hybrids from crosses made within a heterotic group (Akinwale *et al*., 2014). The classification of these inbreds into contrasting heterotic groups will facilitate the development of the low-N tolerant source populations. The high yielding lines identified across environments; CZL 00001, Laposts Seq C7-F18-3-2-1, and CLWN 364 are anti 9071. TDZII 68 is anti 1368. The inbreds in each heterotic group may be recombined to form source populations which could be improved through recurrent selection with subsequent crosses onto the appropriate testers to identify new hybrids.

In order to identify maize cultivars that have stable and high yield performance under low N as well as under high N conditions for commercialization, the GGE biplot was adopted to decompose the GEI. For a hybrid to be released and commercialized, it must demonstrate both high average yield performance and high stability across locations

(Kaya *et al*., 2006; Yan and Tinker, 2006; Jalata, 2011; Badu- Apraku *et al*,. 2011b). The most stable hybrids in this study are CML 494 x 1368(12), TZD II 134 x 9071(8), CML 395/ CML 444 x 9071(6) and TZD II 68 x 1368 (5). These hybrids should be selected for further testing across multiple locations to confirm the consistency of performance and commercialized. The most promising inbreds in this study were identified as CLWN 359(8), TZL Comp 3(23) and CLWN 247(9). These are likely to contribute favorable alleles for grain yield to their progenies. An ideal test environment should effectively discriminate genotypes and represent their mega-environments (Yan & Rajcan, 2002). Based on these criteria, FUML14 (Fumesua, low N 2014) was identified as the ideal test environment for selecting for broad adaptation.

4.5. Conclusions

The objectives of the present study were to identify high yielding maize hybrids under low N and high N growing environments, determine the combining abilities and mode of gene action controlling low N tolerance in intermediate maturing maize inbred lines, classify the inbreds into heterotic groups, assess the performance and stability of the hybrids across low and high N environments, and identify genotypes tolerant to low and high soil nitrogen. The study revealed genetic variability for grain yield and several secondary traits among the inbreds and hybrids. This variability will allow selection for low N tolerant genotypes. CLWN 247 x 9071, ZM523B-29-2-1-1-B*6 x 9071, TZD II 68 x 1368 and P43SCRq Fs100-1-1-8 x 9071 hybrids were identified as the highest yielding, low N tolerant hybrids. These are recommended for further testing for release to farmers for low soil N

environments. Six hybrids (CZL 00001 x 9071, LapostaseqC7-F18-3-2-1 x 9071, CLWN 364 x 9071, CLWN 247 x 9071, CML 395/CML 444 x 9071 and TZD II 68 x 1368 were among the 20 best yielding hybrids under low N, high N and across environments. These are candidates for further testing for commercialization. CML 494 x 1368(12), TZD II 134 x 9071(8), CML 395/ CML 444 x 9071(6) and TZDII 68 x 1368 are the most high yielding and stable hybrids. These hybrids should also be extensively tested in multi-location trials and promoted for adoption. The results confirmed that selection for improved grain yield under low N environments was not effective as the environment played a large part in its expression, therefore secondary traits with high heritability under low N should be used to supplement grain yield data to identify superior genotypes under low N environments.

Significant GCA and SCA effects for grain yield and most other traits were observed across test environments with preponderance of additive gene effects over non- additive gene effects suggesting that inbred lines with high GCA effects for grain yield and other traits are likely to contribute favorable alleles for superior yield performance of their progenies across environments. The inbreds CLWN 247, TZD II 68 and ZM523B-29-2-1-1-B*6 had positive and significant GCA for grain yield and could be useful for contributing favorable alleles for breeding for improved grain yield under low N. The inbred lines identified in the three heterotic groups for each of the three research environments may be crossed within groups to develop source populations for new inbred development. The inbreds in each heterotic group may also be recombined to form populations which could be improved through recurrent selection. Subsequently, inbred lines could be extracted from each population for the production of superior hybrids and synthetics by selfing new inbreds and crossing them onto a tester inbred of opposing

heterotic group. Since farmers' fields in Ghana are rarely characterized by only one abiotic stress a variety must combine tolerance to several abiotic stresses, including drought and low N stress, and have high grain yield potential under favorable conditions to become popular among farmers.

CHAPTER FIVE

5.0 Identification of Quantitative Trait Loci (QTL) for yield and yield related traits under high and low soil nitrogen environments

5.1 Introduction

The surprising increase in crop yield during the past century is attributed to the selection of genotypes with higher yield potential and the increase in the amount of nutrients, particularly nitrogen (N) supplied during the growth cycle (Tuberosa, 2002). Because available soil N is usually the critical factor limiting plant growth, N fertilizer is often applied to croplands and results in marked increases in yield. Low N availability is a major source of yield loss in maize (Pingali and Pandey, 2001). Most maize in developing countries is produced under N-deficient conditions because of limited availability of fertilizer, or low purchasing power of farmers (Bänziger *et al*., 1997). Development of maize cultivars tolerant to low soil N represents a more cost-effective and long-term sustainable alternative to use of additional fertilizer.

Progress in selecting for tolerance to low N is slowed by large genotype x season and genotype x location interactions. The efficiency of selection for yield in low N environments may be improved by selection for correlated, secondary traits (Banziger and Lafitte, 1997; Badu Apraku 2011, 2012). Selection indices based on these traits have been developed and have improved significantly the selection efficiency under stress conditions (Banziger and Lafitte, 1997). The complexity of measuring some of the secondary traits quickly and accurately, however, has limited their routine application in breeding programs (Monneveux and Ribaut, 2006).

The introduction of molecular marker technology and the construction of saturated linkage maps have enabled the detection of the genetic loci associated with complex traits (Kang *et al*., 1998; Li *et al*., 1995; Song *et al*., 2001). Linkage genetic maps and quantitative trait locus (QTL) mapping technology make estimates of the number of loci controlling genetic variation in a segregating population and their characterization with regard to their map positions in the genome (Xiao *et al*., 1996). In maize, the genetic dissection of complex traits for abiotic stress responses has focused principally on drought tolerance (Agrama *et al*., 1996; Ribaut *et al*., 1996: 1997; Tuberosa *et al*., 2002). The advantage of using molecular markers in breeding programs has been evaluated (Morris et al. 2003) and marker-assisted selection experiments to improve grain yield under water limited conditions and low temperatures have been reported (Ribaut and Ragot 2007). Not as much attention has been paid to the understanding of the genetic response of segregating populations to field soil deficiencies such as low P (Reiter et al., 1991) or low N (Agrama *et al*., 1999; Hirel *et al*., 2001).

The genetic basis for nitrogen use efficiency (NUE) has received some research attention. Presterl *et al*. (2003) estimated the quantitative genetic parameters of NUE in European maize. Agrama *et al*. (1999) investigated quantitative trait loci (QTL) for correlated secondary traits of NUE using a set of F2:3 populations derived from two inbred lines, B73 and G79. They identified six QTLs for grain yield at low N availability and five QTLs at high N availability. Hirel *et al*. (2001) reported that QTL for NUE were correlated with agronomic and physiological traits. Maize QTLs for nitrogen-use efficiency (NUE) have been reported for vegetative growth and grain filling periods of a

mapping population grown with N and without N (Bertin and Gallais, 2001; Hirel *et al*., 2001)..

Heritability of GY and other associated traits under low N conditions is generally low with large environmental effects during evaluation. Consequently, the use of marker–assisted selection (MAS) would be a very effective strategy in breeding for tolerance to low N (Zhou, 2010). However, the effectiveness of MAS depends on the precise localization of the QTL using the representative breeding germplasm and identification of tightly linked, easy-to use, molecular markers.

Most of the reported studies in maize have utilized SSRs for linkage map construction and QTL mapping. With the availability of whole sequence genome information in maize (Gore et al., 2009), SNPs are physically anchored and provide an ideal platform for linkage mapping and QTL identification. The use of SNP markers has emerged as a powerful tool for many genetic applications due to the low assay cost, high genomic abundance, locus-specificity, codominant inheritance, simple documentation, potential for high throughput analysis and relatively low genotyping error rates (Rafalski, 2002; Schlotterer, 2004). Numerous SNP markers, mostly developed from DNA sequences of known genes, are now available for use in maize. Their applications in maize breeding include genetic diversity analysis, linkage map construction, marker trait association or quantitative trait locus (QTL) mapping, and MAS (Lu *et al.*, 2009). Markers, shown to be linked to specific genes, may be used to facilitate selection of desired genotypes through MAS. QTLs identified in elite breeding germplasm are of direct relevance for crop improvement through knowledge-based breeding, and can be immediately used for MAS approaches (Wu¨rschum, 2012; Wang *et al*., 2012). The objective of this study was to

map and identify quantitative trait loci (QTL) associated with yield and yield related traits under low N and high N environments.

5.2 Materials and Methods

5.2.1 Germplasm

The two parental lines used in the study differed for their responses to low N stress; CML 494 (highly susceptible to low soil N) and CML 444 (tolerant to low soil N). The lines were selected based on low N evaluation trials conducted during the major cropping season of 2013 at Fumesua, Ejura and Kwadaso (Chapter 4). Crosses were made between the two inbreds during the minor cropping season of 2013 at the CSIR-Crops Research Institute Research fields at Fumesua. The F_1s were backcrossed to CML 494 to obtain the BC_1F_1s and the BC_1F_1s were backcrossed to CML 494 to generate 150 BC_2F_1 families.

5.2.2 Field Evaluation (Phenotyping)

The 150 BC_2F_1, families, the 2 parents, the F_1 hybrid and a check (ENT 70) were evaluated under low N and high N environments during the major and minor cropping seasons of 2014 at the CSIR-Crops Research Institute research fields at Fumesua and Ejura. An 11 x 14 lattice design with two replications was used for the evaluations at the two locations. The low N plots received 30 kg N ha^{-1} while high N plots received 90 kg N ha^{-1} applied in two splits at two and five weeks after planting. Both low N and high N fields received 60 kg P ha^{-1} as single superphosphate (P_20_5) and 60 kg K ha^{-1} as muriate

of potash (K_2O). The planting density in each season was 56 000 plants ha^{-1}. The trial was kept weed-free through the use of Gramozone and Atrazine as pre- and post-emergence herbicides respectively and subsequently by hand weeding.

5.2.3 Data collection

The traits measured in each experiment were averaged across 10 guarded plants selected from each plot. Data were collected on the following traits:

Days to anthesis (DTA) = number of days from planting to the day that 50% of the plants in a plot had shed pollen.

Days to silking (DTS) = number of days from planting to the day that 50% of the plants had silked.

Anthesis-silking interval (ASI) = the difference between days to 50% silking and 50% anthesis.

Plant height (cm) = the distance from the base of the plant to the height of the first tassel branch.

Ear height (cm) = the distance from the base of the plant and the node bearing the upper ear

Number of ears per plant (EPP) = the total number of ears with at least one fully developed grain divided by the number of harvested plants

Stay-green characteristic was scored at 10 weeks after planting on a scale of 1-9 based on the percentage of dead leaf area below the ear as,

1 = 0-10% dead leaf area

2 = 10-20% dead leaf area

3 = 20-30% dead leaf area

4 = 30-40% dead leaf area

5 = 50-60% dead leaf area

6 = 60-70% dead leaf area

7 = 70-80% dead leaf area

8 = 80-90% dead leaf area

9 = 90-100% dead leaf area

For the low N trials, harvested ears from each plot were shelled and the grain weight per plot and the percentage grain moisture were determined. Grain yield in kg ha^{-1} was computed from the shelled grain weight, adjusted to 15% moisture. For the high N plots, a shelling percentage of 80% was assumed for all genotypes and grain yield (obtained from ear weight and converted to kg ha^{-1}) was adjusted to 15% moisture.

5.2.4 Data Analysis

Phenotypic data were analyzed using SAS 9.0 (SAS Institute Inc., NC, USA) with the GLM procedure. Each environment was defined as year x site x nitrogen treatment.

Genotype (G) was treated as fixed, and E and interaction of genotype-by-environment (GEI) as random. The procedure LSMEANS was performed to estimate phenotypic values for genotypes subsequently used for phenotypic and correlation analysis. The procedure VARCOMP was conducted to estimate genotypic variance (σ^2_G), GEI interaction variance (σ^2_{GE}) and error variance (σ^2_E). Restricted maximum likelihood (REML) estimates of the BC_2F_1 families genetic and phenotypic variances were obtained

with SAS PROC Varcomp and were used to compute broad-sense heritability for each trait.

Simple Pearson correlation coefficients were calculated between the traits, using the adjusted means of the BC_2F_1 families. SPSS 16.0 software was used to test the distribution of the traits

5.2.5 SNPs genotyping, Construction of genetic linkage map and QTL mapping

Freeze-dried leaf (two weeks old) of the 153 samples (150 BC_2F_1, parents and hybrid) were sent to LG genomics (formerly KBioscience) for SNP genotyping. Details on the principle and procedure of the DNA assay are available at http://www.kbioscience.co.uk/reagents/KASP . The parental lines were genotyped with a set of 1250 SNP markers, for which KASP assays (Semagn *et al*., 2013), were designed at LGC Genomics Facility in London, UK. In all, 158 SNP markers displayed distinct polymorphism in the parents and were used to genotype the BC_2F_1 population

The complete genotype data obtained was used to construct a genetic linkage map using JoinMap4 (Van Ooijen, 2006). Markers were assigned to linkage groups at independence LOD values > 6.0 and threshold value ranged from 2.0 to 20 with an interval of 1.0. Regression mapping algorithm was used to order the markers and Haldane's mapping function was used to transform estimates of recombination frequency to map distances in centimorgans (cM). Markers that had insufficient linkage data were excluded from the final linkage map. The linkage groups from JoinMap were rearranged into chromosomes according to their order on the reference map.

QTL mapping was done in R/qtl using a single-QTL model. Interval mapping (IM) was used to identify the QTLs. The thresholds of the QTLs (LOD scores) were obtained at p= 0.05 by 1,000 random permutations of the trait values.

5.3 Results

5.3.1 Distribution of grain yield and secondary traits in the BC_2F_1 population

In all environments, the target traits measured in the BC_2F_1 population followed approximately a normal distribution (Figs. 5.1 and 5.2).

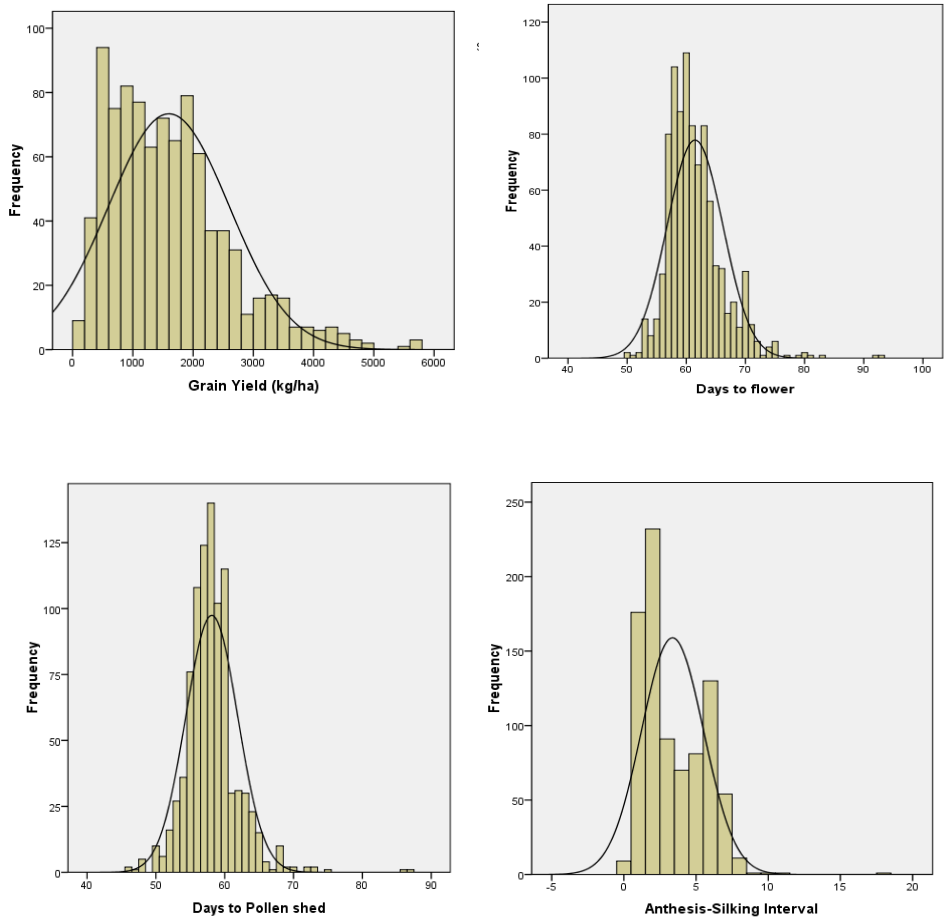


Figure 5. 1 Frequency distribution of eight traits in BC_2F_1 population under high N environment

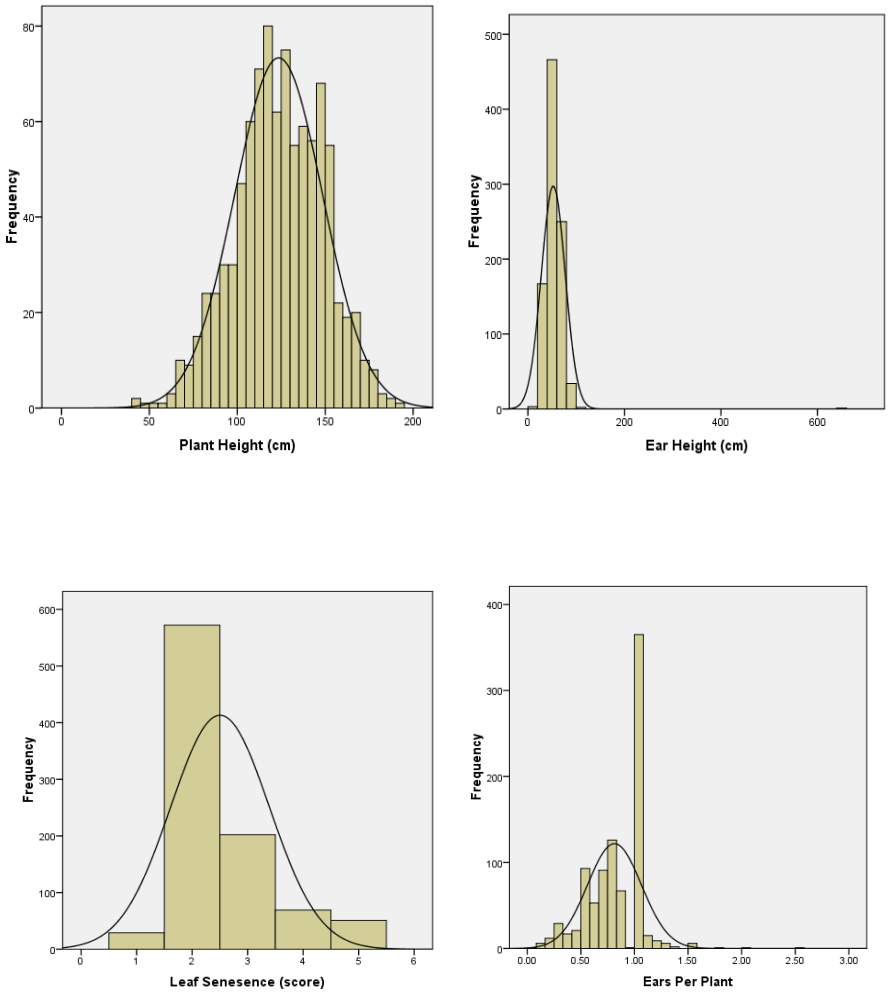


Figure 5. 1 (continued) Frequency distribution of eight traits in BC_2F_1 population under high N environment

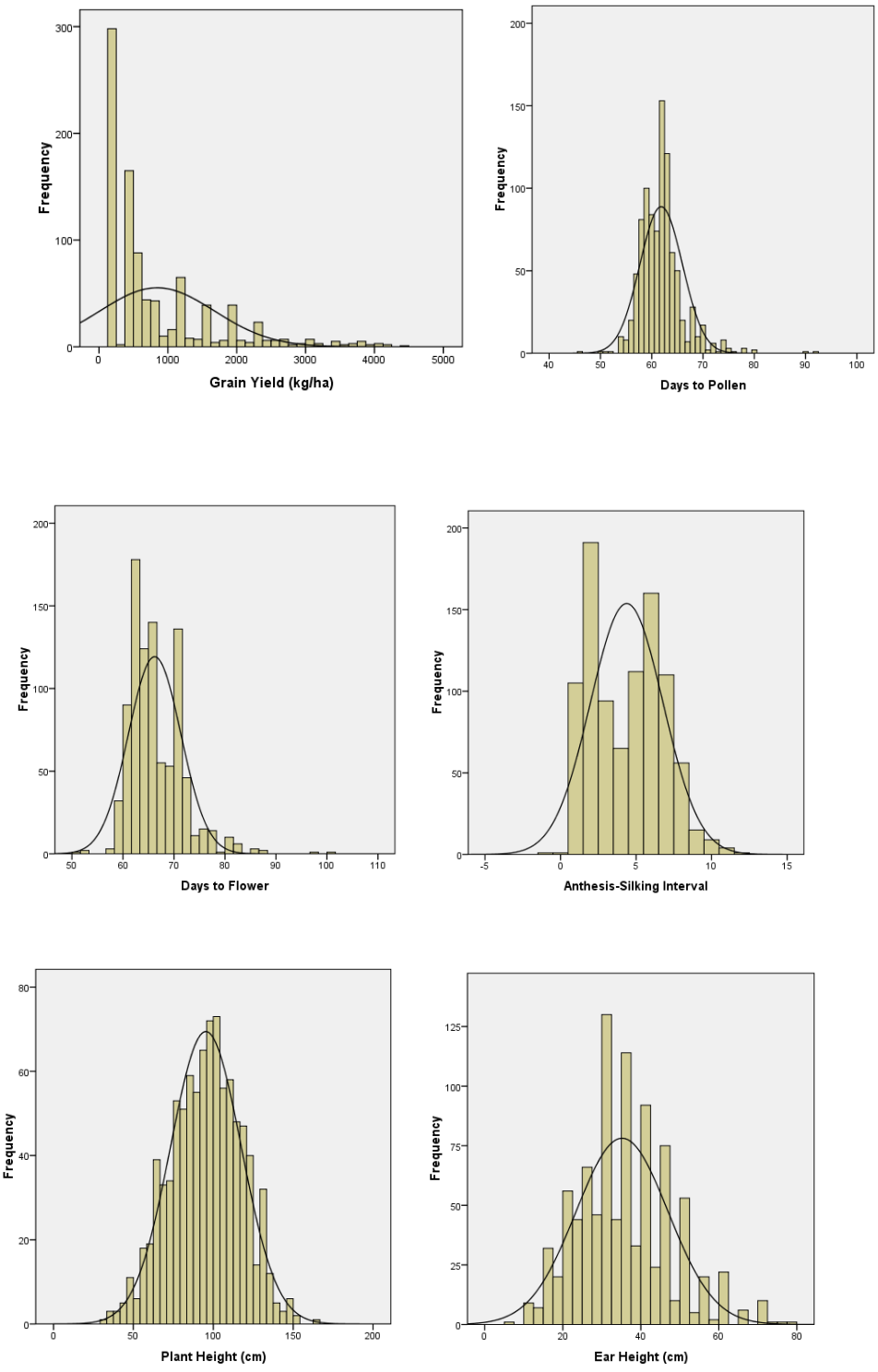


Figure 5.2 Frequency distribution of eight traits in BC_2F_1 population under low N environment

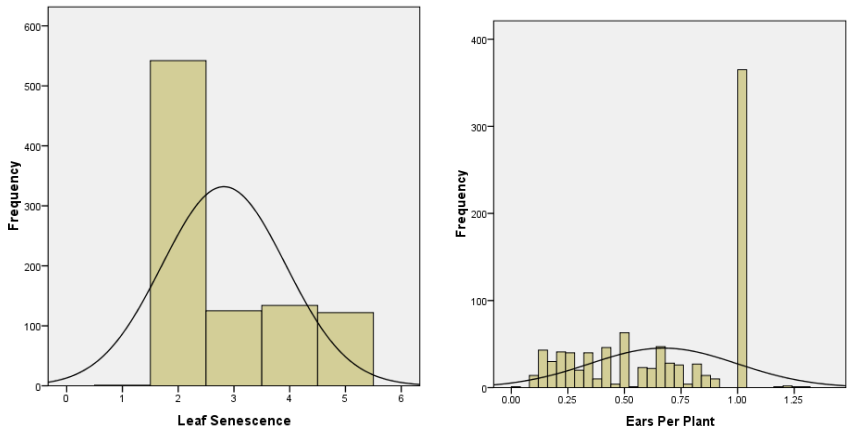


Figure 5.2 (continued) Frequency distribution of eight traits in BC_2F_1 population under low N environment

5.3.2 Evaluation of BC_2F_1 population for yield and yield related traits

There were significant GEI for grain yield, stay green characteristic and ears per plant under low N environment, and for only grain yield under high N environment (Tables 5.1 and5.2). There were significant differences among the genotypes and the environments for all traits under high N and low N environmets.

Broad sense heritability ranged from 8% for ears per plant to 48 % for days to silking under low N, and 32% for ear height to 72% for plant height under high N environment.

Table 5. 1 Mean squares of BC_2F_1 population evaluated across low N environments

Source	DF	GY	DTA	DTS	ASI	EH(cm)	PH(cm)	SG(1-9)	EPP
Envt	2	176548648.9**	55.54**	1805.59**	1314.29**	6653.92**	2933.90**	430.73**	30.93**
Blk(Rep*Envt)	78	552478.4**	30.94**	44.28**	4.50**	297.79**	1309.32**	0.67**	0.04**
Rep(Envt)	3	3745158.4**	675.63**	762.35**	13.30**	178.09ns	1968.28**	0.19**	0.25**
Entry	153	272850.2**	17.07**	22.62**	2.65ns	117.4936**	415.11**	0.26**	0.04**
Envt(Entry)	306	255538.1**	8.45ns	12.27ns	2.52ns	76.89ns	277.826ns	0.22*	0.04*
Error	381	192721.8	9.94872	12.50598	2.234743	72.8488	255.9389	0.185054	0.02898887
h^2		16	48	47	37	35	34	14	8

GY: Grain yield; **DTS**: days to silk; **DTA**: days to anthesis; **ASI**: anthesis silking interval; **PHT**: plant height; **EHT**: ear height; **EPP**: number of ears per plant; **SG**: Stay green characteristic; *, **, Significant at 0.05 and 0.01probability levels, respectively, and ns, not significant **$h^{2;}$** Broad sense heritability

Table 5. 2 Mean squares of BC_2F_1 population evaluated across high N environments

Source	DF	GY	DTS	DTA	ASI	EH(cm)	PH(cm)	EPP	SG(1-9)
Envt	2	189200830.2**	2230.58**	144.66**	1406.34**	25285.19**	59359.99**	7.64**	199.25**
Blk(Rep*Envt)	78	1024333.8**	34.79**	26.71**	2.19**	789.05**	1379.33**	0.06**	0.76**
Rep(Envt)	3	9677253.8**	321.13**	358.14**	15.47**	2943.70**	6490.33**	1.17**	3.48**
Entry	153	756050.8**	18.37**	16.66**	1.52*	558.86ns	434.72**	0.06**	0.29*
Envt(Entry)	306	448258.3*	11.18ns	8.79ns	1.39ns	499.14ns	212.62ns	0.04ns	0.22ns
Error	380	397162.8	10.22	7.75	1.22	462.62	238.98	0.04	0.23
h^2		46	62	69	52	32	72	51	53

GY: Grain yield; **DTS**: days to silk; **DTA**: days to anthesis; **ASI**: anthesis silking interval; **PHT**: plant height; **EHT**: ear height; **EPP**: number of ears per plant; **SG**: Stay green characteristic; *, **, Significant at 0.05 and 0.01probability levels, respectively, and ns, not significant **$h^{2;}$** Broad sense heritability

A total of 23 significant correlations were detected under each environment (Table 5.3 and Table 5.4). Under high N environment, eight significant correlations were negative while 16 were positive. The highest five significant positive correlations were 0.89** (DTA and DTS), 0.65** (ASI and SG), 0.62** (DTS and ASI), 0.53** (EHT and PHT) and 50% (GY and EPP). The highest three significant negative correlations were -0.48% (DTA and PHT), -0.46** (DTS and PHT), and -0.43** (DTA and EHT). The significant positive correlations under low N were 0.89** (DTA and DTS), 0 0.79** (PHT and EHT), 0.62** (EPP and GY) and 0.59** (EPP and SG). The negative correlations were -0.49** (DTA and PHT), - 0.43 (DTA and EHT), and -0.33 (DTS and EHT). Grain yield was significantly and positively correlated with DTS (0.12**), ASI (0.39**), PHT (0.15**), EHT (0.13**) and EPP (0.50**) under high N environment. Under low N environment grain yield was positively correlated with DTS (0.02**), ASI (0.28**), PHT (0.37**), EHT (0.53**) and EPP (0.62**).

Table 5. 3 Correlation among traits under high N environment

	DTA	DTS	ASI	PHT	EHT	EPP	GY	SG
DTA	-							
DTS	0.89**	-						
ASI	0.21**	0.62**	-					
PH	-0.49**	-0.59**	-0.45**	-				
EH	-0.29**	-0.34**	-0.24**	0.53**	-			
EPP	-0.04ns	0.12**	0.35**	0.03ns	0.02ns	-		
GY	-0.19**	0.12**	0.39**	0.16**	0.13**	0.51**	-	
SG	0.24**	0.49**	0.65**	-0.46ns	-0.24ns	0.36**	0.36**	-

GY: Grain yield; **DTS**: days to silk; **DTA**: days to anthesis; **ASI**: anthesis silking interval; **PHT**: plant height; **EHT**: ear height; **EPP**: number of ears per plant; **SG**: Stay green characteristic

Table 5. 4 Correlation among traits under low N environment

	DTA	DTS	ASI	PHT	EHT	EPP	GY	SG
DTA	-							
DTS	0.89**	-						
ASI	0.18**	0.61**	-					
PH	-0.49**	-0.46**	-0.15**	-				
EH	-0.43**	-0.33**	0.03	0.79**	-			
EPP	-0.00ns	0.05	0.13**	0.18**	0.30**	-		
GY	-0.14**	0.02**	0.28**	0.37**	0.53**	0.62**	-	
SG	0.19**	0.40**	0.51**	-0.09ns	0.13**	0.59**	0.53**	-

GY: Grain yield; **DTS**: days to silk; **DTA**: days to anthesis; **ASI**: anthesis silking interval; **PHT**: plant height; **EHT**: ear height; **EPP**: number of ears per plant; **SG**: Stay green characteristic

5.3.3 Genetic linkage map construction

Linkage analysis was performed on 150 BC_2F_1 families genotyped with 158 SNP markers. All the ten maize chromosomes were represented in the linkage map constructed with ten linkage groups (corresponding to the ten chromosomes), spanning a total length of 622.7cM at an average marker interval of 3.9 cM (Table 5.5). Linkage group seven (LG 7) was the smallest (11.3 cM) and had four markers with an average marker density of 2.8 cM. The next smallest linkage group was linkage group three (LG 3) which comprised 14 markers and spanned 17.4 cM with marker density of 1.2 cM. Linkage group nine (LG 9) spanned 19.7 cM with marker density of 2.0 cM. Linkage groups eight and ten (LG 8 and LG 10) had lengths spanning 88.3 cM and 84.2 cM, and marker density of 5.5 cM and 7.0 cM, respectively. Linkage groups four, five and six (LG 4, LG 5 and LG6) were fairly similar in length, spanning 41.5 cM, 58.7 cM and 32.7 cM

respectively. Their marker densities were 3.4cM, 2.9 cM, and 1.6 cM, respectively. Linkage group one (LG 1) spanned 150.4 cM and was the longest with a marker density of 4.3 cM. The second longest was linkage group 2 spanning 118.5 cM with a marker density of 7.9.

Table 5. 5 Genetic map from 158 SNP markers for 150 BC_2F_1 population for CML 444 x CML 494

Linkage group	Length(cM)	Number of mapped markers	Average marker density(Cm)
LG 1 (Chr 1)	150.4	35	4.3
LG 2 (Chr 2)	118.5	15	7.9
LG 3 (Chr 3)	17.4	14	1.2
LG 4 (Chr 4)	41.5	12	3.4
LG 5 (Chr 5)	58.7	20	2.9
LG 6 (Chr 6)	32.7	20	1.6
LG 7 (Chr 7)	11.3	4	2.8
LG 8 (Chr 8)	88.3	16	5.5
LG 9 (Chr 9)	19.7	10	2.0
LG 10 (Chr 10)	84.2	12	7.0
Total/Aveargе	622.7	158	3.9

5.3.4 QTL identification

The QTLs identified are shown in Table 5.6.and Fig. 5.3, respectively. A total of 13 QTL were detected under both low and high N environments with phenotypic variance explained (PVE) ranging from 5% (ASI) to 31 % (DTS). Four QTLs were identified under high N environment and nine QTLs under low N environment.

One QTL was detected for GY under high N environment (*qgy-10-1)* on chromosome 10 flanked by PZA01292_1 and PZB0049_1 at interval of 29.0 cM with LOD of 3.15, and explained 10% of the phenotypic variation. Under low N environment, two QTLs for GY were detected on chromosomes 1 (*qgy-1*) and 10 (*qgy-10-2*). A major QTL, *qgy-1* explained 21% of the phenotypic variation was located between SNP markers PZA02487_1 and PZB02058_1 on chromosome 1 and had marker interval of 0.7. The QTL *qgy-10-2* explained 8% of the phenotypic variation and had a LOD score of 4.12. It was flanked by the same markers that flanked *qgy-10-1* (PZA01292_1 and PZB0049_1), but on a different position (10.3). For *qgy-10-1* the position was (18.2). QTL qgy-10-1 had a marker interval of 29.0cM whiles qgy-10-2 had a marker interval of 0.7CcM.

Three QTL for DTS were identified. One was found under low N environment, it was located on chromosome 1 (*qdts*) between PHM13191_6 and PZB02058_1 with LOD of 3.1 and marker interval of 0.7cM and explained 10.3% of the phenotypic variation. The other two QTLs found under high N were *qdts-5 and qdts-10* and they were located on chromosome 5 and 10. QTL *qdts-5* flanked by markers PZA00980_1 and PZ202792_25, at a marker interval of 9.2, had a LOD score of 2.8 and explained 8% of phenotypic variation. QTL *qdts-10* was flanked by the same markers that flanked QTLs *qgy-10-2* and *qdts-10-1* (PZA01292_1-and PZB0049_1). It explained 31% of the phenotypic variation and had a LOD score of 3.62.

The markers that flanked QTLs (*qgy-10-1,qgy-10-2 and qdts-10*) were the same markers (PZA01292_1 and PZB0049_1) that flanked the QTL (*qasi-10*) detected for ASI on chromosome 10 under low N environment. This QTL explained 5% of phenotypic variation and had a LOD score of 2.8 at marker interval of 29cM. Under high N

environment, the QTL (*qasi-6*) detected for ASI was found on chromosome 6 with marker interval of 4.3cM. It had a LOD score of 4.1 and explained 12% of the phenotypic variation.

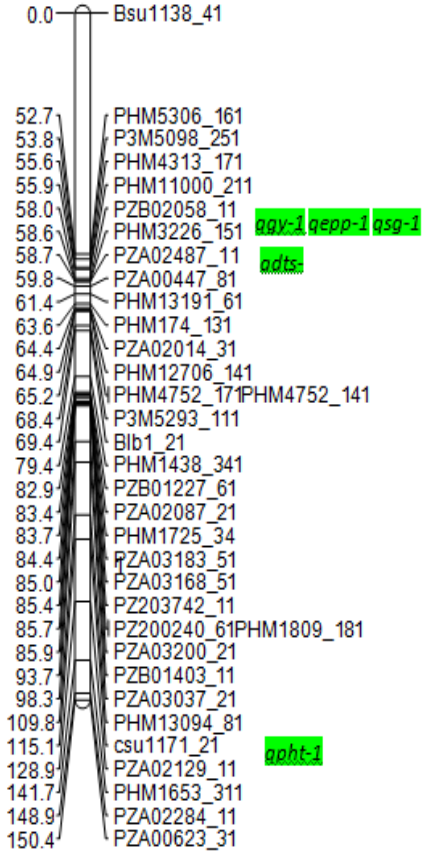
For SG, three QTLs (*qsg-8*) under high N, (*qsg-1 and qsg-4*) under low N were found on chromosome 8, 1 and 4, respectively. QTLs *qsg-8, qsg-1 and qsg-4* explained 12%, 9% and 18% of the phenotypic variation and were flanked by markers PZA02748_3 and PZA01079_1 at 17.8cM, PZA24787_1 and PHM11000_21 at 2.8cM, and PHM3587_6 and PHM3963_33 at 5.2cM, respectively. One QTL each for EPP (*qepp-1*) and PHT (*qpht*) were found on chromosome 1. QTL *qepp-1* was found between the markers PHM174_13 and PHM1100_21 at 7.7cM, the same marker that flanked *qsg-1*. *qepp-1* explained 7% of phenotypic variation, and had a LOD score of 2.7. QTL *qpht-1* also explained 9.6% of phenotypic variation, had a LOD score of 3.2 and was flanked by kPHM16533_31 and PHM13094_8.

Table 5. 6 QTLs identified based on BC_2F_1 population from CML 444 x CML 494 across two nitrogen (N) environments

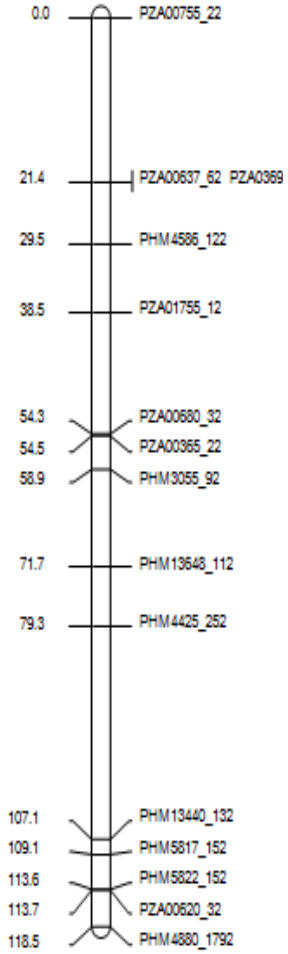
Trait	N level	*QTL*	Chromosome	Markers	Marker Interval	[a]Position	[b]Add	[c]LOD	[d]R^2
GY	HN	*qgy-10-1*	10	PZA01292_1 - PZB0049_1	29	18.2	310.13	3.15	10
	LN	*qgy-1*	1	PZA02487_1 - PZB02058_1	0.7	58.5	-10.4	3.6	21
		qgy-10-2	10	PZA01292_1 - PZB0049_1	29	10.3	-52.8	4.12	8
DTS	LN	*qdts-1*	1	PHM13191_6 - PZB02058_1	0.7	59.3	2.34	3.1	10.3
	HN	*qdts-5*	5	PZA00980_1 - PZ202792_25	9.2	51.2	-1.53	2.8	8
		qdts-10	10	PZA01292_1-PZB0049_1	29	1.3	-2.24	3.62	31
SG	HN	*qsg-8*	8	PZA02748_3-PZA01079_1	17.8	25.3	3.27	3.3	12
	LN	*qsg-1*	1	PZA24787_1-PHM11000_21	2.8	58.5	1.55	3.8	9
		qsg-4	4	PHM3587_6-PHM3963_33	5.2	3.8	0.56	4.13	18
ASI	LN	*qasi-6*	6	PZB00414_2-PHM15251_3	4.3	15.2	0.26	4.1	12
		qasi-10	10	PZA01292_1-PZB0049_1	29	5.8	1.2	2.8	5
EPP	LN	*qepp-1*	1	PHM174_13-PHM1100_21	7.7	58.5	0.2	2.7	7
PHT	LN	*qpht-1*	1	PHM16533_31-PHM13094_8	31.9	128.9	9.06	3.2	9.6

GY: Grain yield; **DTS**: days to silk; **DTA**: days to anthesis; **ASI**: anthesis silking interval; **PHT**: plant height; EHT: ear height; **EPP**: number of ears per plant; **SG**: Stay green characteristic**; HN** = high N**; LN** = low N · [a]Position of peak marker in centMorgans· [b]**Add**=Additive effect; - and + alleles indicate that alleles that increased trait values came from CML494 (-) and CML444 (+), respectively [c]**LOD** = log10 of odds ratio [d]**R^2** Percentage of phenotypic variation explained by QTL.

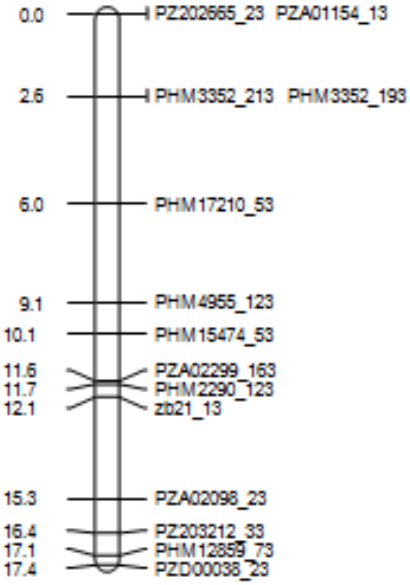
Chromosome 1

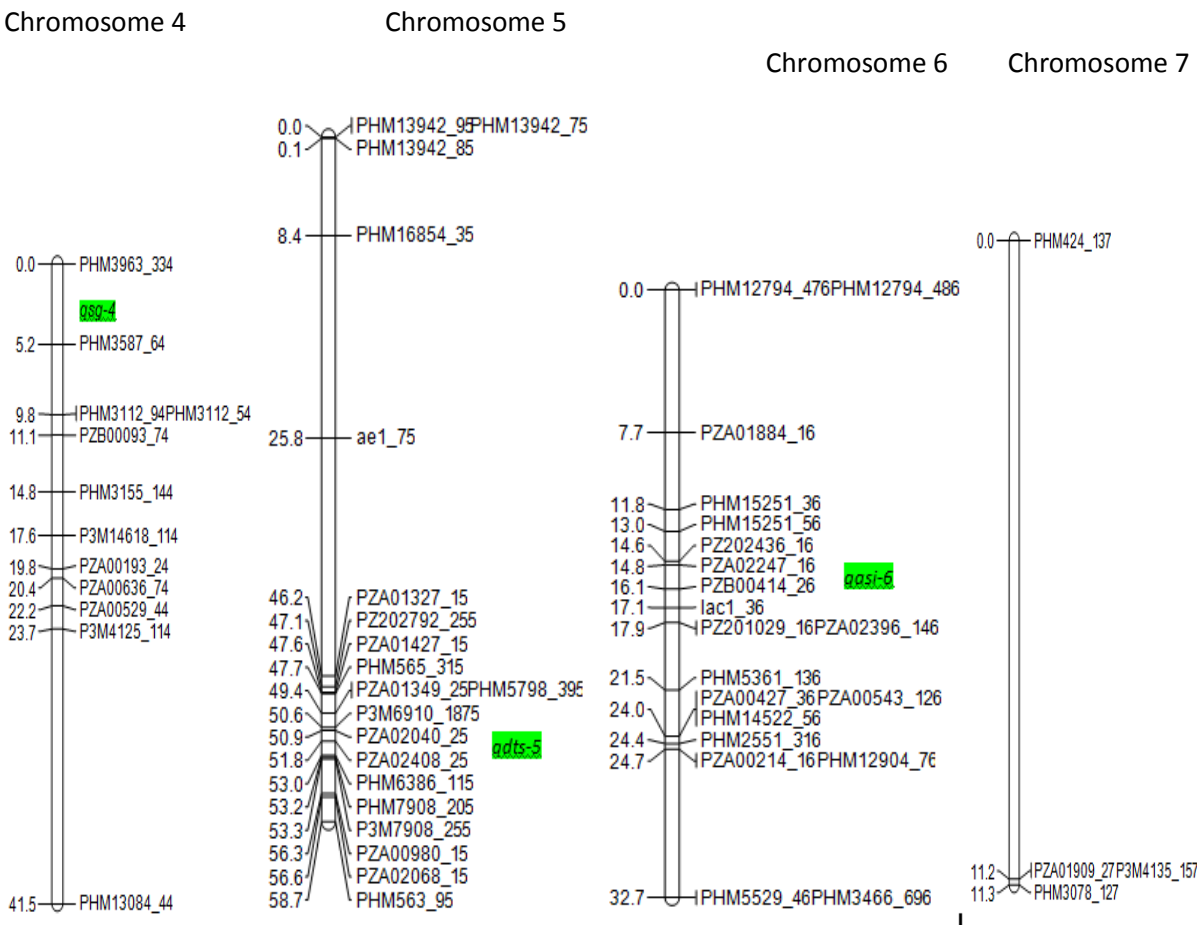


Chromosome 2



Chromosome 3





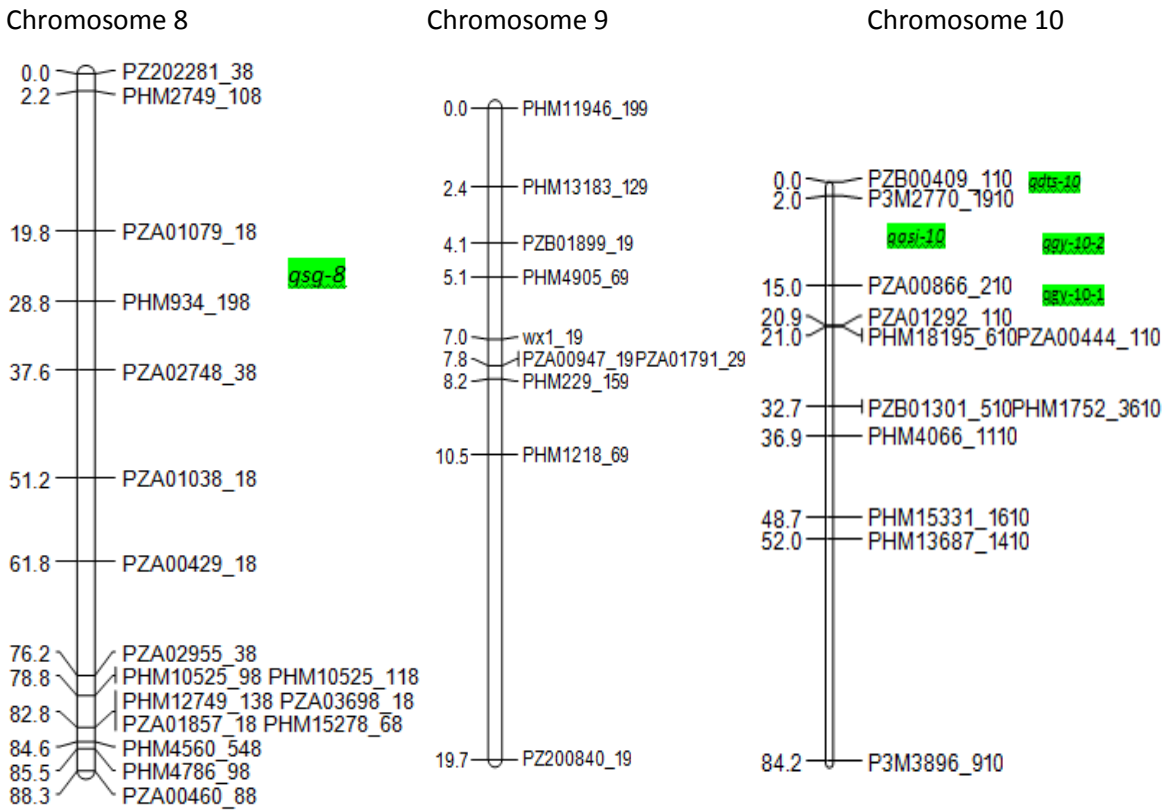


Figure 5. 3 Linkage map showing QTL on chromosomes 1, 4,5,6,8 and 10 for six traits (GY, ASI, DTS, SG, EPP and PHT)

5.4 Discussion

Low N is one of the major constraints militating against the achievement of the full yield potential of maize in sub-Saharan Africa. In depth understanding of this complex trait will be beneficial for the development of stress resilient cultivars. Precise and consistent phenotyping of such complex traits is very difficult due to highly fluctuating environmental and soil conditions. Selection and release of new varieties based on unreliable and inconsistent phenotypic data often leads to failure in adoption by the farmers. Under such circumstances, integration of genomics tools with conventional genetic improvement would facilitate the development of improved cultivars with high yield under low N conditions. The target traits measured in the BC_2F_1 population in the present study followed approximately a normal distribution suggesting a suitable phenotypic segregation for QTL mapping. Results showed significant environmental variation for grain yield and other measured traits indicating differences in the test environments. This observation is in agreement with the findings of Badu-Apraku *et al.* (2007a). Several researchers have previously reported variations in response of maize to environmental stress (Betran *et al*., 2003d, e; Mosisa *et al*., 2007; Derera *et al*., 2008). The few traits that had significant GEI indicate that most individual families responded similarly in all environments. Similar results were reported by Makumbi (2011), who found significant GEI in only two traits under low N. Although the heritability estimates were lower for GY under both environments, some secondary traits had substantially higher heritability estimates indicating their potential to aid in indirect selection for increased grain yield under these environments. This result is consistent with the findings

of Ifie (2013) and Mafouasson (2014). Besides heritability, strong correlation of the secondary traits with GY is an important attribute that would enable their routine integration in breeding programmes (Banzinger *et al*., 2000). In the present study, significant phenotypic correlations were observed between GY and all the measured traits. This finding is in agreement with the results of other researchers (Mafouasson, 2014; Bolanos and Edmeads, 1996; Ribaut *et al*., 1997; Zheng *et al*., 2009; Lu *et al*., 2011; Ifie, 2013).

A linkage map that spanned 622.7 cM in length and covers all 10 chromosomes of maize was constructed using 158 SNP markers out of the 1250 SNP markers used in genotyping the parents. The few polymorphic markers used resulted in less coverage of the map and may have led to large intervals between markers of detected QTL. The relatively large gap suggested that some QTLs may have remained undetected due to the shortage of available markers in the corresponding regions (Li *et al*., 2007). However, it has been reported that with markers spaced about 10 cM to 15 cM apart, it is possible to identify few markers associated with the trait of interest if phenotypic data and QTL analysis were well done (Bernardo, 2008).

The mapping of SNPs on the linkage groups was not different from what were mapped by Zaidi *et al*. (2015) and Almeida *et al*. (2014) using similar markers. However, the orientation, size and distances differed. This could be attributed to the type and size of the population and number of markers used. The length of the linkage map was smaller than that reported by other authors (Zaidi *et al*., 2015; Almeida *et al*., 2014). It was, however, bigger than that reported by Simic *et al.* (2009), who found a linkage map length of 484.6

cM using SSR markers. The differences in the sizes of the linkage maps may probably be attributed to the type of marker and the number of markers used.

QTL analysis resulted in identification of a total of 13 QTLs for six different traits under low N environments (9 QTLs) and high N environments (4 QTLs). Some QTLs detected for different traits were overlapping in some specific genomic regions. Chromosome 10 harbored overlapping QTL for GY, DTS and ASI. They were all flanked by the SNP marker PZA01292_1 and PZB0049_1 but, had different peak marker positions. These QTLs may have had pleiotropic effects thus explaining the correlation observed among the traits. Similar overlapping genomic regions for GY and ASI on chromosomes 10 were reported by Ribaut *et al*. (1997) and Malosetti *et al*. (2008). This explains the reason for the strong correlation of ASI with GY across a broad range of germplasm suggesting the possibility of a cluster of tightly linked loci orchestrating low N tolerance through coordinated expression of these traits. Higher heritability was recorded for ASI and DTS than for GY for both low N and high N environments. This suggests that understanding the genetic basis of ASI and DTS will aid in designing efficient marker-based breeding strategies for enhanced GY under low N environments. Some earlier studies have reported QTL for yield and secondary traits on chromosome 10 under optimal and water stress conditions (Li *et al*., 2010; Zheng *et al*., 2009)

QTLs for GY, SG and EPP were mapped to the same location on chromosome 1 at a marker peak of 58.0. This could probably be due to the physiological relationship and strong correlation between them. Close linkage between GYP and EPP has been reported by numerous researchers using classical analysis (Agrama., 1996; Ifie, 2013;

Mafouasson, 2014). The mapping of the traits in the same region could indicate that this region might be a hotspot for yield traits and transferring this region will lead to varieties with multiple traits. Agrama *et al.* (1999) and Ribaut *et al.* (2007) detected QTLs for GY under low N on chromosomes 1. QTL detected for EPP on chromosome 1 under low N was also reported by Ribaut *et al*. (1997) under drought stress. The identification of common genomic regions for the regulation of some traits under drought and low N conditions has important implications for breeding maize. Many drought areas are located in developing countries, where, for economic reasons, N supply is insufficient. Based on phenotypic data, Banziger *et al*. (2002) and Badu- Apraku *et al* (2013b) suggested that selection for tolerance to mid-season drought stress led to morphological and physiological changes that increased yield under N deficiency.

The quest for stress resistance, high yield and good quality is unending for crop breeders, so the desirable crop production characteristics of functional stay-green genotypes make them very attractive. Beavis *et al*. (1994), using restriction fragment length polymorphisms (RFLP) markers, identified three and five stay-green QTLs in an F_4 and a top- cross maize population generated from B73_Mo17. Zheng *et al*. (2009), using SSR markers detected 14 QTLs in an F_2 population. In the present study, 3 QTLs were identified for SG; one, under high N on chromosome 8 and two under low N on chromosomes 1 and 4. The few QTLs detected in this study may probably be due to the differences in the parental lines used, the segregation population, genetic map or agro-ecological conditions. Wang *et al* (2012) also identified QTLs for SG on chromosomes 1 and 4 indicating that chromosomes 1 and 4 were important in controlling SG.

A QTL for plant height (*qpht*) was detected on chromosome 1 with a phenotypic variation of 9.6%. Plant height was also shown to be correlated with yield, hence, it is an important trait for selection. Other researchers have mapped this QTL at other locations. For example, Ribaut *et al.* (2007) mapped five QTLs for PHT on chromosomes 3, 4, 6, 9, and 10. The QTL identified in this study was different from that found by Ribaut *et al*.,(2007), suggesting that it belongs to a new chromosome associated with PHT in maize and that plant height in maize is controlled by polygenes. Due to additive effects, 9 QTLs *(qgy-10-1, qdts-1, qsg-8, qsg-1, qsg-4, qasi-6, qasi-10, qepp-1, qpht-1*) could increase phenotypic values of traits, while the other 4 (qgy-1, qgy-10-2, qdts-5, qdts-10) could decrease them to some extent.

5.5 Conclusions

A total of 13 QTLs were identified on a linkage map spanning a total length of 622.7 cM, with marker density of 3.9 cM. The localization of grain yield QTLs with some of the yield related traits is an excellent indication of strong association. Identification of QTLs of secondary traits that improve crop growth and performance especially under low N environments will certainly assist breeders in rapid introgression of these genomic regions into desired elite germplasm. Five QTLs (*qgy-1, qts-1, qsg-1, qsg-4 and qasi-6*) for GY, DTS, SG and ASI respectively, were close to their adjacent markers, with an interval of 0.7 to 5.2cM between them and this explains phenotypic variance from 9% to 21%, suggesting that the markers are linked with the gene controlling the trait, and could be considered for marker assisted selection (MAS). Other QTLs identified were far from their linked markers, greater than or equal to 10 cM, and thus, it is necessary to find more

molecular markers for these given chromosomal regions. The low density of markers used for the mapping resulted in wide QTL intervals, possibly encompassing many genes, therefore, increasing marker density using recently available low-cost, high-throughput genotyping methods could resolve in the QTL intervals closer to a single-gene QTL and possible reduce interaction effect with nearby genes that are inadvertently selected. Also, fine mapping of the QTLs with high p-values should be done to increase the possibility of using the significant marker for marker-assisted breeding. Finally, validation of these QTL in another mapping population is necessary before their use in MAS.

CHAPTER SIX

6.0 Conclusions and recommendations

Maize is one of the most important staple food crops in SSA and contributes significantly to consumer diets in Ghana. Unfortunately, however, its yield has remained below 1.8 t ha^{-1} because production is faced with major challenges. Drought and low soil fertility are the most important threats to sustainable crop production and thus the most important obstacles to achieving food security in SSA countries including Ghana. Low soil fertility (characterized by low phosphorus and nitrogen availabilities) is believed to become, in the long run, a more serious concern than the lack of moisture in West Africa (Bationo and Mokwunye, 1991, Gruhn *et al*., 2000) because of soil deficiency coupled with continuous cropping of the land over decades with no restoration. Therefore, germplasm improvement for low N tolerance remains a high priority in the country. Low N tolerance is required for farmers to achieve high and stable maize yields, especially for farmers who cannot afford to buy fertilizer and are located in poor soil areas. This study was thus conducted to (1) assess maize production constraints of Ghanaian maize farmers, and their perceptions and knowledge on soil fertility, (2) determine heterotic patterns and combining ability of grain yield for intermediate maturing maize inbred lines under low and high N environments, (3) determine the mode of gene action conditioning grain yield under low N, (4) evaluate the testcrosses (single cross hybrids) for high yield, stability and tolerance to low and high N and (5) identify and map Quantitative Trait Loci (QTL) for grain yield (GY) and secondary traits under low and high N environments.

In order to incorporate the preferences of farmers into the breeding programmes a survey was conducted among 120 maize farmers in six major maize growing communities of Ghana including Akrobi, Aframso, Teacherkro, Awisa, Amponsakro and Adiembra.

Low soil fertility was identified as a major challenge to farmers. Other major challenges faced by farmers include drought, pests and diseases. Fertilizer application is the main method used by farmers to manage low soil fertility. Fertilizer is expensive and it is not available sometimes. This means low levels of application which invariably result in low yield. Other alternatives such as application of manure and land rotation have been scarcely utilized, probably because these techniques have not been sufficiently disseminated. Most of the farmers had not heard about hybrid seeds, or preferred their local or improved seeds to hybrid seeds. It therefore was not surprising that the released hybrids in Ghana were not widely adopted and grown by these farmers. Maize farmers in the districts indicated preference for low N tolerant, drought tolerant maize varieties with good storability, disease and insect resistant and little need for inputs. In addition, they opted for varieties with slender cobs, light in weight with lots of grains.

The presence of genetic variability is of prime importance for good progress in improving a trait in a selection program. There were significant differences among genotypes for most traits under low N and high N and this meant that adequate genetic variation existed among the hybrids and inbreds which could allow good progress from selection under contrasting environments. It also indicated good probability for the improvement of these genotypes under stress and optimal conditions across a range of environments. Significant environmental variation for grain yield and all other traits for both inbreds and hybrids under low and high N environments indicate that each environment was

unique and different. The performance of hybrids for most traits was not consistent across environments since genotype x environment effects were significant for most traits of the hybrids. GEI effects, however, were significant for only a few traits of the inbreds suggesting that the inbreds responded similarly for most of the traits studied at the different locations. Moderate heritability estimates for grain yield, days to silking and anthesis, anthesis-silking interval, plant height, ear height, stay-green characteristic, and chlorophyll content suggested that early generation testing for these traits to improve low N tolerance would be effective.

For farmers in low soil N environments four hybrids CLWN 247 x 9071 , ZM523B-29-2-1-1-B*6 x 9071, TZD II 68 x 1368 and P43SCRq Fs100-1-1-8 x 9071 were identified as highest yielding and recommended for further testing for release. Farmers cultivate maize under varying soil fertility levels, therefore, high yield under low N and high N conditions is desirable. Seven hybrids (CZL 00001 x 9071, LapostaseqC7-F18-3-2-1 x 9071, CLWN 364 x 9071, CLWN 247 x 9071, CLWN 247 x 87036, TZD II 68 x 1368, and CML 395/CML 444 x 9071) had inherent ability for outstanding performance under both low N and high N conditions and are therefore candidates for further testing for commercialization.

Since the performance of inbreds is not a good predictor of their performance in hybrid combinations, the combining ability of the inbred lines for tolerance to low N and the stability of the hybrids were investigated under low N, and high N environments. Information on the combining abilities of inbred lines is important in identifying productive hybrids for commercial hybrid production. In the present study, significant GCA and SCA effects for grain yield and most measured traits were found under low N,

high N and across test environments with a predominance of GCA effects over SCA effects**.** This suggests that grain yield and the other traits are controlled predominantly by additive gene action than non additive gene action. The breeding implication of the significant GCA and SCA effects for grain yield and other traits is that appreciable breeding progress could be made using hybridization, backcrossing, and recurrent selection for the development of hybrids and synthetic varieties as well as in population improvement.

Inbred lines with favorable GCA effects for grain yield and other traits are likely to transmit their characteristics to the progeny and could be useful in a breeding program. Such inbreds could be used as parents to form a synthetic population that could be improved for stress environments and ultimately be crossed onto an inbred that is of a different heterotic type to get hybrid vigor that drives maximum yield The significant positive GCA effects observed for grain yield of inbreds; CLWN 247, TZD II 68 and ZM523B-29-2-1-1-B*6 suggests that these lines could be useful for contributing favourable alleles for breeding for improved grain yield under low environments, also CLWN 247 could be combined with 9071 and TZD 11 68 with 1368. The consistency in performance for CLWN 247 in exhibiting good GCA effects for grain yield under low N, high N and across environments makes it a good general combiner for grain yield across all environments and also a good specific combiner with 9071.

For a commercially successful hybrid program, there is a need for information on the heterotic patterns of the parental lines used in the program. Classification of inbreds into heterotic groups is of utmost importance for determining the potential usefulness of parental lines for the development of high yielding hybrids and synthetics. In the present

study heterotic classification was based on three different methods; (SCA, HGSCA, and HGCAMT). Most of the inbred lines were classified into the same heterotic gropus based on the different methods, indicating that there was close correspondence among the methods used. The inbreds in each heterotic group may be recombined to form source populations which could be improved through recurrent selection with subsequent crosses onto the appropriate testers to identify new hybrids

CML 395/ CML 444 x 9071 and TZD II 68 x 1368 were identified as the high yielding and stable hybrids based on the stability analysis and should be extensively tested in multi-locations and promoted for adoption

The complexity of measuring some of the secondary traits correlated to yield quickly and accurately under low N, has made its use limited in breeding programs. The identification of QTLs tolerant to low N will therefore be beneficial if they are used in MAS. A linkage map spanning total length of 622.7 cM was generated using 158 SNP markers. The average marker density was 3.9 cM. Thirteen QTLs were identified for six different traits under low N environments (9 QTLs) and high N environments (4 QTLs). Five QTLs (*qgy-1, qts-1, qsg-1, qsg-4 and qasi-6*) for GY, DTS, SG and ASI respectively, were close to their adjacent markers, with an interval of 0.7 to 5.2cM between them and this explains phenotypic variance from 9% to 21%, suggesting that the markers are linked with the gene controlling the trait, and could be considered for marker assisted selection (MAS).

6.1 Recommendations

It is obvious from this study that low soil N is an important challenge that needs to be addressed. A solution to this problem would offer more opportunities for farmers to increase productivity. It is, therefore, recommended that;

- Farmers should be constantly involved in the selection of varieties to take care of their preferences that are specific to their needs
- The adoption of hybrid varieties should be encouraged
- Promising hybrids identified in this study should be extensively tested and promoted for adoption.
- Further selection should be done under low and high N environments to increase the number of high-yielding tolerant hybrids in Ghana
- The maize national programme should utilize lines identified as having good GCA as parental lines in the hybrid breeding programme.
- The heterotic groups should be used for line improvement and development of high yielding hybrids.
- Further validation of identified QTLs and use of the QTLs in maize improvement via marker assited breeding will accelerate the breeding cycle.

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APPENDICES

Appendix 3.1 Questionnaire on farmers' knowledge of low soil fertility and their perception and adoption of maize hybrids

Region ……
District…….……..……

Village……

Latitude.....South…… Longitude……East
..…….……..

Interviewed by……Date…….…….……

A. Background information

1. Sex of respondent 1. Male 2. Female
2. Age of respondent
1. 16-35 Years 2. 35-65 Years 3. Above 65 Years
3. Are you a member of any farmer group:

1. Yes (Specify) ……2. No (Why not)…….

4. Educational level :

1. None 2. Primary School 3. Secondary School 4. Above Secondary School

5. Household composition

Options	Age group	Total number
5.1	Under 5 years	
5.2	5-15 years	
5.3	15-65 years	
5.4	65 years and above	
5.5	Total	

B. Crop enterprise (What types of crops do you cultivate? List and rank them)**.**

No	Crops	Rank (in order of importance) 1 – 6
6.1		
6.2		
6.3		
6.4		
6.5		

C. **Farm size and cropping system for maize**

7. what is the size of your farm

1. Less than 2ha 2. 2 - 5ha, 3. More than 5ha 4. Other, please specify……..

8. What is the type of cropping system you use?

1. Mono cropping 2. Mixed cropping 3. Others (please specify)

9. Farming capital

1. Own saving 2. Credit (loan) 3. Turn over 4. Others (please specify)

10. Adequacy of land for farming by the household (tick only 1)

1. Yes 2. Fair (if rented) 3. No. 4. Other (please specify)

11. Method of land preparation is done by: (tick only 1, if more then rank them)

1. Tractor 2. Hand tillage using a hoe 3. No tillage 4. Other (specify)…….

12. Planting on time (tick only 1)

1. Always on time 2. Sometimes on time 3. Always late

13. If late, reasons for late planting (tick only 1, if more then rank them)

1. Seed not available 2. Lacks of labor 3. Lack of fertilizer 4.Other (specify) …….

14. Seed availability of maize in your area (tick only 1)

1. Good 2.Fair 3. Poor

15. Participation in a field day in the last 3 years (tick only one)

1. Yes 2. No

E. Crop calendars and labor demand

Activity	Period	Labor demand (1=low,

		2=moderate, 3=high)
16.1 land preparation		
16.2 Planting		
16.3 Harvesting		
16.4 Storage and marketing		

17. Maize varieties currently grown and why?

List and rank them on their qualities (eg. yield, early/late maturing, disease resistance, tolerance to drought, tolerance to low soil fertility etc.)

Variety	**Rank**	**Reason (Qualities)**	**Yield**

18. What are the major constraints to maize production?

1. Diseases 2. Pest 3. Low soil fertility 4. Drought 5. Others……

F. Soil Fertility

19. Is your soil fertile?
1. Yes 2. No 3. Don't know

20. If yes, what informs your decision?
1. Color of soil 2. Texture of soil 3. High yield

21. If no, what informs your decision?
1. Color of soil 2. Texture of soil 3. Reduced yield 4. Weed infestation
5. Symptoms on the crop 6. Others (Please specify)
……

22. What, in your opinion, causes low soil fertility
1. Continuous cropping 2. Excessive rainfall 3. Lack of crop rotation
4. Non application of fertilizer 5. Other (please specify)……

23. What are the effects of low soil fertility on your crop? (CHECK OUT ON LOW N SYMPTOMS)

…….. ……..
……..

24. What do you do to improve the fertility of your soil? (check all that apply)

1. Fertilizer 2. Manuring 3. Crop rotation 4. Land rotation 5. Fallow 6. Others, specify……..

25. If the answer to '23' is crop rotation, what crop do you use?

……

26. What are the challenges you face on the type of soil fertility improvement method used? tick

	Expensive	Non availability	Other, specify
Fertilization			
Manuring			
Crop rotation			
Land rotation			
Fallow			
Other, specify			

27. Do you apply fertilizers in cropping system?

1. Yes 2. No

28. From whom did you get the information about fertilizer?

1. MOFA 2. Research institute 3. Other project (NGO, donor) 4. Farmers' organization
5. Other farmer, 6. Input supplier/trader, 7. Trader/buyer, 8.Certified seed producer,
9. Other (specify)…….

29. Do you know about the different types of fertilizer?

1. Yes 2.No

29. What kind of fertilizer do you use? (check all that apply)

1. NPK 2. Ammonia 3. Urea 4. Manure 5. Other (Please specify)…….

30. How often do you apply fertilizer in one cropping system?

1. Once 2. Twice 3. Thrice

31. How many bags of fertilizer were you buying five years ago?

1. NPK……. 2. Ammonia…….3. Urea……4. Manure……5. Other…..

32. How many bags of fertilizer do you buy now?

2. 1. NPK……. 2. Ammonia……..3. Urea……4. Manure…….5. Other…..

33. What is your rate of fertilizer application?

3. NPK……. 2. Ammonia…….3. Urea……4. Manure……5. Other…..

34. What is the price of fertilizer you buy from the market?

	Subsidized	Unsubsidized
NPK		
Ammonia		
Urea		
Other, specify		

35. Where do you buy/ get your fertilizer?

36. Distance to source of fertilizer, (if quoted in miles state in other)

1. Within 5km 2. 5-20km 3. 20-50km 4. More than 50km 5. Other….

37. Do you know about Nitrogen fertilizer? (prompt by asking if farmer uses ammonia or urea)

1. Yes 2.No 3. Don't know

38. Why do you use ammonia /urea……..

39. How did you acquire information about ammonia/urea

1. MOFA 2. Research 3. NGO 4. Farmer 5. Input dealer 6. other

40. What are the major challenges of using fertilizer?

1. Expensive 2. Non Available 3. Health Implication 4. Others (Please specify)…….

41. Can you identify symptoms of low fertilizer application (note yellowing of leaves to be low N)

1. Yes 2.No 3. Don't know

42. What are the symptoms?

1. Yellow Leave 2. Stunted growth 3. Brown Leaves 4. Others (Please specify)…….

43. Can low soil fertility be solved? 1. Yes 2. No 3. Don't know

44. If yes, how do you think it can be solved?

.....

45. If no, why can't it be solved?

……..

46. Would you prefer to grow your crops with small fertilizer application?

1. Yes 2. No 3. Don't know

G. Technology (Seed) Specific Attributes

47. Do you know about hybrid seeds?

1. Yes 2. No 3. Don't know

48. Have you ever grown hybrid seeds before?

1. Yes 2. No 3. Don't know

49. If yes, compare your local and hybrid maize seed in terms of yield/acre

Local …….. Improved ……

50. Rank 2 most critical problems encountered if you use local seed maize

No.	**Item**	**1=biggest problem, 5= least problem**
1	Distance to local seed source	
2	No local seed source	
3	Failure to buy seed	
4	Low yields	
5	Fertilizer not available	
6	Fail to buy fertilizer (expensive)	
7	Disease problem	
8	Lack cash or credit	
9	Post harvest losses	
10	Lack labor	
11	Lack grain market	
12	Other (specify	
13	None	

51. Choose the most critical problem encountered in provision of improved maize seed (hybrid) in your area.

No.	**Item**	**1=biggest problem, 5=least problem**

1	Improved seed arrive late	
2	Few farmers buy improved seed	
3	Farmers prefer to plant local seeds	
4	Long distance to sources of seed	
5	High seed price	
6	Lack fertilizer	
7	None	

52. What was the source of maize seeds you grew last season (2011/12)?

52.1 Variety name	**52.2 seed type, refer to code**	**52.3 Seed source**

Seed type, 1=Improved seeds, 2=Local seeds, 3=Recycled seeds
Seed source, 1. Own saved improved seed, 2. Own saved local seed, 3. Private seed trade, 4. Gift seed local, 5. Bought local seed, 6. Provided improved seeds by relative and friends, 7. Relief seed by government or NGOs

H. Marketing of maize

53. Why do you cultivate maize?

1. For sale 2. Home consumption 3. Both 4. Others

54. If both, what percentage of maize do you sell……

55. Who is your main purchaser of your maize? 1. Market women 2. Kenkey sellers 3. Consumers 4. Government agency 5. NGO 6. Others (Please specify)…….

56. Is it difficult to sell the maize you produce? 1. Yes 2. No 3. Don't know

57. If yes, what is the major reason for the lack of market for the maize you produce?

1. Transport 2. Bad Road 3. Pricing 4. Storage 5. Lack of market 6. Others.....

58. Do you make profits? 1. Yes 2. No 3. Don't know

Appendix 4.1 Grain yield and other agronomic traits of hybrids across low N environments in 2013 and 2014

Entry	GY	DTA	DTS	ASI	PHT	EHT	EPP	SG	PA	CA	HC	CC	Base index	% yield reduction
TZDII 68 X 1368	2717.53	54.48	58.06	3.58	168.95	64.30	0.84	3.28	2.84	2.42	2.69	18.89	7.97	37.45
ZM523B-29-2-1-1-B*6 X 9071	2679.76	57.51	61.54	4.03	173.94	85.81	0.83	3.26	2.68	2.29	2.73	20.17	8.12	17.83
CLWN 247 X 9071	2596.03	58.43	61.96	3.53	156.91	93.48	0.92	3.37	2.66	2.01	2.58	16.58	10.53	44.00
TZM501 X KU1414 X TZM501 X 9071	2563.99	57.98	62.64	4.66	152.01	81.27	0.82	3.41	2.54	2.26	2.61	19.72	6.40	33.53
P43SCRq Fs100-1-1-8 X 9071	2522.71	59.05	63.27	4.22	180.26	86.79	0.76	2.96	2.63	2.43	2.46	17.41	6.84	33.31
CLWN 359 X 9071	2463.30	58.30	62.57	4.27	167.03	78.58	0.66	3.28	2.55	2.44	2.70	15.67	4.50	39.74
TZd II 134 X 9071	2404.34	57.81	62.57	4.77	183.31	102.15	0.74	3.42	2.64	2.54	2.68	20.97	2.87	41.91
Laposta Seq C7-F18-3-2-1 X 9071	2375.79	57.50	61.69	4.19	172.97	80.02	0.73	3.25	2.49	2.63	2.50	19.98	4.96	52.23
CZL 00001 X 9071	2355.33	58.50	63.27	4.76	170.61	79.25	0.71	3.07	2.29	2.50	2.49	22.77	5.45	55.96
TZD II 68 X 9071	2338.68	55.65	60.54	4.89	193.85	87.93	0.81	3.32	2.63	2.62	2.37	16.01	3.32	42.75
CLWN 364 X 9071	2329.46	58.86	63.10	4.25	170.04	80.20	0.79	3.09	2.61	2.45	2.67	13.53	5.77	51.91
CML 395/ CML 444 X 9071	2314.64	57.49	62.36	4.87	162.69	79.76	0.84	3.02	2.66	2.82	2.51	18.71	3.75	47.72
TZDII 68 X 87036	2303.58	54.02	58.25	4.23	176.59	73.07	0.73	2.71	2.48	2.77	2.48	20.58	5.84	33.27
CML 198/ LPSC X 9071	2264.67	58.53	62.41	3.88	161.29	77.53	0.74	3.40	2.57	3.09	2.56	21.41	2.67	46.78
TZd II 141 X 9071	2247.65	58.65	63.30	4.66	171.02	95.71	0.75	3.52	2.72	2.54	2.71	16.18	1.67	44.48
ZM523B-29-2-1-1-B*6 X 1368	2198.53	55.93	59.81	3.88	164.12	76.94	0.75	3.14	2.86	2.68	2.64	21.56	3.08	31.25
M131 X 1368*	2161.00	57.17	61.59	4.42	167.33	77.76	0.93	3.10	2.67	2.87	2.68	20.35	4.55	37.46
CLWN 247 X 87036	2141.09	57.42	61.47	4.04	171.87	84.59	0.70	3.22	2.80	2.81	2.78	19.90	1.42	53.59
CZL 068 X 1368	2126.67	55.31	59.32	4.01	149.71	63.50	0.69	2.73	2.64	2.43	2.59	23.26	5.25	47.72
CLWN 240 X 9071	2116.21	58.41	63.18	4.78	177.48	97.76	0.80	3.62	2.54	3.08	2.48	11.53	0.22	50.24
Laposta Seq C7-F71-1-1-2 X 9071	2073.41	56.70	61.28	4.58	178.42	86.98	0.69	2.98	2.34	2.56	2.39	20.68	4.12	33.21
CML 444 X 9071	2072.42	59.39	64.14	4.75	168.72	88.58	0.64	3.44	2.69	2.65	2.62	12.52	-0.64	48.33
Laposta Seq C7-F71-1-1-2 X 1368	2056.68	54.23	58.06	3.83	159.97	67.81	0.78	2.94	2.69	2.90	2.54	24.52	3.82	28.08

Appendix 4.1 Continued

CZL 0713 X 1368	2034.63	55.99	59.69	3.71	164.43	78.78	0.78	3.09	2.48	2.67	2.26	22.60	5.39	50.40
CLWN 364 X 87036	2029.31	56.10	60.94	4.83	166.49	72.98	0.66	3.10	2.65	2.91	2.70	19.11	-0.33	50.09
J -16-1 X 9071	2027.95	58.27	63.18	4.91	170.69	87.50	0.63	3.48	2.56	2.87	2.58	13.50	-1.61	50.49
CML 494 X 9071	2019.59	58.59	63.20	4.61	165.82	87.80	0.73	3.21	2.65	2.50	2.52	17.58	1.98	53.47
CML 395/ CML 444 X 1368	2012.48	56.64	60.68	4.04	157.91	74.42	0.79	2.66	2.93	2.98	2.67	21.26	2.63	54.49
TZd II 140 X 9071	2005.00	58.95	63.28	4.33	182.24	84.32	0.66	3.53	2.92	2.92	2.93	16.23	-2.39	53.66
CLWN 238 X 9071	1996.89	57.89	61.34	3.44	166.80	88.50	0.70	3.46	2.68	2.94	2.56	15.47	1.47	54.89
M131 X 9071	1983.64	57.42	62.59	5.17	168.95	85.06	0.80	3.35	2.67	2.96	2.68	17.43	-0.67	48.69
CLWN 240 X 1368	1979.79	57.61	61.78	4.17	162.68	78.48	0.66	3.69	2.62	2.72	2.57	15.77	-0.45	40.79
CLWN 247 X 1368	1973.27	57.69	61.43	3.74	165.80	77.11	0.77	3.54	2.92	2.53	2.90	18.59	1.38	56.21
TZd II 134 X 87036	1941.14	56.24	60.06	3.82	176.53	87.06	0.82	3.04	2.55	2.59	2.34	24.87	5.27	47.02
CZL 068 X 9071	1934.72	57.07	61.95	4.89	160.14	71.57	0.74	2.98	2.63	3.20	2.62	18.43	-0.39	52.65
CML 198/ LPSC X 1368	1915.50	56.86	61.17	4.31	159.37	73.11	0.70	3.53	2.49	2.83	2.48	21.81	0.37	38.19
CZL 068 X 87036	1891.50	55.56	60.39	4.82	157.05	69.03	0.84	2.58	2.54	2.92	2.52	23.02	3.57	49.62
87036 X 9071	1875.84	57.01	60.46	3.45	162.80	80.73	0.67	3.02	2.37	2.99	2.43	20.41	3.59	47.27
CML 395/ CML 444 X 87036	1869.05	54.53	58.54	4.01	164.05	75.80	0.72	2.59	2.37	2.34	2.23	23.32	6.63	54.56
TZd II 141 X 87036	1847.57	56.06	59.42	3.36	167.04	81.39	0.63	3.04	2.84	3.02	2.89	19.30	0.28	53.90
CLRCW 36 X 9071	1825.50	59.06	63.64	4.57	174.15	85.52	0.80	2.99	2.62	3.01	2.69	15.02	1.20	46.58
CLWN 341 X 87036	1820.12	58.28	62.16	3.89	159.68	76.63	0.70	2.76	2.63	2.67	2.40	17.09	3.22	43.26
Laposta Seq C7-F18-3-2-1 X 1368	1817.35	56.29	59.82	3.53	173.91	77.12	0.75	3.31	2.67	3.27	2.54	19.47	0.48	44.24
CML 442 X 9071	1812.79	56.75	61.60	4.85	160.28	79.63	0.74	2.98	2.46	3.33	2.57	23.80	-0.48	49.24
CML 494 X 1368	1812.17	57.16	61.18	4.03	151.02	72.43	0.80	3.29	2.79	2.91	2.65	18.33	0.60	61.20
ZM523B-29-2-1-1-B*6 X 87036	1805.35	55.44	59.50	4.06	170.03	77.00	0.71	3.03	2.74	2.91	2.73	19.03	0.64	46.28
CZL 03007 X 9071	1803.56	58.50	62.05	3.56	161.98	77.14	0.84	3.03	2.66	2.70	2.44	17.92	4.39	46.90
Laposta seq C7-F18-3-2-1 X 87036	1788.46	54.53	57.75	3.22	175.51	81.46	0.73	2.51	2.55	2.61	2.48	27.14	6.38	43.00
CLWN 240 X 87036	1787.74	56.61	61.12	4.51	170.37	77.63	0.69	3.18	2.64	2.79	2.68	19.57	-0.27	54.18
TZL comp 3 X 9071	1776.14	58.99	63.11	4.12	157.33	77.53	0.71	3.18	2.81	3.01	2.68	15.73	-0.92	46.76

Appendix 4.1 Continued

TZL comp 3 X 87036	1766.63	57.46	61.08	3.62	141.86	74.50	0.73	2.78	2.75	2.70	2.58	18.79	3.07	44.84
CML 254 X 87036	1759.18	56.80	61.07	4.26	176.53	87.44	0.73	3.28	2.61	2.91	2.67	25.79	0.04	44.15
CZL 03007 X 1368	1744.55	57.67	62.13	4.46	162.70	74.60	0.76	3.72	2.97	2.61	2.77	14.68	-2.64	48.22
CLWN 359 X 87036	1744.43	56.17	60.06	3.89	167.58	71.78	0.69	2.86	3.15	2.71	2.85	22.20	-0.65	42.92
CLWN 238 X 87036	1740.78	56.63	60.50	3.87	166.47	81.25	0.77	3.51	2.56	2.62	2.48	17.37	1.73	50.86
CML 444/CML 395/DTPWC8F31 X 87036	1728.96	56.33	60.19	3.87	152.26	70.29	0.72	2.77	2.41	2.91	2.34	22.20	3.48	49.06
ZM 521 B -66-4-1-1 X 87036	1713.24	55.02	59.18	4.16	165.15	77.19	0.70	2.88	2.67	2.70	2.48	20.56	1.48	46.60
CZL 00001 X 1368	1698.03	56.56	60.84	4.28	173.24	81.32	0.76	3.21	2.51	2.94	2.47	20.69	0.76	52.41
CML 442 X 87036	1685.32	54.89	59.58	4.69	156.14	70.76	0.73	2.70	2.75	3.08	2.72	24.90	-0.65	52.80
CLWN 341 X 1368	1681.60	57.64	61.74	4.10	162.99	78.13	0.77	3.67	2.80	3.04	2.77	17.17	-2.42	57.06
CML 254 X 9071	1668.76	58.54	63.06	4.51	176.77	87.62	0.72	3.45	2.65	2.82	2.68	18.57	-1.63	60.20
P43SRCq Fs100-1-1-8 X87036	1665.55	56.61	60.42	3.81	163.59	71.88	0.74	3.06	2.49	2.86	2.31	20.99	2.32	60.38
CML 494 X 87036	1645.57	56.46	60.86	4.40	145.35	70.31	0.65	2.70	2.40	3.22	2.34	18.86	0.33	53.50
CML 442 X 1368	1635.92	55.40	59.23	3.84	154.46	68.27	0.66	3.06	2.60	3.03	2.50	24.76	-0.15	47.54
CML 444/ CML 395/DTPWC 8F31 X 9071	1627.79	58.52	62.88	4.36	152.49	81.08	0.72	3.24	2.79	3.39	2.60	15.48	-3.41	61.50
CLRCW 36 X 87036	1622.06	58.83	63.83	5.00	164.02	75.64	0.68	3.29	2.68	2.94	2.77	18.11	-3.36	59.99
TZM 501 X KU 1414 X43 TZM 501 X 87036	1608.14	55.63	60.79	5.16	162.21	72.80	0.72	3.18	2.62	3.04	2.47	21.30	-2.97	53.85
Laposta Seq C7-F71-1-1-2 X 87036	1593.62	55.65	59.50	3.85	162.75	69.15	0.71	2.75	3.00	3.16	2.82	24.80	-1.25	58.23
M131 X 87036	1583.43	56.84	61.34	4.51	169.21	76.86	0.70	3.19	2.67	3.29	2.52	18.84	-3.03	58.61
ZM 521B-66-4-1-1 X 9071	1574.07	58.13	61.50	3.37	159.80	76.40	0.67	3.01	2.65	3.53	2.65	19.22	-1.11	47.35
CLWN 349 X 87036 *	1561.72	56.37	60.70	4.33	167.50	66.30	0.66	3.03	2.59	3.08	2.68	21.37	-1.57	61.01
TZd II 140 X 1368	1559.78	56.94	61.58	4.64	165.79	73.80	0.67	3.72	2.99	3.44	2.98	18.27	-7.99	53.51
CML 444 X 1368	1541.39	58.54	62.83	4.29	156.38	79.86	0.63	3.34	2.74	2.87	2.51	16.96	-3.21	53.77
CML 444 X 87036	1520.28	56.76	60.72	3.96	174.20	90.12	0.77	3.47	2.81	2.94	2.61	19.24	-1.88	56.00
TZd II 141 X 1368	1518.68	56.90	61.12	4.22	170.32	76.23	0.61	3.82	2.88	2.54	2.78	18.12	-4.70	55.69
P43SCRq Fs100-1-1-8 X 1368	1513.01	57.24	60.85	3.60	160.83	72.86	0.72	3.11	2.53	2.96	2.37	18.98	0.98	62.75

Appendix 4.1 Continued

CLWN 341 X 9071	1504.48	58.69	63.63	4.94	161.28	79.27	0.76	3.19	2.68	2.97	2.60	15.66	-2.59	63.94
CZL 00001 X 87036*	1494.50	55.50	59.89	4.38	152.00	67.83	0.84	3.07	2.30	2.58	2.38	21.54	3.28	57.13
CZL 0713 X 87036	1489.26	54.86	58.46	3.60	176.69	86.02	0.67	2.81	2.38	2.65	2.29	24.25	3.07	61.93
TZd II 140 X 87036 *	1482.09	56.27	60.46	4.18	157.54	72.86	0.66	3.31	2.96	3.32	2.87	24.44	-5.45	54.16
CLWN 349 X 9071	1476.37	58.70	63.45	4.74	167.34	76.17	0.78	3.28	2.75	3.00	2.68	19.18	-2.90	61.30
CZL 0713 X 9071	1471.01	59.22	64.11	4.88	165.37	84.40	0.62	3.11	2.87	2.79	2.83	20.14	-4.68	60.19
1368 X 87036	1461.75	57.09	61.76	4.67	157.80	73.18	0.57	3.27	2.75	3.04	2.68	18.75	-5.53	50.32
CML 198/LPsc X 87036	1453.02	56.48	60.63	4.15	157.71	65.35	0.65	3.17	2.89	3.02	2.70	22.16	-3.81	58.22
CLWN 364 X 1368	1448.83	57.51	61.71	4.20	162.45	71.85	0.56	3.25	2.54	2.89	2.50	17.52	-2.88	65.18
J-16-1 X 1368	1439.53	56.96	61.19	4.23	166.24	71.07	0.61	3.68	3.08	2.95	2.80	18.67	-7.11	49.05
CZL 03007 X 87036	1436.13	56.71	61.15	4.44	167.76	76.06	0.72	3.37	2.70	3.03	2.62	18.22	-3.29	62.79
J-16-1 X 87036	1413.77	56.33	60.75	4.41	170.77	76.89	0.58	2.79	2.76	2.86	2.69	19.98	-2.84	56.26
CLWN 349 X 1368	1372.88	57.66	61.26	3.60	161.66	63.99	0.79	3.28	2.86	3.05	2.70	17.28	-1.56	51.62
CLWN 359 X 1368	1328.56	56.76	60.89	4.14	160.16	65.20	0.61	3.53	2.65	3.29	2.60	19.20	-5.74	69.22
1368 X 9071	1320.14	55.78	60.86	5.08	169.33	76.72	0.58	3.14	2.42	2.81	2.39	18.36	-3.93	59.06
CML 444/CML 395/DTPWC8F31 X 1368	1307.70	57.93	61.92	3.98	152.61	69.87	0.63	3.70	2.85	3.22	2.77	17.77	-6.74	64.27
TZd II 134 X 1368	1251.94	55.74	60.64	4.90	163.23	83.55	0.63	3.50	3.01	2.63	2.72	18.39	-7.18	66.54
Etubi – (Check)	1124.41	56.83	61.05	4.22	149.60	69.83	0.57	2.94	2.83	2.95	2.36	21.48	-5.22	64.29
CML 254 X 1368	1115.88	57.50	62.13	4.64	158.46	79.13	0.61	3.61	2.75	2.79	2.61	20.20	-7.03	61.61
ZM 521 B-66-4-1-1 X 1368	1105.24	56.02	59.73	3.70	148.34	70.40	0.63	3.23	2.90	3.11	2.76	22.27	-5.43	52.77
CLRCW 36 X 1368	1073.84	58.67	63.14	4.47	164.86	76.95	0.57	3.31	2.83	3.23	2.64	16.80	-8.22	69.17
TZM501 X KU1414 X TZM501 X 1368	1037.20	58.95	62.57	3.62	148.46	65.65	0.70	3.42	2.75	3.13	2.80	18.40	-4.53	71.40
CLWN 238 X 1368	744.52	59.37	63.21	3.84	141.51	71.07	0.54	3.34	3.07	3.38	3.00	20.39	-10.67	77.51
TZL Comp3 X 1368	528.36	58.42	62.81	4.38	139.53	63.97	0.54	3.48	3.08	3.81	2.72	16.11	-15.03	80.24

Appendix 4.2 Grain yield and other agronomic traits of hybrids across high N environments in 2013 and 2014

Entry	GY	DTS	DTA	ASI	PHT(cm)	EHT(cm)	EPP	SG	PASP	EASP	HC	CC
CZL 00001 X 9071	5347.69	59.99	57.49	2.50	194.86	95.05	0.76	2.44	2.24	2.20	2.48	27.56
Laposta Seq C7-F18-3-2-1 X 9071	4973.61	57.85	56.43	1.41	181.37	87.55	0.66	2.16	2.29	2.05	2.32	33.03
CLWN 364 X 9071	4844.14	57.65	56.05	1.60	186.11	92.06	0.64	1.96	1.99	2.19	2.01	26.24
CML 494 X 1368	4670.56	57.26	55.82	1.44	173.21	78.09	0.79	2.29	2.27	2.29	2.14	30.38
CLWN 247 X 9071	4635.60	58.66	56.70	1.96	184.28	98.57	0.75	2.83	2.27	2.17	2.24	27.78
CLWN 247 X 87036	4613.79	56.54	54.95	1.59	184.28	90.52	0.66	2.26	2.66	2.66	2.19	33.30
CLWN 247 X 1368	4506.51	57.86	56.37	1.49	179.15	96.80	0.67	2.68	2.28	1.94	2.34	31.05
CML 395/ CML 444 X 9071	4427.80	56.84	54.97	1.87	177.85	91.59	0.66	2.06	2.23	2.60	2.54	27.66
CLWN 238 X 9071	4426.70	60.90	57.84	3.06	181.58	97.50	0.59	2.60	2.40	2.41	2.52	25.14
CML 395/ CML 444 X 1368	4421.70	56.75	54.98	1.77	171.42	84.37	0.72	1.99	2.10	2.26	2.07	30.15
TZDII 68 X 1368	4344.40	55.16	53.20	1.96	185.17	78.74	0.60	2.38	2.16	2.27	2.21	31.80
CML 494 X 9071	4340.45	58.69	56.60	2.09	182.30	93.80	0.67	2.38	2.36	2.15	2.39	29.92
TZd II 140 X 9071	4326.64	57.47	55.61	1.86	181.59	91.25	0.60	2.32	2.34	2.40	2.80	27.32
CLWN 359 X 1368	4316.22	56.58	55.13	1.46	181.16	81.67	0.70	2.26	2.49	2.42	2.63	33.62
CML 198/ LPSC X 9071	4255.33	58.87	56.94	1.94	182.78	89.56	0.70	2.54	2.24	2.20	2.11	33.01
CLWN 240 X 9071	4252.90	58.90	56.84	2.06	185.53	97.05	0.64	2.49	2.14	2.05	2.37	24.89
CML 444/ CML 395/DTPWC 8F31 X 9071	4227.84	58.86	57.09	1.77	181.79	93.90	0.65	2.28	2.03	2.46	2.05	25.62
P43SRCq Fs100-1-1-8 X87036	4203.94	56.40	54.98	1.42	182.06	84.74	0.72	1.89	2.03	2.15	1.69	28.79
CML 254 X 9071	4192.69	59.64	57.47	2.18	177.77	100.44	0.67	2.22	2.45	2.33	2.38	33.61
CLWN 341 X 9071	4172.07	61.08	58.32	2.76	183.99	90.18	0.71	2.42	2.30	1.81	2.36	28.44
CLWN 364 X 1368	4161.42	56.78	55.39	1.39	180.44	85.58	0.87	2.68	2.54	2.26	2.17	27.88
TZd II 134 X 9071	4139.32	58.47	56.66	1.81	192.48	101.08	0.66	2.02	2.55	2.44	2.66	31.64

Appendix 4.2 continued

CML 395/ CML 444 X 87036	4112.86	54.69	53.44	1.25	186.20	88.43	0.74	1.83	1.94	2.29	2.02	36.14
CZL 0713 X 1368	4102.23	56.10	54.65	1.45	172.23	88.72	0.70	2.33	2.45	2.35	2.58	34.91
J -16-1 X 9071	4096.21	58.46	56.30	2.16	188.60	96.87	0.64	2.20	2.13	2.06	2.00	32.49
CLWN 359 X 9071	4087.54	58.80	57.13	1.67	191.30	94.41	0.61	2.15	1.85	2.23	1.99	27.16
CZL 068 X 9071	4085.61	57.16	54.80	2.36	173.26	80.58	0.67	2.11	2.12	2.81	2.35	29.36
TZD II 68 X 9071	4084.97	57.19	55.89	1.30	187.58	94.22	0.66	2.15	2.60	2.22	2.56	25.61
CZL 068 X 1368	4068.04	54.48	52.62	1.86	164.42	74.42	0.69	2.19	2.26	2.46	2.13	34.15
CLWN 364 X 87036	4065.87	56.22	54.46	1.76	178.35	82.60	0.65	2.07	2.02	2.81	2.21	28.18
P43SCRq Fs100-1-1-8 X 1368	4061.86	56.04	55.19	0.85	175.85	78.58	0.66	2.56	2.31	2.36	2.33	33.09
CLRCW 36 X 87036	4054.10	58.01	56.35	1.66	183.22	86.45	0.72	2.16	2.12	2.13	2.32	25.44
TZd II 141 X 9071	4048.51	57.88	56.66	1.22	196.12	105.06	0.64	2.61	2.32	2.16	2.49	27.81
CML 444 X 9071	4011.04	59.69	58.00	1.69	188.87	104.14	0.64	2.32	2.26	2.26	2.35	26.55
TZd II 141 X 87036	4008.12	55.68	54.35	1.33	183.97	86.11	0.75	2.14	2.28	2.15	2.18	33.21
CLWN 349 X 87036 *	4005.94	57.01	55.00	2.01	180.31	81.08	0.61	2.47	2.43	2.59	2.42	30.27
CLWN 341 X 1368	3915.72	58.95	57.60	1.35	174.33	81.38	0.65	2.62	2.57	2.36	2.28	22.12
CZL 0713 X 87036	3911.74	54.36	53.13	1.24	183.71	93.41	0.75	1.95	1.99	2.02	2.24	43.16
CLWN 240 X 87036	3901.88	56.73	55.18	1.55	175.26	86.04	0.65	2.29	2.62	2.56	2.37	32.59
M131 X 9071	3866.20	58.19	56.62	1.57	185.35	94.92	0.71	2.43	2.28	2.35	2.26	32.43
CZL 03007 X 87036	3859.12	55.73	54.48	1.25	184.11	90.59	0.68	1.98	2.11	2.23	1.78	30.39
TZM501 X KU1414 X TZM501 X 9071	3857.27	60.17	57.96	2.22	180.94	89.48	0.67	2.54	2.23	1.98	2.34	24.94
M131 X 87036	3825.56	58.77	56.45	2.32	171.27	91.93	0.54	2.43	2.61	2.77	2.34	34.12
Laposta Seq C7-F71-1-1-2 X 87036	3815.43	55.43	53.94	1.49	170.29	77.29	0.67	1.90	2.32	2.62	2.45	41.19
CLWN 349 X 9071	3815.30	59.12	57.02	2.10	182.98	87.11	0.74	2.32	2.35	2.50	2.34	25.30
P43SCRq Fs100-1-1-8 X 9071	3782.67	59.70	57.87	1.82	187.36	90.82	0.63	2.57	2.39	2.52	2.41	24.50
CZL 068 X 87036	3754.70	55.77	53.79	1.98	168.32	74.75	0.70	2.03	2.20	2.60	2.26	34.82
TZd II 134 X 1368	3741.08	57.31	55.20	2.11	188.78	97.46	0.56	2.66	2.59	1.99	2.55	31.28
CZL 0713 X 9071	3694.78	58.50	56.54	1.97	176.58	99.58	0.63	2.00	2.08	1.96	2.24	29.66
TZd II 134 X 87036	3664.23	55.01	53.89	1.12	179.40	88.87	0.72	2.13	2.14	2.29	2.51	40.67

Appendix 4.2 continued

CML 444/CML 395/DTPWC8F31 X 1368	3660.05	58.34	56.77	1.58	168.32	81.90	0.61	2.70	2.54	2.59	2.63	28.28
TZM501 X KU1414 X TZM501 X 1368	3627.13	58.25	56.63	1.62	174.28	78.85	0.59	2.68	2.49	2.59	2.46	30.29
CML 442 X 9071	3571.20	57.50	55.32	2.18	165.01	76.97	0.67	2.26	2.38	2.73	2.28	35.25
CML 442 X 87036	3570.43	56.05	54.65	1.39	170.89	84.31	0.71	2.30	2.51	2.92	2.46	35.93
CZL 00001 X 1368	3567.88	56.79	55.41	1.39	175.39	79.84	0.55	2.61	2.32	2.41	2.49	31.69
CLWN 238 X 87036	3542.74	56.86	55.03	1.83	183.10	97.97	0.73	2.16	2.19	2.37	1.69	29.30
CML 494 X 87036	3538.52	55.73	53.93	1.81	175.30	86.48	0.70	2.32	2.14	2.22	2.35	34.01
CZL 00001 X 87036	3486.15	56.73	55.09	1.64	178.65	81.39	0.58	2.10	2.15	2.49	2.11	33.30
TZM 501 X KU 1414 X43 TZM 501 X 87036	3484.55	54.98	53.42	1.55	180.46	78.94	0.66	2.27	2.37	2.47	1.87	36.29
CLRCW 36 X 1368	3483.05	59.05	57.43	1.63	180.81	80.32	0.60	2.71	2.58	2.36	2.43	26.27
CML 198/LPsc X 87036	3477.39	56.49	54.12	2.37	172.04	77.01	0.65	2.17	1.94	2.92	2.24	38.21
M131 X 1368	3455.47	58.02	55.62	2.40	182.10	90.97	0.62	2.43	2.36	2.77	2.26	30.81
CML 444 X 87036	3454.94	58.23	56.45	1.78	185.85	97.46	0.61	2.19	2.43	2.80	2.54	31.34
TZDII 68 X 87036	3451.96	54.34	52.72	1.62	178.96	87.12	0.70	2.13	2.67	2.51	2.20	33.97
TZd II 141 X 1368	3427.41	58.02	55.99	2.03	171.09	83.68	0.57	2.66	2.63	2.69	2.61	27.02
CLRCW 36 X 9071	3417.40	60.92	57.91	3.01	190.94	98.36	0.60	2.38	2.20	2.17	2.13	24.48
CZL 03007 X 9071	3396.44	58.65	56.70	1.95	178.35	84.54	0.68	2.52	2.35	2.38	2.29	33.25
CML 444/CML 395/DTPWC8F31 X 87036	3393.97	56.13	54.88	1.26	171.48	83.10	0.62	2.23	1.93	2.65	2.21	30.81
CZL 03007 X 1368	3369.33	56.54	55.02	1.52	162.68	78.14	0.70	2.65	2.52	2.33	2.34	24.20
ZM523B-29-2-1-1-B*6 X 87036	3360.65	55.84	54.22	1.62	180.63	87.11	0.68	2.23	2.45	2.62	2.39	38.26
TZd II 140 X 1368	3354.96	56.43	55.15	1.28	179.79	80.14	0.71	2.37	2.27	2.46	2.39	28.11
CLWN 240 X 1368	3343.47	58.26	56.62	1.63	176.71	88.91	0.58	2.29	2.23	1.90	2.43	28.13
TZL comp 3 X 9071	3335.90	59.93	58.18	1.75	170.60	87.60	0.64	2.33	2.29	2.30	2.32	27.64
CML 444 X 1368	3334.37	58.47	56.88	1.59	178.76	93.73	0.58	2.48	2.27	2.74	2.20	26.61
CLWN 238 X 1368	3309.74	59.13	57.42	1.71	152.53	81.08	0.65	2.57	2.67	2.63	2.65	23.61
ZM523B-29-2-1-1-B*6 X 9071	3261.37	59.69	57.39	2.29	173.70	90.85	0.64	2.41	2.68	2.84	2.82	28.82

Appendix 4.2 continued

Laposta Seq C7-F18-3-2-1 X 1368	3259.31	55.06	53.72	1.34	187.24	82.82	0.71	2.23	1.95	2.33	2.23	37.15
TZd II 140 X 87036 *	3233.35	56.60	54.79	1.81	175.65	83.48	0.75	2.20	2.37	2.76	2.51	33.86
J-16-1 X 87036	3232.24	55.49	54.04	1.46	184.46	76.09	0.72	2.42	1.94	2.17	2.32	37.24
ZM 521 B -66-4-1-1 X 87036	3208.04	56.08	54.09	1.99	175.58	86.90	0.76	2.46	2.50	2.65	2.42	36.08
CLWN 341 X 87036	3207.70	58.90	57.14	1.76	174.15	85.99	0.67	2.34	2.52	2.27	2.07	28.37
TZL comp 3 X 87036	3202.70	56.59	54.97	1.62	172.57	87.43	0.63	2.36	2.24	2.58	2.10	35.55
ZM523B-29-2-1-1-B*6 X 1368	3198.05	55.20	54.10	1.10	171.79	83.24	0.81	2.40	2.60	2.66	2.05	31.73
CML 254 X 87036	3149.70	57.09	55.10	1.99	174.92	93.52	0.62	2.50	2.22	2.82	2.10	30.95
Laposta seq C7-F18-3-2-1 X 87036	3137.71	55.01	53.62	1.38	185.78	85.51	0.60	1.96	2.48	2.73	2.18	39.72
CML 442 X 1368	3118.58	55.45	54.29	1.15	166.24	77.86	0.65	2.45	2.58	2.52	2.40	37.32
Laposta Seq C7-F71-1-1-2 X 9071	3104.60	57.96	55.94	2.02	184.53	90.36	0.62	2.57	2.49	2.91	2.77	33.38
CML 198/ LPSC X 1368	3098.94	56.26	55.09	1.16	168.48	75.82	0.59	2.58	2.58	2.81	2.42	35.19
CLWN 359 X 87036	3056.04	56.76	55.13	1.62	176.53	79.91	0.58	2.23	2.45	2.20	2.72	34.62
ZM 521B-66-4-1-1 X 9071	2989.80	58.61	56.36	2.25	171.52	86.42	0.60	2.33	2.94	3.12	2.77	28.75
CML 254 X 1368	2906.62	57.82	56.15	1.67	171.11	85.11	0.53	2.73	2.53	2.15	2.03	33.86
Laposta Seq C7-F71-1-1-2 X 1368	2859.66	56.47	55.21	1.26	168.31	77.80	0.59	2.48	2.35	2.56	1.96	32.26
CLWN 349 X 1368	2837.42	57.83	55.99	1.84	182.81	78.95	0.59	2.42	2.30	2.48	2.36	32.74
J-16-1 X 1368	2825.11	55.60	53.86	1.74	171.07	78.31	0.57	2.91	2.77	2.79	2.90	33.46
TZL Comp3 X 1368	2673.48	59.26	57.56	1.70	145.54	69.35	0.54	2.91	3.20	3.01	3.25	28.34
ZM 521 B-66-4-1-1 X 1368	2340.25	57.61	55.65	1.96	157.75	77.86	0.54	2.45	2.84	3.11	2.68	33.12
Etubi – (Check)	3148.90	56.29	54.87	1.42	164.10	74.63	0.56	2.26	2.74	3.24	2.61	36.69
1368 X 87036	2942.19	56.94	55.12	1.82	173.64	84.87	0.55	2.51	2.53	2.94	2.34	31.61
1368 X 9071	3224.92	58.50	56.16	2.34	177.17	84.50	0.59	2.22	2.25	2.35	2.31	32.74
87036 X 9071	3557.51	57.53	55.32	2.21	177.30	88.54	0.58	2.51	2.31	2.76	2.33	30.86
Means	3712.89	57.42	55.64	1.76	177.86	86.77	0.65	2.35	2.35	2.45	2.34	31.22
Max	5347.69	61.08	58.32	3.06	196.12	105.06	0.87	2.91	3.20	3.24	3.25	31.22
Min	2340.00	54.34	52.62	0.85	145.54	69.35	0.53	1.83	1.85	1.81	1.69	22.12
SE	284.00	0.54	0.44	0.29	4.75	3.55	0.06	0.16	0.16	0.23	0.16	2.05

Appendix 4.3 Grain yield and other agronomic traits of hybrids across environments in 2013 and 2014

Entry	GY	DTS	DTA	ASI	PHT	EHT	EPP	SG	PASP	EASP	HC	CC
CZL 00001 X 9071	3851.51	60.63	57.99	2.63	182.74	87.15	0.73	2.75	2.27	2.35	2.48	25.17
Laposta Seq C7-F18-3-2-1 X 9071	3674.70	58.77	56.96	1.80	177.17	83.78	0.70	2.71	2.39	2.34	2.41	26.51
CLWN 247 X 9071	3615.81	59.31	57.57	1.74	170.59	96.02	0.83	3.10	2.47	2.09	2.41	22.18
CLWN 364 X 9071	3586.80	59.38	57.45	1.92	178.07	86.13	0.71	2.53	2.30	2.32	2.34	19.89
TZDII 68 X 1368	3530.96	55.61	53.84	1.77	177.06	71.52	0.72	2.83	2.50	2.34	2.45	25.34
CLWN 247 X 87036	3377.44	58.01	56.19	1.82	178.07	87.55	0.68	2.74	2.73	2.74	2.49	26.60
CML 395/ CML 444 X 9071	3371.22	58.60	56.23	2.37	170.27	85.68	0.75	2.54	2.44	2.71	2.52	23.19
CLWN 359 X 9071	3275.42	59.69	57.71	1.97	179.17	86.50	0.64	2.72	2.20	2.34	2.35	21.41
TZD II 134 X 9071	3271.83	59.52	57.23	2.29	187.90	101.61	0.70	2.72	2.59	2.49	2.67	26.30
CML 198/ LPSC X 9071	3260.00	59.64	57.73	1.91	172.04	83.55	0.72	2.97	2.40	2.64	2.34	27.21
CML 494 X 1368	3241.36	58.22	56.49	1.74	162.11	75.26	0.80	2.79	2.53	2.60	2.40	24.36
CLWN 247 X 1368	3239.89	58.64	57.03	1.61	172.47	86.95	0.72	3.11	2.60	2.23	2.62	24.82
CML 395/ CML 444 X 1368	3217.09	57.72	55.81	1.91	164.67	79.39	0.75	2.32	2.52	2.62	2.37	25.70
TZD II 68 X 9071	3211.82	57.87	55.77	2.10	190.72	91.08	0.74	2.73	2.62	2.42	2.46	20.81
CLWN 238 X 9071	3211.79	60.12	57.87	2.25	174.19	93.00	0.65	3.03	2.54	2.67	2.54	20.30
TZM501 X KU1414 X TZM501 X 9071	3210.63	60.40	57.97	2.44	166.48	85.37	0.74	2.98	2.39	2.12	2.47	22.33
CLWN 240 X 9071	3184.55	60.04	57.62	2.42	181.51	97.40	0.72	3.05	2.34	2.57	2.43	18.21
CML 494 X 9071	3180.02	59.94	57.59	2.35	174.06	90.80	0.70	2.80	2.51	2.33	2.45	23.75
TZd II 140 X 9071	3165.82	59.38	57.28	2.09	181.92	87.78	0.63	2.93	2.63	2.66	2.87	21.78
P43SCRq Fs100-1-1-8 X 9071	3152.69	60.48	58.46	2.02	183.81	88.81	0.69	2.77	2.51	2.47	2.43	20.95
TZd II 141 X 9071	3148.08	59.59	57.65	1.94	183.57	100.39	0.70	3.06	2.52	2.35	2.60	21.99
CZL 068 X 1368	3097.35	55.90	53.97	1.94	157.06	68.96	0.69	2.46	2.45	2.45	2.36	28.71
CZL 0713 X 1368	3068.43	56.89	55.32	1.58	168.33	83.75	0.74	2.71	2.46	2.51	2.42	28.75
J -16-1 X 9071	3062.08	59.82	57.29	2.54	179.65	92.19	0.64	2.84	2.34	2.46	2.29	22.99

Appendix 4.3 continued

CLWN 364 X 87036	3047.59	57.58	55.28	2.29	172.42	77.79	0.65	2.58	2.33	2.86	2.46	23.64
CML 444 X 9071	3041.73	60.91	58.70	2.22	178.80	96.36	0.64	2.88	2.47	2.45	2.48	19.53
CZL 068 X 9071	3010.17	58.56	55.93	2.62	166.70	76.07	0.71	2.55	2.37	3.01	2.49	23.89
CML 395/ CML 444 X 87036	2990.95	55.62	53.99	1.63	175.12	82.12	0.73	2.21	2.16	2.31	2.13	29.73
ZM523B-29-2-1-1-B*6 X 9071	2970.56	59.61	57.45	2.16	173.82	88.33	0.74	2.83	2.68	2.57	2.78	24.49
P43SRCq Fs100-1-1-8 X87036	2934.74	57.41	55.80	1.61	172.83	78.31	0.73	2.48	2.26	2.50	2.00	24.89
CML 254 X 9071	2930.72	60.35	58.01	2.35	177.27	94.03	0.69	2.84	2.55	2.57	2.53	26.09
TZD II 141 X 87036	2927.85	56.55	55.20	1.35	175.50	83.75	0.69	2.59	2.56	2.59	2.53	26.25
CML 444/ CML 395/DTPWC 8F31 X 9071	2927.82	59.87	57.81	2.06	167.14	87.49	0.68	2.76	2.41	2.92	2.32	20.55
M131 X 9071	2924.92	59.39	57.02	2.37	177.15	89.99	0.76	2.89	2.47	2.66	2.47	24.93
TZDII 68 X 87036	2877.77	55.29	53.37	1.92	177.77	80.10	0.71	2.42	2.57	2.64	2.34	27.28
CLWN 240 X 87036	2844.81	57.93	55.90	2.03	172.82	81.84	0.67	2.74	2.63	2.67	2.53	26.08
CLWN 341 X 9071	2838.28	61.36	58.51	2.85	172.63	84.72	0.73	2.80	2.49	2.39	2.48	22.05
CLRCW 36 X 87036	2838.08	59.92	57.59	2.33	173.62	81.05	0.70	2.73	2.40	2.54	2.55	21.77
CZL 068 X 87036	2823.10	57.08	54.68	2.40	162.68	71.89	0.77	2.30	2.37	2.76	2.39	28.92
CLWN 359 X 1368	2822.39	57.74	55.94	1.80	170.66	73.43	0.66	2.90	2.57	2.86	2.61	26.41
M131 X 1368*	2808.23	58.81	56.39	2.41	174.72	84.37	0.78	2.77	2.52	2.82	2.47	25.58
CLWN 364 X 1368	2805.13	58.25	56.45	1.79	171.45	78.72	0.72	2.96	2.54	2.57	2.34	22.70
TZd II 134 X 87036	2802.68	56.53	55.06	1.47	177.96	87.97	0.77	2.59	2.35	2.44	2.42	32.77
CLWN 341 X 1368	2798.66	59.35	57.62	1.73	168.66	79.76	0.71	3.15	2.69	2.70	2.52	19.64
P43SCRq Fs100-1-1-8 X 1368	2787.43	57.44	56.22	1.23	168.34	75.72	0.69	2.83	2.42	2.66	2.35	26.03
CLWN 349 X 87036	2783.83	57.85	55.69	2.17	173.91	73.69	0.64	2.75	2.51	2.84	2.55	25.82
Laposta Seq C7-F71-1-1-2 X 87036	2704.53	56.46	54.80	1.67	166.52	73.22	0.69	2.33	2.66	2.89	2.64	32.99
M131 X 87036	2704.50	59.06	56.64	2.41	170.24	84.40	0.62	2.81	2.64	3.03	2.43	26.48
CZL 0713 X 87036	2700.50	55.41	53.99	1.42	180.20	89.72	0.71	2.38	2.19	2.33	2.26	33.70
ZM523B-29-2-1-1-B*6 X 1368	2698.29	56.51	55.02	1.49	167.96	80.09	0.78	2.77	2.73	2.67	2.34	26.64
CML 442 X 9071	2692.00	58.55	56.04	2.51	162.64	78.30	0.70	2.62	2.42	3.03	2.42	29.53
CLWN 240 X 1368	2661.63	59.02	57.11	1.90	169.69	83.70	0.62	2.99	2.43	2.31	2.50	21.95

Appendix 4.3 continued

CZL 03007 X 87036	2647.62	57.44	55.60	1.85	175.94	83.33	0.70	2.67	2.40	2.63	2.20	24.31
CLWN 349 X 9071	2645.83	60.28	57.86	2.42	175.16	81.64	0.76	2.80	2.55	2.75	2.51	22.24
CLWN 238 X 87036	2641.76	57.68	55.83	1.85	174.78	89.61	0.75	2.83	2.38	2.49	2.08	23.33
CZL 00001 X 1368	2632.96	57.81	55.98	1.83	174.31	80.58	0.66	2.91	2.42	2.68	2.48	26.19
CML 442 X 87036	2627.88	56.81	54.77	2.04	163.52	77.53	0.72	2.50	2.63	3.00	2.59	30.41
CLRCW 36 X 9071	2621.45	61.28	58.49	2.79	182.54	91.94	0.70	2.69	2.41	2.59	2.41	19.75
CZL 03007 X 9071	2600.00	59.35	57.60	1.75	170.17	80.84	0.76	2.77	2.51	2.54	2.37	25.59
CML 494 X 87036	2592.05	57.30	55.19	2.10	160.32	78.40	0.67	2.51	2.27	2.72	2.35	26.44
Laposta Seq C7-F71-1-1-2 X 9071	2589.01	58.62	56.32	2.30	181.48	88.67	0.65	2.77	2.41	2.73	2.58	27.03
ZM523B-29-2-1-1-B*6 X 87036	2583.00	56.67	54.83	1.84	175.33	82.05	0.70	2.63	2.59	2.76	2.56	28.65
CZL 0713 X 9071	2582.90	60.30	57.88	2.42	170.97	91.99	0.62	2.56	2.48	2.38	2.53	24.90
CML 444/CML 395/DTPWC8F31 X 87036	2561.46	57.16	55.60	1.56	161.87	76.70	0.67	2.50	2.17	2.78	2.27	26.50
CZL 03007 X 1368	2556.94	58.33	56.35	1.99	162.69	76.37	0.73	3.19	2.75	2.47	2.56	19.44
TZL comp 3 X 9071	2556.02	60.52	58.58	1.94	163.97	82.57	0.67	2.75	2.55	2.65	2.50	21.68
TZM 501 X KU 1414 X43 TZM 501 X 87036	2546.35	56.89	54.53	2.36	171.34	75.87	0.69	2.73	2.50	2.76	2.17	28.79
Laposta Seq C7-F18-3-2-1 X 1368	2538.33	56.44	55.01	1.43	180.57	79.97	0.73	2.77	2.31	2.80	2.39	28.31
CLWN 341 X 87036	2513.91	59.53	57.71	1.82	166.91	81.31	0.68	2.55	2.57	2.47	2.23	22.73
CML 198/ LPSC X 1368	2507.22	57.71	55.98	1.73	163.92	74.46	0.65	3.06	2.53	2.82	2.45	28.50
TZd II 134 X 1368	2496.51	57.97	55.47	2.50	176.00	90.51	0.59	3.08	2.80	2.31	2.64	24.84
CZL 00001 X 87036*	2490.33	57.31	55.30	2.01	165.33	74.61	0.71	2.59	2.22	2.54	2.24	27.42
CML 444 X 87036	2487.61	58.47	56.61	1.87	180.03	93.79	0.69	2.83	2.62	2.87	2.58	25.29
TZL comp 3 X 87036	2484.67	57.84	56.22	1.62	157.22	80.96	0.68	2.57	2.49	2.64	2.34	27.17
CML 444/CML 395/DTPWC8F31 X 1368	2483.88	59.13	57.35	1.78	160.46	75.88	0.62	3.20	2.70	2.90	2.70	23.02
TZD II 141 X 1368	2473.05	58.57	56.45	2.12	170.71	79.96	0.59	3.24	2.76	2.61	2.70	22.57
CML 198/LPsc X 87036	2465.21	57.56	55.30	2.26	164.88	71.18	0.65	2.67	2.41	2.97	2.47	30.18
Laposta seq C7-F18-3-2-1 X 87036	2463.08	55.38	54.08	1.30	180.64	83.49	0.66	2.24	2.51	2.67	2.33	33.43
ZM 521 B -66-4-1-1 X 87036	2460.64	56.63	54.56	2.07	170.36	82.05	0.73	2.67	2.58	2.67	2.45	28.32

Laposta Seq C7-F71-1-1-2 X 1368	2458.17	56.26	54.72	1.54	164.14	72.80	0.69	2.71	2.52	2.73	2.25	28.39
TZd II 140 X 1368	2457.37	58.00	56.05	1.96	172.79	76.97	0.69	3.05	2.63	2.95	2.69	23.19
CML 254 X 87036	2454.44	58.08	55.95	2.13	175.72	90.48	0.68	2.89	2.41	2.86	2.39	28.37
CML 444 X 1368	2437.88	59.65	57.71	1.94	167.57	86.79	0.60	2.91	2.50	2.80	2.35	21.79
CLWN 359 X 87036	2400.24	57.41	55.65	1.75	172.05	75.84	0.63	2.55	2.80	2.45	2.78	28.41
CML 442 X 1368	2377.25	56.34	54.84	1.49	160.35	73.07	0.65	2.76	2.59	2.77	2.45	31.04
TZD II 140 X 87036 *	2357.72	57.53	55.53	2.00	166.59	78.17	0.71	2.75	2.66	3.04	2.69	29.15
TZM501 X KU1414 X TZM501 X 1368	2332.17	59.41	57.79	1.62	161.37	72.25	0.64	3.05	2.62	2.86	2.63	24.35
J-16-1 X 87036	2323.00	57.12	55.18	1.94	177.61	76.49	0.65	2.60	2.35	2.52	2.51	28.61
ZM 521B-66-4-1-1 X 9071	2281.94	59.05	57.25	1.81	165.66	81.41	0.64	2.67	2.80	3.33	2.71	23.99
CLRCW 36 X 1368	2278.45	60.10	58.05	2.05	172.83	78.63	0.58	3.01	2.71	2.79	2.53	21.53
J-16-1 X 1368	2132.32	57.40	55.41	1.98	168.66	74.69	0.59	3.30	2.93	2.87	2.85	26.06
CLWN 349 X 1368	2105.15	58.55	56.82	1.72	172.23	71.47	0.69	2.85	2.58	2.76	2.53	25.01
CLWN 238 X 1368	2027.13	60.17	58.39	1.78	147.02	76.08	0.60	2.96	2.87	3.01	2.83	22.00
CML 254 X 1368	2011.25	58.98	56.82	2.15	164.79	82.12	0.57	3.17	2.64	2.47	2.32	27.03
ZM 521 B-66-4-1-1 X 1368	1722.75	57.67	55.84	1.83	153.05	74.13	0.58	2.84	2.87	3.11	2.72	27.70
TZL Comp3 X 1368	1600.92	60.03	57.99	2.04	142.53	66.66	0.54	3.19	3.14	3.41	2.98	22.23
Etubi – (Check)	2136.65	57.67	55.85	1.82	156.85	72.23	0.56	2.60	2.79	3.10	2.48	29.08
1368 X 9071 (check)	2272.53	58.68	55.97	2.71	173.25	80.61	0.59	2.68	2.34	2.58	2.35	25.55
1368 X 87036 (check)	2201.97	58.35	56.10	2.25	165.72	79.02	0.56	2.89	2.64	2.99	2.51	25.18
87036 X 9071(check)	2716.68	58.00	56.16	1.83	170.05	84.63	0.63	2.77	2.34	2.88	2.38	25.63
Means	2765.77	58.37	56.38	1.99	171.24	82.31	0.69	2.77	2.52	2.65	2.47	25.27
Max	3851.51	61.36	58.70	2.85	190.72	101.61	0.83	3.30	3.14	3.41	2.98	33.70
Min	1600.92	55.29	53.37	1.23	142.53	66.66	0.54	2.21	2.16	2.09	2.00	18.21
SE	156.60	0.41	0.34	0.22	3.60	2.43	0.43	0.13	0.12	0.16	0.11	1.34