Prospects for marker assisted improvement of African tropical maize germplasm for low nitrogen tolerance

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by

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DECLARATION

I Berhanu Tadesse Ertiro hereby declare that this thesis, prepared for the degree Philosophiae Doctor in Plant Breeding, which was submitted by me to the University of the Free State, is my own original work and has not previously in its entirety or in part been submitted to any other university. All sources of materials and financial assistance used for the study have been duly acknowledged. I also agree that the University of the Free State has the sole right to the publication of this thesis.

SUMMARY

Nitrogen (N) is one of the most yield limiting nutrients in maize. However, farmers in sub-Saharan Africa (SSA) use very little N due to low income. Nitrogen Use Efficient (NUE) varieties can provide a partial solution to the problem through efficient N uptake and utilisation. Designing an effective breeding strategy for improving any trait of interest requires knowledge of quantitative genetic parameters, genomic regions associated with the traits and the use of efficient selection methods. The objectives of this study were to 1) assess the efficiency of indirect selection for grain yield under low N stress conditions through grain yield under optimum N conditions and through secondary traits under low N conditions, 2) identify single nucleotide polymorphism (SNP) marker loci significantly associated with grain yield and secondary traits under low N and optimum conditions, 3) map and characterize the quantitative trait loci (QTL) for grain yield and some secondary traits under optimum and low N stressed conditions, and 4) evaluate the accuracy of genomic selection for improvement of grain yield and other secondary traits under optimum and low N stressed environments. Results showed that genetic variance for grain yield was highly affected by low N stress, more than secondary traits, and low correlation was observed between optimum and low N environments for grain yield. This lead to low relative efficiency of indirect selection for grain yield under low N using grain yield under optimum conditions. The efficiency of indirect selection for grain yield under low N through secondary traits under low N conditions was also low. The efficiency of selection could be enhanced through identification of genomic regions and associated markers linked with grain yield under low N. A total of 158 putative protein coding genes associated with significant SNPs, of which seven linked with four known genes, were identified through a genome-wide association study. Markers associated with the putative and known genes could be used for marker assisted selection (MAS) in NUE breeding. In addition, a total of 155 significant QTL were identified for grain yield and six secondary traits under optimum and low N stress conditions in five doubled haploid (DH) lines derived from bi-parental lines. Interestingly, for grain yield, plant height, ear height and leaf senescence, the highest number of QTL were found under low N stressed environments compared to optimum conditions, indicating the availability of QTL under low N. However, no common QTL between optimum and low N stressed conditions

were identified for grain yield and anthesis silking interval. Lack of significant QTL for grain yield common across populations and between management conditions indicates that MAS cannot be an efficient method for selection of grain yield under both optimum and low N conditions. An alternative to MAS is genomic selection, which uses information from all markers. In this study, the magnitude of both genome-wide and phenotypic predictions was negatively affected by low N stress, and phenotypic prediction ability was always higher than genome-wide prediction ability for all traits under both N conditions. Low N stress had a larger effect on the prediction accuracy for grain yield than other secondary traits. In general, genomic selection that uses information from all markers is a promising method for the improvement of the selection efficiency for grain yield under low N.

Key words: Low N stress, genomic selection, maize, marker assisted selection, nitrogen use efficiency, QTL

DEDICATION

This work is dedicated to my late Mother, Almaz Solomon Bela.

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Abbreviations and symbols

A	Main season
AD	Anthesis date
Add	Additivity
ASI	Athesis-silking interval
B	Off-season
BLUP	Best linear unbiased prediction
bp	Base pair
C/N	Carbon to nitrogen ratio
CIMMYT	International Maize and Wheat Improvement Centre
cM	Centi morgan
CML	CIMMYT maize line
CTAB	Cetyl trimethyl ammonium bromide
DAP	Diammonium phosphate
D’	Coefficient of linkage disequilibrium (D)
DH	Doubled haploid
DNA	Deoxyribonucleic acid
EH	Ear height
ENV	Environment
EPO	Ear position
EPP	Ears per plant
FIE1	Fertilization Independent Endosperm 1
FAOSTAT	Food and Agriculture Organization Corporate Statistical Database
Fav	Favourable
FarmCPU	Fixed and random model Circulating Probability Unification
GBS	Genotyping by sequencing
GCA	General combining ability
GD	Genotypic data
GEBV	Genomic estimated breeding value
GM	Genotypic map
GS	Genomic selection
GWAS	Genome wide association study
GXE	Genotype by environment interaction
GY	Grain yield
h^2	Broad sense heritability
H4C7	Histone H4 gene
ha	Hectare
ha^{-1}	Per hectare
HG	Heterotic group
h_{HN}	Square root of heritability under optimum N
h_{LN}	Square root of heritability under low N
ICIM	Inclusive interval mapping
IMAS	Improved maize for Africa
IR	Indirect selection
kg	Kilogram
LD	Linkage disequilibrium
LEAP	Leadership Enhancement in Agriculture Program
LN	Low nitrogen

LNM	Low N during main season (moderate N stress)
LNO	Low N during off-season (severe N stress)
LOD	Logarithm of odds
LOESS	Localized regression curves
LPS	LaPostaSeqC7-F64-2-6-2-2-B-B
m	meter
M	Marker effect
m^2	meter square
MAF	Minor allele frequency
MAS	Marker assisted selection
Mbp	Mega base pair
META-R	Multi-environment trial analysis with R
Mgt	Management
MLM	Mixed Linear Model
N	Nitrogen
NOT1	Neighbor of tga1
NUE	Nitrogen use efficiency
OPT	Optimum N
P_2O_5	Phosphorus pentoxide
PCA	Principal componet axis
PH	Plant height
PSY2	A putative phytonene synthase
PVE	Phenotypic variance explained
QQ	quantile-quantile
QTL	Quantitative trait loci
QTN	Quantitative trait nucleotides
R	Statistical software for data analysis
RE	Relative efficiency
REP	Replication
REML	Restricted maximum likelihood
r_g	Genetic correlation
r_G (LN.HN)	Genetic correlation between grain yields under optimum and low N environments
r^2	Squared allele frequency correlation coefficient
R_g	Response to selection based on genomic value
r_{MG}	Prediction accuracy
r_{MP}	Genome wide prediction
RNAi	Ribonucleic acid (RNA) interference
r_P	Phenotypic prediction
R_p	Response to selection based on phenotypic value
RPS8	Ribosomal protein S8
rrBLUP	Ridged regression of best linear unbiased prediction
SEN	Leaf senescence
SNP	Single nucleotide polymorphism
SSA	Sub-Saharan Africa
TASSEL	Trait analysis by association, evolution and linkage
TGA1	Teosinte Glume Architecture 1
TPVE	Total phenotypic variance explained
UFS	University of the Free States
USAID	United States agency for international development

WEMA	Water efficient maize for Africa
Y	Phenotypic data
δ_E^2	Error variance
δ_G^2	Genotypic variance
δ_{GE}^2	Genotype x environment interaction variance
%	Percentage
µ	Mean

CHAPTER 1

Introduction

Maize is one of the most important cereal crop used for food. In 2014, an estimated area of 184 million hectares of the total world cultivated land was allotted for maize production, surpassed only by wheat. The total production of maize obtained in 2014 was the highest of all cereals. In this year, the total world maize production was estimated at 1 037 791 518 ton (FAOSTAT, 2017). Maize is the staple food in most parts of Africa. In 2014, Africa contributed about 20% of the world's maize area (FAOSTAT, 2017) indicating the importance of the crop on the continent. However, Africa contributes only 8% to the total world maize production. This is mainly due to low productivity of the crop (2.1 t ha^{-1}) as compared to the world average production of 5.6 t ha^{-1}. Several biotic and abiotic constraints play together to affect the productivity of maize.

Poor soil fertility, including low nitrogen (N) stress, is among widespread abiotic factors affecting maize production in sub-Saharan Africa. N is one of the yield limiting nutrients. Because of its role in photosynthesis and transport, plants require N in large quantities to attain normal growth and development. The total world N nutrient consumption in 2014 was estimated at 108,937,126 tonnes of which only 4% was used in Africa (FAOSTAT, 2017). On average, African small holder farmers use less than 10 kg of fertilizer per hectare of crop land (Shiferaw et al., 2011).

Low income of small scale farmers is the main factor limiting African farmers from using the recommended amount of N fertilizer. Thus, N deficiency has become a widespread production constrain on the continent. The traditional approach to overcome the problem is through increasing the application of organic and inorganic fertilizers. Decisions to increase inorganic fertilizers, particularly N, involves both environmental and economic challenges (Presterl et al., 2003). An alternative approach is the use of nitrogen use efficient (NUE) varieties. This approach has been advocated by several scholars as the remedy to address low productivity in sub-Saharan Africa due to economic reasons (Bänziger et al., 1997) and environmental

challenges in the developed world due to excessive use of N fertilizers (Presterl et al., 2003). Developing and growing maize varieties with high NUE will reduce farmers' risk associated with crop failure, provide incentives to invest in inputs like other fertilizers, and allow them to attain food security on a smaller area. Other benefits of high NUE varieties include high yield per unit area, frees up land and labour to grow cash crops, and reduce the risk of forest clearing and fallow cultivation in search of increased yield (Shiferaw et al., 2011). Therefore, high NUE varieties have both economic and environmental advantages.

Like all traits, developing NUE varieties requires genetic variability and an efficient method of selection to achieve gain from selection. Ample literature is available on genetic diversity of maize for NUE and its components (Moll et al., 1987; Lafitte and Edmeades, 1994; Bänziger et al., 1997; 2000; Presterl et al., 2003; Worku et al., 2007; 2012). These studies consistently reported genetic variability for low N tolerance in both tropical and temperate maize germplasm. Some of these studies compared the efficiency of direct selection under low N environments vs. indirect selection under optimum environments (Bänziger et al., 1997; Presterl et al., 2003). These studies found higher efficiency of direct selection under low N environments for improvement of NUE because of different mechanisms under low N and optimum conditions for grain yield (GY). Due to low heritability and genetic variation of GY under low N environments compared to optimum environments, some authors suggested the incorporation of some secondary traits like anthesis silking interval (ASI), plant height (PH) and ears per plant (EPP) for selection of GY under low N conditions. These traits have high heritability; they are easy to measure and highly correlated with GY under low N environments. Though some progress has been made through these approaches, new techniques like molecular markers are believed to, in future, further enhance the efficiency of selection.

Marker-based selection can be used to enhance the efficiency of selection and thereby increase gain from selection. In this study, the efficiency of different selection methods for the improvement of GY under low N conditions were investigated with the objective of identifying cost effective selection methods. This study aimed to investigate the efficiency of indirect selection for GY under low N and identification of quantitative trait loci (QTL) associated with GY and secondary traits under both optimum and low N

conditions. Identifying QTL underlying GY and secondary traits under low N conditions are believed to increase the efficiency of gain from selection using marker assisted selection (MAS). The last aim focused on the use of all markers for increasing selection efficiency, as MAS relies only on a few and significant marker effects. This behaviour of MAS is often criticised as it is not suited for quantitative traits controlled by many small effect QTL. Genomic selection (GS) is advocated as the best for such traits as it uses marker effects from all markers to estimate the genomic estimated breeding values (GEBVs) of inbred lines. The GEBVs from the training set is then used to estimate the breeding value of other untested lines. Using this approach, GS can improve the efficiency of the breeding programme. Therefore, the major objective of this study was to identify the most efficient selection method for GY improvement under low N conditions, by comparing conventional and marker based approaches.

The specific objectives were to:

i. Estimate the efficiency of indirect selection for GY under low N conditions through GY under optimum conditions and through secondary traits under low N conditions
ii. Identify QTL underlying GY under low and optimum N conditions using traditional linkage analysis
iii. Identify marker trait associations for GY and secondary traits under optimum and low N through a genome-wide scan
iv. Estimate the efficiency of genomic selection for GY and secondary traits under optimum and low N conditions

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CHAPTER 2

Literature review

2. 1. Maize

Maize (*Zea mays* L.), also known as corn, is a cereal grain first domesticated by indigenous people in southern Mexico about 10 000 years ago (Paliwal, 2000a). Maize is a diploid species with a basic set of ten (n=10) chromosomes (Paliwal, 2000b). The spread of maize from its origin to various parts of the world has been remarkable and rapid. Native inhabitants of various “indigenous” tribes took this food plant to other regions and countries of Latin America, the Caribbean and then to the United States and Canada. European explorers took maize to Europe and traders later took it to Asia and Africa (Paliwal, 2000a). Currently, maize has a very wide environmental adaptation ranging from temperate to tropical environments, from sea level to above 3000 meters above sea level (masl) and cultivated on diversified soil types.

Maize production plays a significant role in world agriculture. In 2016, maize was grown for grain or silage on more than 188 million hectares worldwide. In the same year, the total production was 1060 million ton with average yield of 5.6 ha^{-1} (http://www.fao.org/faostat/en/#data/QC). The production and productivity of maize is affected by several biotic and abiotic factors, of which low soil fertility is the major one (Sanchez, 2002). Nitrogen is one of the nutrients plants require in large quantities. Application of high dose of fertilizer is not feasible in both the developing and developed world due to economic reasons and environmental concerns, respectively (Bänziger et al., 1997; Presterl et al., 2003; Weber et al., 2012). Therefore, cultivation of nitrogen use efficient (NUE) varieties is often recommended to achieve reasonable yield from lower doses of N application and to reduce ground water pollution due to excessive N application (Bänziger et al., 1997; Presterl et al., 2003; Weber et al., 2012).

2.2. Nitrogen use efficiency

Depletion of soil fertility, mainly N, along with other biotic and abiotic factors are one of the main reasons for low productivity of maize in sub-Saharan Africa (Sanchez, 2002). Nutrients lost due to crop production and other reasons (leaching, denitrification, etc) are often compensated through the application of inorganic fertilizers. Small scale farmers who are the main producers of food in Africa can hardly afford the high price of inorganic fertilizers (Lafitte and Edmeades, 1994; Sanchez, 2002, Weber et al., 2012). In agriculture based economies of Eastern Africa (Ethiopia, Kenya, Tanzania and Uganda) for example, small holder farming accounts for 75% of agricultural production and over 75% of employment (Salami et al., 2010). Fertilizer application in sub-Saharan Africa is negligible, accounting for less than 1% of the global N fertilizer application. The development of improved maize germplasm with NUE is a cost effective and environmentally friendly approach that could increase yields and have a major impact on livelihoods, food security and sustainability in sub-Saharan Africa. Low N stress affects GY and several traits related to GY (Bänziger et al., 1997; 2000; 2006; Presterl et al., 2003; Worku et al., 2007a; 2008; 2012) and grain quality (Borrás et al., 2002; Duarte et al., 2005; Worku et al., 2007b; Ngaboyisonga et al., 2012). The problem of low soil N is not limited to only eastern and southern Africa, but also the west and central African sub-region (Ajala et al., 2018). Improvement of the NUE is economically and environmentally a sound method for increasing food production in sub-Saharan Africa.

Moll et al. (1987) defined NUE for grain maize as “the grain yield per unit of N from soil” including N fertilizer. Liang and MacKenzie (1994) defined NUE as the total plant N divided by the amount of N applied. NUE is a complex trait that has two major components. It is the product of N uptake efficiency (N uptake per N from soil), and N utilisation efficiency (yield per N uptake) (Gallais and Hirel, 2004; Worku et al., 2007a). For a given N fertilization, NUE is strictly related to GY, and N uptake efficiency is strictly related to total N uptake. NUE is an expression of the carbon to N ration (C/N); for grain, it can also be defined as the product of N utilization efficiency (NUtE) at the whole plant level and the harvest index; it is then directly related to N partitioning, such as post anthesis N-remobilisation. In 16 tropical hybrids evaluated in Zimbabwe and Kenya, Worku et al. (2007a) found that high GY under low N was consistently

associated with higher post-anthesis N uptake, increased grain production per unit N accumulated, and an improved N harvest index. To develop varieties with improved NUE it is thus necessary to have genetic variability in the germplasm collection for N uptake efficiency and for N utilisation efficiency. It is also important to know the relationships of such traits to agronomic traits such as GY (Gallais and Hirel, 2004) and to identify appropriate testing environments where the germplasm are evaluated for GY and associated traits.

Many studies confirmed the presence of considerable genetic variability for NUE in both tropical and temperate maize germplasm (Bänziger et al., 1997; Bertin and Gallais, 2000; Presterl et al., 2003; Weber et al., 2012; Ajala et al., 2018). Generally, the extent of genetic variances under low N was lower than under optimum conditions (Bertin and Gallais, 2000). Bänziger et al. (1997) evaluated lowland tropical germplasm in 14 replicated trials under optimum and managed low N stress environments in CIMMYT, Mexico. The study found lower GY and genetic variances for GY under low N than optimum environments. A similar study was conducted with temperate germplasm and contrasting results were found for GY genetic variance with untransformed and transformed data: high genetic variance was seen under low N using untransformed data and low genetic variance under low N conditions using transformed data (Presterl et al., 2003). The genetic variability for GY in maize germplasm reflects differences in GY under low N conditions. The genetic variation in the maize germplasm could be favourably exploited for the development of NUE for low N stress environments through testing in appropriate environments.

Studies which assessed the efficiency of selection environments for low N conditions indicated higher efficiency of direct selection under low N conditions than indirect selection under optimum conditions. In a study of Bänziger et al. (1997), prediction efficiency of indirect selection for low N under high N conditions was significantly lower than direct selection under low N conditions, particularly when relative yield reduction due to low N stress was high (> 43%) for lowland tropical germplasm. Similar results were reported for temperate germplasm. The efficiency of indirect selection for low N under optimum was reported to be 70% of direct selection under low N stressed environments. Generally, direct selection under low N stressed conditions is the most efficient approach for predicting performance under low N (Bänziger et al., 1997;

Presterl et al., 2003; Weber et al., 2012). According to Bänziger et al. (2000) and Chapman and Edmeades (1999), the genetic variation for tolerance to stress conditions is revealed to a greater extent when genotypes are planted under managed stress conditions than random drought or optimum conditions, and therefore they proposed the evaluation of genotypes under managed stress conditions. With high N-input, genetic variation in NUE was explained by variation in N uptake, whereas with low N-input, NUE variability was mainly due to differences in NUE (Gallais and Hirel, 2004).

Efforts to improve the NUE have been underway through the evaluation of germplasm under both optimum and low N environments. In addition to indirect selection, other secondary traits correlated with GY have been identified and used to facilitate the improvement of GY under low N. Research results indicated higher importance of anthesis silking interval (ASI), sencescence (SEN), ears per plant (EPP) as the most important secondary traits for selection of high yielding genotypes under low N (Lafitte and Edmeades, 1994). The advent of molecular markers also brought a new opportunity for efficient and cost effective selection tools for the improvement of NUE. QTL identification through conventional and genome-wide association mapping studies are widely used for the dissection of genomic regions underlying GY and other secondary traits under low N condition (Ribaut et al., 2007).

2.3. Marker based approaches to improve nitrogen use efficiency

Marker based approaches can offer significant advantages, particularly for expensive or difficult traits, for traits controlled by multiple genes and recessive genes (Bernardo, 2008). In addition to reducing costs of conventional breeding, it has the potential to generate time savings. The use of markers for crop improvement starts with knowing the exact location of the genes involved in the control of given traits and identifying diagnostic markers. QTL mapping/analysis can be used to understand the genetic architecture of quantitative traits, thereby relating specific genetic loci with the biological mechanisms associated with desirable phenotypes (Agrama, 2006).

2.3.1. QTL mapping for grain yield and related traits under low N conditions

Plant breeders achieved considerable improvement of yield and other economically important traits mainly through visual selection coupled with statistical inference (Agrama, 2006). Use of additional selection tools such as molecular markers help breeders achieve further improvement in GY and abiotic stress tolerance. Molecular markers enable breeders to exercise selection that is based on genotypic or DNA-based differences rather than phenotypic differences, and they therefore have the potential to greatly increase selection efficiency. Incorporation of molecular markers for improvement of a trait requires the identification of genomic regions associated with the trait of interest of the target species. High yield and better performance of other yield related traits under low N conditions are an indication of better NUE. The genetic mechanisms for GY under optimum and low N conditions are distinct, where genotypes that are high yielding under optimum conditions may not necessarily perform the same under low N conditions. Dissecting the genomic regions involved in the control of GY under low N conditions helps to pave the way towards the implementation of MAS for high yield under low N conditions (Agrama, 2006; Ribuat et al., 2007).

The most common method of QTL detection is the use of bi-parental mapping populations. Despite large numbers of publications on QTL detection for abiotic stress tolerance on maize, only a few were done for low N stress conditions (Ribaut et al., 1996; 2007; Agrama et al., 1999; Almeida et al., 2013; 2014, Semagn et al., 2013; 2014; Fan et al., 2015; Zaidi et al., 2015). Ribaut et al. (2007) used 240 $F_{2:3}$ families and identified eight QTL for GY under low N conditions, of which two were also detected under optimum conditions. Using 413 introgression lines Liu et al. (2012) identified 33 QTL for GY and yield components under N limiting conditions. To better understand quantitative genetic basis of NUE, Hirel et al. (2001) developed a quantitative genetic approach by associating metabolic functions and agronomic traits with DNA markers. QTL analysis for GY and various physiological traits identified several loci related to the traits on the genetic map of maize and observed QTL associations between GY and glutamine synthetase and nitrate reductase activity. Based on this information, Hirel et al. (2001) hypothesized that leaf nitrate accumulation and the reactions catalysed by glutamine synthetase and nitrate reductase are co-regulated and represent key elements controlling NUE in maize.

The studies conducted so far are important for the understanding of genetic architecture NUE in maize. However, the use of the QTL identified so far are limited because of several challenges associated with QTL mapping. The need for building mapping populations distinct from breeding populations limit the size of mapping populations and, consequently, the accuracy of QTL position and effect estimates (Dekkers and Hospital, 2002). In addition, allelic diversity and genetic background effects that are present in a breeding programme will not be captured with a single bi-parental population. Therefore, accurate estimation of QTL requires multiple mapping populations from diverse sources, which entails high cost. After identifying the QTL, validation of the results in locally adapted germplasm is another key step. Failure to carry out these will lead to gains from MAS that are inferior to traditional phenotypic selection because of poor estimates of the numerous small effect QTL (Bernardo, 2001). The resources required for QTL detection coupled with validation and effect re-estimation limit the effectiveness of bi-parental population derived QTL for MAS in plant breeding populations (reviewed by Holland, 2004).

2.3.2. Genome-wide association studies

To avoid the disconnect between bi-parental and breeding populations, linkage disequilibrium (LD) based mapping was proposed for dissecting complex traits in breeding populations (Rafalski, 2010; Jannink et al., 2010). This strategy avoids the need to develop mapping populations other than the breeding population that impose an additional burden on breeding programmes. Also, mapping within breeding populations will allow for QTL identification and allelic value estimates that can be directly utilised by MAS without the need for extensive validation (Breseghello and Sorrells, 2006; Holland, 2004). Essentially, association mapping exploits historical and evolutionary recombination at the population level. Association mapping offers three advantages over linkage analysis: much higher mapping resolution; greater allele number and a broader reference population; and less research time in establishing an association (Flint-Garcia et al., 2003). Linkage analysis and association mapping, however, are complimentary to each other in terms of providing prior knowledge, cross-validation, and statistical power (Wilson et al., 2004).

Based on the scale and focus of a study, association mapping is generally grouped into two broad categories: candidate-gene association mapping and genome-wide

association mapping. Candidate-gene association mapping relates polymorphisms in selected candidate genes that have purported roles in controlling phenotypic variation for specific traits (Zhu et al., 2008). Genome-wide association mapping (or genome scan), on the other hand, surveys genetic variation in the whole genome to find signals of association for various complex traits. While researchers interested in a specific trait or a suite of traits often exploit candidate-gene association mapping, a large consortium of researchers might choose to conduct comprehensive genome-wide analyses of various traits by testing hundreds of thousands of molecular markers distributed across the genome for association (Zhu et al., 2008).

Association mapping analysis is performed based on the principle of linkage disequilibrium. The terms “association mapping” and “linkage disequilibrium” are often used interchangeably. However, in the strictest sense, the two terms have different meanings and explain different phenomena. While association mapping refers to significant marker-trait association, linkage disequilibrium is the non-random association of alleles, markers or genes/QTL between genetic/marker loci (Flint-Garcia et al., 2003; Gupta et al., 2005; Yu and Buckler, 2006). In this context, association mapping is one of several uses of linkage disequilibrium (Gupta et al., 2005) and the comparatively high-resolution provided by association mapping is dependent upon the structure of linkage disequilibrium across the genome.

The number of markers required for association mapping and the mapping resolution are determined by the extent of LD decay over physical distance in a population (Flint-Garcia et al., 2003). For example, if LD decays rapidly, then a higher marker density is required to capture markers located close enough to functional sites. Flint-Garcia et al. (2003) reviewed the extent of LD levels varying both within and between species. LD extends less than 1000 bp for maize landraces, 2000 bp for diverse maize inbred lines, and 100 kb for commercial elite inbred lines. The diversity in elite and commercial inbred lines is less than in maize landraces due to inbreeding and selection. LD decay can also vary considerably from locus to locus. For example, significant LD was observed up to 4 kb for the Y1 locus (encoding phytonene synthase), but was seen at only 1 kb for PSY2 (a putative phytonene synthase) in the same maize population (Yu and Buckler, 2006). Many genetic and non-genetic factors,

including recombination, drift, selection, mating pattern, and admixture, affect the structure of LD (Flint-Garcia et al., 2003; Gaut and Long, 2003).

Several approaches are available for measuring the magnitude of LD (Flint-Garcia et al., 2003; Gupta et al., 2005). Of all measures, D' and r^2 are the most preferred and common measures in plants. The choice between the two common methods depends on the objectives of the study. D' measures only recombination differences while r^2 summarises recombination and mutation history. The r^2 also indicates how markers may be correlated with the QTL of interest, therefore for association studies, r^2 is often preferred (Flint-Garcia et al., 2003; Gupta et al., 2005). LD based association studies on maize identified genomic regions and putative genes underlying GY and yield related secondary traits on maize (Flint-Garcia et al., 2003; Gupta et al., 2005).

Increasing the biological knowledge of the inheritance and genetic architecture of quantitative traits and identifying markers for selection of a complex trait (Bernardo, 2008) are the general objectives of QTL mapping studies. The latter objective is more related to plant breeding and leads to MAS to facilitate rapid gains from selection. Despite several reports on QTL, model genes and markers associated with traits of interest in different crop species over the last three decades, most are not adequately exploited in breeding programmes (Bernardo, 2008). MAS has several limitations that prevent their routine use in plant breeding programmes (Jannink et al., 2010). Jannink et al. (2010) summarised the major limitations of QTL identification methods that can make MAS poorly suited to crop improvement. These are (i) use of bi-parental populations that are not representative and do not have the same level of allelic diversity and phase as the breeding programme as a whole; (ii) the high cost of generating mapping populations; (iii) the requirement of the validation of the identified QTL that requires additional resources and efforts; (iv) the separation of QTL identification from estimation, means that estimated effects will be biased, and small-effect QTL will be missed entirely as a result of using stringent significance thresholds. Now new methods are available that eliminate most the limitations of MAS.

2.3.3. Genomic selection

MAS has several limitations that restricts its application for routine selection in plant breeding programmes. The use of MAS has been limited to the improvement of simple and monogenic traits. Of all the limitations, the use of bi-parental populations that are not representative of the breeding population (used for detection of QTL) hinder the application of MAS the most. The bi-parental populations used for QTL mapping are not representative of the breeding population and do not capture the gene diversity and germplasm background differences present in the breeding population. In addition, the statistical methods used in MAS are not suited to the polygenic nature of quantitative traits (Jannink et al., 2010). In statistical analysis, MAS first identifies significant QTL and then estimates their effects (Jannink et al., 2010).

Association mapping applied directly to breeding populations has been proposed to mitigate the lack of relevance of bi-parental populations in QTL identification (Rafalski, 2010). However, low heritability, small population sizes, few large-effect QTL, confounding population structure, and arbitrary significance thresholds found in current association mapping efforts allow identification of only a few QTL with overestimated effects (Schön et al., 2004).

Genomic selection is a form of MAS that simultaneously estimates all marker effects across the entire genome to calculate GEBVs (Meuwissen et al., 2001). Unlike MAS, there is no defined subset of significant markers used for genomic selection (Meuwissen et al., 2001; Heffner et al., 2009). In GS, all markers are fitted simultaneously to avoid biased marker effects and capture all the small effects (Heffner et al., 2009). In genome-wide selection, the population is divided into two parts: training and test sets. The training set is both genotyped and phenotyped while the test set is only genotyped. The training set is used to estimate marker effects. Then genotypic values of individuals in a test population are predicted from the marker effects estimated from the training population. The central process of GS is the calculation of GEBVs for individuals having only genotypic data (Meuwissen et al., 2001). These GEBVs are then used to select the individuals for advancement in the breeding cycle. Therefore, selection of an individual without phenotypic data can be performed by using a model to predict the individual's breeding value (Meuwissen et

al., 2001). This process of predicting the performance of individuals which are not phenotyped but genotyped, decreases the breeding cycle time and increases genetic gain per unit time (Zhang et al., 2014). To maximise GEBV accuracy, the training population must be representative of selection candidates in the breeding programme to which GS will be applied.

Simulation (Bernardo and Yu, 2007) and empirical (Massman et al., 2013) studies on maize have shown 14 to 50% higher gains with genome-wide selection than with QTL-based selection (marker assisted recurrent selection). Genome-wide selection studies on maize (Dawson et al., 2013; Jacobson et al., 2014; Krchov et al., 2015), wheat (Dawson et al., 2013) and rice showed relatively higher prediction accuracy of genome-wide selection for GY and secondary traits of economic importance. Also, De los Campos et al. (2009), Malosetti et al. (2007) and Crossa et al. (2011), using extensive empirical maize and wheat data, demonstrated that using low-to-intermediate marker density and pedigree information increased the prediction accuracy of unobserved phenotypes. Most studies reported, however, were conducted under optimally managed experimental conditions. Some studies which assessed the accuracy of genome-wide prediction under water stressed and well-watered conditions verified the higher advantage of genomic selection. Zhang et al. (2014) estimated the prediction accuracy of genome-wide selection in 19 tropical maize bi-parental population and reported consistently lower and variable prediction accuracy under stress conditions than optimum conditions for all the target traits. They attributed the low prediction accuracy to poor heritability under stress conditions. In a study that compared GS and marker assisted recurrent selection under drought stress condition, Beyene et al. (2015) found higher genetic gain through genome-wide selection for GY after two cycles of genome-wide selection under drought stress environments. Because of the consideration of all information from all markers, genomic selection is believed to be more important for stress environments including low N stress conditions than the traditional MAS.

The accuracy of genomic prediction is affected by the composition and number of individuals in a training population. One of the most important applications of genomic selection in maize breeding is to predict and identify the best untested lines from bi-parental populations, when the training and validation sets are derived from the same

cross (Lian et al., 2014). In this approach, both the training and the test sets are drawn from the same bi-parental population. Despite high prediction accuracy, it has the limitation of failure to know the accuracy prior to planting the prediction set. An alternative to this is the general combining ability (GCA) approach. In the GCA model, the performance of the prediction set can be estimated without the need for evaluating them. For example, if the training population is composed of the A/B and C/D bi-parental populations, the values of another bi-parental population, for example A/C, can be predicted without the need to phenotype A/C. This method was successful in 969 bi-parental populations in temperate germplasm under optimum conditions (Jacobson et al., 2014). Different methods such as best linear unbiased prediction (BLUP), ridge regression, Bayesian regression, kernel regression and machine learning methods have been proposed to develop prediction models for genome-wide selection that overcome the problems associated with over fitting of models (Meuwissen et al., 2001; Heffner et al., 2009).

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CHAPTER 3

Manuscript 1: Efficiency of indirect selection for grain yield under low N stress through secondary traits and grain yield under optimum conditions

3.1 Abstract

Small scale maize farmers in SSA use meager amounts of N in their maize crops mainly due to low income. NUE varieties can provide a solution to the problem of low N conditions through efficient N uptake and utilization. Designing an effective breeding strategy for improving any trait of interest requires knowledge on quantitative genetic parameters and the use of efficient selection methods. The objectives of this study were to: 1) compare the quantitative genetic parameters of GY and secondary traits under optimum and low N environments and 2) assess the efficiency of indirect selection for GY under low N stress through GY under optimum N and through secondary traits under low N stress. DH lines derived from five bi-parental populations were planted in replicated trials under optimum N and low N field. The low N fields were depleted for several seasons and no N fertilizer was applied. Genotype effect for GY and secondary traits was significant ($P \leq 0.05$) in all optimum and low N sites. Low N stress reduced mean GY and plant and ear heights. Genetic variance for GY was, on average, reduced by 17% under moderate N stress and 63% under severe N stress conditions, while genetic variances for days to anthesis and plant height increased under both moderate and severe low N stress conditions. The heritability of most secondary traits was consistently higher under both management conditions compared to the heritability of GY. Phenotypic and genetic correlations of GY with plant and ear height was positive under low N conditions. Genotypic correlations were higher than phenotypic correlations for all traits under both N conditions. The relative efficiency of indirect selection for GY under low N using GY from optimum environments ranged from 0.14 to 0.74 with an overall average of 0.45. The efficiency of indirect selection for GY through secondary traits was less than one for most traits. It was concluded that despite reduction in genetic variances under stress conditions, there was genetic variability for GY and other secondary traits under low N conditions.

Direct selection for GY under low N rather than under optimum conditions was more efficient for improvement of yield under low N conditions. The use of an index of secondary traits could result in higher efficiency of GY improvement rather than selection for only GY under low N conditions.

Key words: Nitrogen, NUE, indirect selection, low N, phenotypic correlation, genetic correlation

3.2. Introduction

N is one of the nutrients required by plants in comparatively large amounts. Its role is critical in photosynthesis, protein synthesis and in virtually every other aspect of plant physiology. Despite its importance in plant physiology and thus productivity, farmers in developing countries have limited access to N fertilizers, mainly due to unavailability or high cost of fertilizer (Lafitte and Edmeades, 1994, Weber et al., 2012). According to the World Bank Report (2015), fertilizer consumption (kilogram per hectare of arable land) for SSA was only 15 kg ha^{-1} compared to 157.2 kg ha^{-1} for the European Union countries in the same period. Other estimates indicate much lower rates of fertilizer application: African small holder farmers use less than 10 kg of fertilizer per hectare of crop land (Shiferaw et al., 2011). Contrary to the on-farm conditions in most of Africa, most maize varieties developed are bred under optimally managed environments (well-fertilized) that are not representative of the target growing environments.

Selection for GY performance under low N stress conditions can be done through one of three ways: i) selection under optimum conditions, ii) selection under low N conditions or iii) selection under both optimum and low N conditions. The choice of any of the three methods is mainly dictated by the magnitude of the relationship between the two environments. The correlation between optimally managed and low N stress environments was reported to be positive but low (Bänziger et al., 1997; Presterl et al., 2003). Simultaneous selection under optimum and low N stress environments has resulted in improved GY under both optimum and low N environments (Lafitte and Edmeades, 1994) showing the potential for simultaneous improvement of GY under both management conditions. NUE, which is both N uptake

and utilisation efficiency (Hirel et al., 2001; Gallais and Hirel, 2004), is an important characteristic for achieving simultaneous improvement under optimum and low N conditions. A large body of literature is available on the presence of large genetic variability in maize germplasm for NUE (Lafitte and Edmeades, 1994; Bänziger et al., 1997; Hirel et al., 2001; Presterl et al., 2002; 2003; Gallais and Hirel, 2004; Worku et al., 2007; 2008; 2012).

Designing an efficient breeding strategy for improving any trait of interest requires knowledge of quantitative genetic parameters (such as variances, heritability and correlated response of traits) and the stability of these parameters across target environments and different genetic backgrounds. In tropical maize germplasm with a different selection history under low N environments, Bänziger et al. (1997) reported higher heritability for GY under optimum N conditions, similar error variances under low and optimum N, and positive genetic correlation between optimum and low N conditions. They also observed decreased efficiency for indirect selection for yield under low N conditions with increased levels of stress. Presterl et al. (2003) found higher variances for genotype, genotype by location interaction and error under low N stress compared to optimum conditions using untransformed data, but the opposite when the data was transformed, in temperate maize. Among full-sib families forming part of two selection cycles (C_0 and C_2) of a recurrent selection scheme in the tropical maize population “Across 8328 BN”, Lafitte and Edmeades (1994) reported stronger genetic correlation (rg = 0.51) than phenotypic correlation. Availability of high genetic variance, and correlation between traits or environments, are among determinant factors for doing direct or indirect selection through correlated traits or environments.

Indirect selection for GY based on secondary traits is an easy, fast and cheap approach compared to direct selection for GY (Bernardo, 2002) due to relatively high heritability of secondary traits and high genetic correlation between secondary traits and GY under low N. Due to low cost and effectiveness, indirect selection for GY under low N based on GY under optimum conditions, or through secondary traits, could increase gain because indirect selection is relatively quicker and cheaper than direct selection in the target environment or for the primary traits (Bänziger and Lafitte, 1997; Bänziger et al., 1997). Indirect selection for primary traits based on secondary traits was reported to be successful for perennial ryegrass under optimum management

(Conaghan et al., 2008). Bänziger and Lafitte (1997) studied the efficiency of secondary traits to improve maize yield for low N target environments. The results showed that among secondary traits, ears per plant and leaf senescence discriminated high-yielding genotypes the best, while leaf chlorophyll concentration, and in some instances ASI, provided information on environmental variation within experiments. The authors concluded that secondary traits can increase the efficiency of selection for GY in maize breeding programmes targeting low N environments. Ziyomo and Bernardo (2013) found indirect selection for GY under drought through secondary traits (ASI, SEN or chlorophyll content) in maize to be less efficient.

Indirect selection for GY under low N stress based on GY under optimum conditions has an advantage in terms of cost and the anticipated gain from selection. Comparison of indirect selection in a particular set of growing environments (such as optimum N in a conventional system) with direct selection in another set of environments (low N stress and organic systems) was inefficient for GY in maize (Bänziger et al., 1997; Presterl et al., 2003; Lorenzana and Bernardo, 2008; Ziyomo and Bernardo, 2013) and wheat (Brancourt-Hulmel et al., 2005). However, most of the studies used diverse maize germplasm and progenies with a different selection history in target environments or single bi-parental populations. The objectives of this study were: 1) to compare the quantitative genetic parameters (heritability, variance and genetic correlation) of GY and secondary traits under optimum and low N environments and 2) to assess the efficiency of indirect selection for GY under low N stress through GY under optimum N and through secondary traits under low N.

3.3. Materials and methods

3.3.1. Plant materials

Seven hundred and six DH lines derived from five bi-parental populations obtained from CIMMYT were used for this study. All DH lines were developed through the *in vivo* DH technique (Geiger and Gordillo, 2009). Population 1 (CML494/CML550), population 2 (CML504/CML550) and population 3 (CML511/CML550) were represented by 108, 219 and 111 heterotic group B DH lines, respectively. These DH lines were initially developed for marker assisted recurrent selection for low N stress breeding under the Improved Maize for African Soils (IMAS) project. Population 4

(CML505/LaPostaSeqC7-F64-2-6-2-2) and population 5 (CML536/LaPostaSeqC7-F64-2-6-2-2) represented by 159 DH lines and 109 DH lines, respectively, both from heterotic group A, were developed under the Water Efficient Maize for Africa (WEMA) project. CML550, LaPostaSeqC7-F64-2-6-2-2 and CML494 are among the top 20 low N donor inbred lines identified from a 412 panel of inbred lines tested under low N while CML504, CML505 and CML536 were low N sensitive inbred lines. DH lines derived from population 1 were test crossed to CML312, and those DH lines derived from population 2 and 3 were test crossed to CML312/CML443, and population 4 and 5 to CML395/CML444. Low N tolerant donor inbred lines (CML494, CML550 and LaPostaSeqC7-F64-2-6-2-2) combined with other low N sensitive inbred lines were used to form tolerant by tolerant and tolerant by susceptible bi-parental populations which were used for development of DH lines.

3.3.2. Trial management and data collection

Testcross progenies from the DH lines derived from the five populations were organized into five different trials and planted across one to ten sites in Kenya and Rwanda between the main season (A) of 2014 and off-season (B) of 2015 (Table 3.1; Appendix Tables 2-16). In each trial, three to seven commercial checks were tested along with testcross progenies. All optimum trials were evaluated during main seasons. For low N trials, some were evaluated during main seasons while others were evaluated during the off-season to capture the seasonal variability of N availability. All trials were laid out in an alpha lattice design (Patterson and Williams, 1976) each with two replications except one site for each population 2 and population 3, that had three replications at the low N site in Kiboko during the 2014 off-season. In all sites, plots were hand planted with inter and intra row spacing of 0.75 m and 0.25 m, except Kiboko. At Kiboko, a row length of 4 m, with inter and intra row spacing of 0.75 m and 0.2 m were used under both optimum and managed low N stress sites. Two seeds per station were used in all sites to ensure optimum plant populations. Three weeks after germination, plots were thinned to one plant per station to achieve a final plant density of 53 000 plants per hectare. At planting, only triple phosphate (46% P_2O_5) was applied to all low N trials at a rate of 50 kg P_2O_5 ha^{-1}. For optimum trials, Diammonium phosphate (DAP) fertilizer was used at the recommended rate for each location. Four weeks after planting, all optimum trials were top dressed with urea

fertilizer. The rate and type of fertilizer applied was the same during main and off-seasons.

Table 3.1 Trial management information and quantitative genetic parameters for testcross progenies of five DH bi-parental populations evaluated in five trials under optimum and low N conditions in Kenya and Rwanda from seasons 2014A to 2015B

Trial Name	Site	Year*	Number Entries	Number Checks	Mgt	Rep	Mean GY	σ^2g	σ^2e	h^2
CML494/CML550; Tester: CML312										
15B-EMB-8	Embu	2015B	110	2	LNO	2	2.24	0.12	0.43	0.36
15A-KKM-9	Kakamega	2015A	110	2	Opt	2	7.13	0.63	1.32	0.49
15A-RWA-3	Rwanda	2015A	110	2	Opt	2	6.72	0.65	2.11	0.38
15A-KBK-1	Kiboko	2015A	110	2	Opt	2	8.88	0.47	1.35	0.41
15A-KBK-2	Kiboko	2015A	110	2	LNM	2	4.00	0.31	0.76	0.45
15B-KBK-5	Kiboko	2015B	110	2	LNO	2	2.05	0.10	0.23	0.48
15A-KIT-7	Kitale	2015A	110	2	LNM	2	5.43	0.24	0.93	0.34
15A-MTW-4	Mtwapa	2015A	110	2	LNM	2	4.10	0.09	0.51	0.25
CML504/CML550; Tester: CML312/CML443										
14A-ALU-9	Alupe	2014A	224	5	LNM	2	3.60	0.35	0.81	0.46
14A-EMB-5	Embu	2014A	224	5	LNM	2	4.22	0.15	0.61	0.34
14A-KKM-3	Kakamega	2014A	224	5	**LNO**	2	2.40	0.07	0.39	0.27
14A-KKM-4	Kakamega	2014A	224	5	**Opt**	2	5.35	0.87	0.50	0.78
14B-KBK-1	Kiboko	2014B	224	5	LNO	3	**2.92**	0.24	0.47	0.61
14B-KBK-2	Kiboko	2014B	224	5	LNO	3	2.39	0.14	0.44	0.50
14A-KBK-1	Kiboko	2014A	224	5	Opt	2	**6.49**	0.20	0.75	0.35
14A-KBK-2	Kiboko	2014A	224	5	Opt	2	10.60	0.95	1.52	0.56
14A-KIT-10	Kitale	2014A	224	5	Opt	2	5.67	0.37	1.41	0.35
14A-KIT-8	Kitale	2014A	224	5	Opt	2	4.91	0.48	2.58	0.27
CML511/CML550; Tester: CML312/CML443										
14A-ALU-9	Alupe	2014A	116	6	LNM	2	2.65	0.25	0.40	0.56
14A-KKM-3	Kakamega	2014A	116	6	**LNM**	2	2.72	0.13	0.49	0.35
14A-KKM-4	Kakamega	2014A	116	6	**Opt**	2	4.99	0.49	0.42	0.70
14B-KBK-1	Kiboko	2014B	116	6	LNO	3	2.12	0.11	0.37	0.47
14B-KBK-2	Kiboko	2014B	116	6	LNO	3	**2.83**	0.15	0.38	0.53
14A-KBK-2	Kiboko	2014A	116	6	Opt	2	10.40	0.79	0.94	0.63
14A-KBK-5	Kiboko	2014A	116	6	Opt	2	**6.07**	0.34	0.91	0.42
14A-KIT-10	Kitale	2014A	116	6	Opt	2	5.19	0.79	1.68	0.49
14A-KIT-8	Kitale	2014A	116	6	Opt	2	5.35	1.01	2.39	0.46
14A-MTW-6	Mtwapa	2014A	116	6	LNM	2	2.47	0.42	0.61	0.58
CML505/LaPostaSeqC7-F64-2-6-2-2-B-B; Tester: CML395/CML444										
WET15A-EVALITC-08-1	Kakamega	2015A	174	6	Opt	2	7.53	0.75	1.75	0.46
WET15A-EVALITC-08-2	Kiboko	2015A	174	6	Opt	2	5.81	0.17	0.75	0.31
WET15A-EVALITC-08-5	Kiboko_LN	2015A	174	6	LNM	2	4.29	0.17	1.03	0.25
WET15A-EVALITC-08-6	Kiboko2_LN	2015A	174	6	Opt	2	5.12	0.04	1.17	0.06
WET15A-EVALITC-08-8	Kiboko3_LN	2015B	174	6	LNO	2	1.11	0.05	0.10	0.51
CML536/LaPostaSeqC7-F64-2-6-2-2-B-B; Tester: CML395/CML444										
WET15A-EVALITC-11-1	Kakamega	2015A	130	8	Opt	2	8.58	1.10	2.26	0.49
WET15A-EVALITC-11-2	Kiboko	2015A	130	8	Opt	2	5.00	0.60	0.94	0.56
WET15A-EVALITC-11-5	Kiboko_LN	2015A	130	8	LNM	2	4.33	0.41	1.08	0.43
WET15A-EVALITC-11-6	Kiboko2_LN	2015A	130	8	Opt	2	5.71	0.46	1.44	0.39
WET15A-EVALITC-11-8	Kiboko3_LN	2015B	130	8	LNO	2	1.25	0.13	0.20	0.56

A, main season; B, off-season; LN, low N; opt, optimum N; LNM, low N site during main season; LNO, low N during off-season (severe low N stress); Mgt, management; Rep, replication; σ^2g, genotypic variance; σ^2e, error variance; h^2, broad sense heritability

Optimum and low N trials at Kiboko were irrigated as required throughout the growing season to avoid moisture stress, but trials on all other sites were rain fed. Except for N fertilization, the same management was applied to trials planted under optimum and low N stress sites. The low N trial fields were depleted for several seasons and no N fertilizer was applied. Measurements were taken for male flowering date as the number of days from planting to when 50% of plants shed pollen, plant and ear heights, as the distance in centimeters from the base of the plant to the first branch of the tassel and the upper most ear, respectively. GY per hectare for each plot was estimated from the field weight by adjusting the grain moisture to 12.5%. At harvest, edge plants from both sides of rows were removed from all trials to avoid border effects.

3.3.3. Data analysis

META-R (Multi-Environment Trial Analysis), software developed on R for Windows (R Core Team, 2016) by CIMMYT (Alvarado et al., 2015) was used for data analysis. First, data from individual low N and optimum sites were analysed followed by combined analysis across sites by management. The software generated BLUPs for all the traits at individual sites as well as across sites for each management condition. Variance components (genotypic, error, location and genotype by location interactions) and heritability were estimated on combined (across sites) entry mean basis for all traits in all trials. Restricted maximum likelihood (REML) estimates of genetic components were obtained with the lme4 package (Bates et al., 2015) embedded in META-R software (Alvarado et al., 2015; R Core Team, 2016). Broad sense heritability, which is an estimate of the extent to which phenotypes were determined by the genotypes for each trait, was estimated on testcross means as described by Hallauer and Miranda (1989) for all sites and across sites for each management condition as:

$$h^2 = \frac{\delta_G^2}{\delta_G^2 + \frac{\delta_{GE}^2}{ENV} + \frac{\delta_E^2}{REP\ \mathrm{X}\ ENV}}$$

where h^2 is the heritability of a trait; δ_G^2 is the genotypic variance, δ_{GE}^2 is the variance of the genotype by environment interaction; δ_E^2 is the residual variance; ENV is the number of sites and REP is the number of replications.

The efficiency of indirect selection for GY under low N based on GY from optimum management was calculated from trials planted under both optimum and low N conditions in the same site. The relative efficiency (RE) of indirect selection for low N was predicted using the formula proposed by Falconer and Mackay (1996) assuming equal selection intensities in both N levels as:

$$RE = \frac{h_{HN}\ X\ r_G\ (LN.HN\ )}{h_{LN}}$$

where RE is the relative efficiency of indirect selection for low N under optimum conditions, h_{HN} and h_{LN} are the square roots of heritability under optimum and low N conditions, respectively and r_G (LN.HN) is the genetic correlation between GYs under optimum and low N environments. The same formula (Ziyomo and Bernardo, 2013) was used for estimating the relative efficiency of indirect selection for GY under low N stress based on secondary traits under low N.

3.4. Results

3.4.1. Mean, variance and heritability of GY and secondary traits

Genotype effect for GY and secondary traits was significant (*P≤0.05*) in all optimum and low N sites (Table 3.1) and combined across sites by management. Mean GY for low N trials during main and off-seasons was significantly different in some populations (Table 3.1). Therefore, combined analysis for low N trials during main and off-seasons was performed separately. Compared to GY under optimum conditions, low N stress during the off-season resulted in higher mean GY reduction (71%) than in the main season (39%), indicating the severity of stress during the off-season compared to the main season (Table 3.2). Low N stress during the main and off-seasons also resulted in shorter plant and ear heights. Except for the magnitude of effect, the direction of effect of low N stresses during both main and off-seasons were similar on GY and the secondary traits.

Genotypic variance for GY was higher under optimum conditions than both main (moderate) and off-season (severe) low N stressed conditions. On average, the genetic variance for GY across the five populations was reduced by 17% under moderate N stress and 63% under severe N stress conditions compared to optimum conditions. Contrary to this, the genetic variances for AD and PH increased under both moderate and severe low N stress compared to optimum N environments. However, the higher genetic variance for GY under optimum conditions did not translate into higher heritability. The average estimates of heritability for GY across the five populations were on par under optimum and severely N stressed conditions. Under moderately N stress conditions, the heritability was reduced by 17% compared to the heritability under optimum conditions. The heritability of most secondary traits was consistently higher than that of GY under both management conditions.

Table 3.2 The ratio of low N (both moderate, LNM and severe, LNO) to optimum for heritability, components of variance and grand mean for grain yield (GY), anthesis date (AD), plant height (PH) and ear height (EH) in five trials consisting of test cross progenies evaluated under optimum and low N conditions in Kenya and Rwanda

Parameter	Population 1				Population 2				Population 3				Population 4				Population 5			
	GY	AD	PH	EH	GY	AD	PH	EH	GY	AD	PH	EH	GY	AD	PH	EH	GY	AD	PH	EH
	LNM to optimum																			
Heritability	0.76	0.92	0.99	0.89	0.70	1.02	0.96	0.86	0.9	1.0	0.7	0.6	0.92	0.75	0.64	0.73	0.91	0.67	1.03	0.71
Loc Variance	0.59	1.65	2.61	28.23	0.03	0.11	0.75	1.04	0.0	1.0	3.2	4.5								
GenoVariance	0.30	1.71	2.09	0.62	0.55	0.99	1.54	1.46	0.5	1.3	0.4	0.6	1.70	1.20	1.0	0.90	1.16	1.17	1.84	1.00
GXE Variance	0.46	1.28	8.28	7.85	0.34	0.40	0.65	0.00	0.4	0.9	6.71 E+11	3.7								
ResVariance	0.46	2.53	1.85	1.20	0.54	0.36	0.94	1.32	0.4	0.7	0.9	1.5	1.04	1.16	1.35	1.40	0.71	1.89	1.45	1.58
Grand Mean	0.60	0.97	0.84	0.63	0.59	0.97	0.93	0.83	0.4	0.9	0.7	0.6	0.79	1.02	0.86	0.90	0.67	0.99	0.92	0.93
	LNO to optimum																			
Heritability	0.45	1.04	0.63	0.69	0.98	1.02	0.97	0.94	1.2	1.1	0.8	0.8	1.90	0.80	0.80	0.80	1.18	0.88	1.02	0.65
Loc Variance	0.00	2.11	0.02	0.14	0.02	0.29	0.93	1.00	0.0	0.0	0.0	0.0								
GenoVariance	0.11	3.73	0.95	0.37	0.36	0.74	0.50	0.35	0.5	1.0	0.5	0.4	0.52	1.71	1.69	0.79	0.36	1.01	2.07	0.49
GXE Variance	0.26	0.57	0.00	0.00	0.20	0.64	1.72 E+11	0.87	0.0	0.0	2.68 E+11	0.8								
ResVariance	0.21	2.98	1.65	1.13	0.33	0.47	0.55	0.45	0.3	0.4	0.6	0.6	0.10	1.48	1.56	1.12	0.13	0.80	1.65	0.93
Grand Mean	0.28	1.03	0.59	0.42	0.39	0.96	0.65	0.50	0.4	0.9	0.6	0.5	0.20	0.93	0.60	0.57	0.19	0.90	0.67	0.61

Loc, location; geno, genotype; GXE, genotype by environment; Res, residual; population 1, CML494/CML550; population 2, CML504/CML550; population 3, CML511/CML550; population 4, CML505/LP; population 5, CML536/LP

3.4.2. Phenotypic and genetic correlations between grain yield and secondary traits

Phenotypic and genetic correlation of GY with PH and EH was positive and significant under optimum as well as the two N levels (Table 3.3). Remarkably, the magnitude and the direction for the correlation between GY and AD was changed with an increase in stress level. Under optimum conditions, the average correlation coefficient between GY and AD was positive, but was negative under severe low N stress conditions. Generally, genotypic correlations had higher magnitude than phenotypic correlations for all traits under optimum and both N stress conditions.

Table 3.3 Genetic and phenotypic correlation between grain yield and three secondary traits

Traits	Population 1		Population 2		Population 3		Population 4		Population 5		Average	
	Phe	Geno	Phe	Gen	Phe	Gen	Phe	Gen	Phe	Gen	Phe	Gen
Optimum												
AD	-0.10	0.06	0.16*	0.36^{+}	0.04	0.32^{+}	-0.11	0.23**	0.19*	0.48^{+}	0.04	0.29
PH	0.10	0.25*	0.53^{+}	0.85^{+}	0.47^{+}	0.70^{+}	0.16*	0.19**	0.45^{+}	0.25^{+}	0.34	0.45
EH	0.12	0.24*	0.57+	0.88^{+}	0.46^{+}	0.68^{+}	0.11	0.17*	0.45^{+}	0.33^{+}	0.34	0.46
Low N - main season												
AD	-0.22*	-0.06	0.15*	0.34^{+}	-0.35^{+}	-0.36^{+}	-0.21**	0.17*	-0.07	0.56^{+}	-0.14	0.13
PH	0.20*	0.07	0.37+	0.43^{+}	0.41^{+}	0.38^{+}	0.12	-0.22^{+}	0.39^{+}	0.41^{+}	0.30	0.21
EH	0.23**	0.29^{+}	0.29+	0.37^{+}	0.32^{+}	0.17	0.13	-0.18*	0.46^{+}	0.67^{+}	0.29	0.26
Low N – off-season												
AD	-0.30^{+}	-0.40^{+}	-0.03	0.03	-0.03	0.03	-0.17*	0.02	-0.34^{+}	-0.39^{+}	-0.18	-0.14
PH	0.50^{+}	1.00^{+}	0.24+	0.23^{+}	0.54^{+}	0.62^{+}	0.43^{+}	0.59^{+}	0.47^{+}	0.62^{+}	0.44	0.61
EH	0.47^{+}	1.00^{+}	0.17**	0.16**	0.41^{+}	0.39^{+}	0.46^{+}	0.89^{+}	0.52^{+}	0.81^{+}	0.41	0.65

Phe, phenotypic; Geno, genotypic; *P≤0.05; **P≤0.01; +P≤0.001; population 1, CML494/CML550; population 2, CML504/CML550; population 3, CML511/CML550; population 4, CML505/LP; population 5, CML536/LP

3.4.3. Efficiency of indirect selection for grain yield under low N

Indirect selection for GY under low N could be made through secondary traits under low N or/and based on GY from optimum conditions. The degree to which growing environments are related would dictate the use of direct or indirect selection for quantitative traits. The efficiency of indirect selection for GY under low N using GY from optimum environments, was calculated using seven pairs of optimum-low N sites for all populations (Table 3.4). The average heritability of GY from these sites was 0.5 under optimum and 0.41 under low N environments. Genetic correlation between GY from optimum and low N conditions ranged from 0.19 to 0.56 with an overall average of 0.37. The relative efficiency of indirect selection for GY under low N using GY from optimum environments ranged from 0.14 to 0.74 with an overall average of 0.45. The

lowest efficiency was observed between 14A-KBK-1 (main season) and 14B-KBK-1 (off-season) in population 2. Efficiency of indirect selection was relatively higher for low and optimum trials planted in the same growing season (main season in this case) than those grown in different growing seasons.

Table 3.4 The efficiency of indirect selection for grain yield under low N through grain yield under optimum N conditions

Population	Trial name	Heritability		r_G	Efficiency
		Optimum	Low N		
1	15A-kbk-1 vs 15A-kbk-2	0.41	0.45	0.34	0.32
2	14A-kkm-3 vs 15A-kkm-4	0.78	0.27	0.42	0.71
2	14A-kbk-1 vs 14B-kbk-1	0.35	0.61	0.19	0.14
3	14A-kkm-4 vs 14A-kkm-3	0.70	0.35	0.53	0.74
3	14A-kbk-5 vs 14B-kbk-2	0.42	0.53	0.27	0.24
4	kiboko vs Kiboko_LN	0.31	0.25	0.56	0.62
5	kiboko vs kiboko_LN	0.56	0.43	0.32	0.37
Average		0.50	0.41	0.37	0.45

r_G, genetic correlation; A, main season; B, off-season

The efficiency of indirect selection for GY through secondary traits was less than one (Table 3.5), signifying the higher efficiency of direct selection for GY under low N than through secondary traits under the same management conditions. The only exception was PH and EH in populations which showed higher efficiency of indirect selection for GY.

Table 3.5 The efficiency of indirect selection for grain yield under low N through secondary traits under low N conditions

Traits	**Population 1**	**Population 2**	**Population 3**	**Population 4**	**Population 5**	**Average**
Low N - main season						
AD	0.08	0.44	0.47	0.26	0.62	0.37
ASI	0.49	0.41	0.31	0.33	0.43	0.39
PH	0.10	0.56	0.43	0.28	0.44	0.36
EH	0.44	0.45	0.19	0.22	0.73	0.41
Low N – off-season						
AD	0.79	0.03	0.04	0.02	0.43	0.26
ASI	0.00	0.37	0.02	0.53	0.92	0.37
PH	1.51	0.26	0.64	0.59	0.58	0.72
EH	1.76	0.17	0.41	0.79	0.74	0.77

Low N, low nitrogen

3.5. Discussion

3.5.1. Mean, variances and heritability of traits in trials

Low N stress is one of the most widespread maize production challenges for small-holder farmers across SSA (Bänziger et al., 1997; Worku et al., 2007; 2008; 2012). High percentages of GY reduction under low N conditions observed in this study signifies the importance of N in maize production. As low N sites simulate small scale farms in SSA, the yield reduction in managed low N sites reflect the amount of yield lost due to shortage of N. The negative effect of low N stress on GY production can be managed with the application of enough N fertilizer and other agronomic management practices such as intercropping and/or rotation. However, most small-holder farmers in SSA can hardly afford the use of the required amount and type of fertilizers for their maize crop. Genetic improvement of maize is a cost effective and environmentally friendly approach that makes use of the large genetic variation present in maize germplasm (Bänziger et al., 1997; Hoisington et al., 1999). Significant differences among entries for GY and other secondary traits in this study showed the presence of genetic diversity among DH lines derived from crosses of tropical inbred lines. This shows the effectiveness of the DH technique in creating genetic variation that can be exploited in hybrid breeding for low N stress tolerance. Genetic variance among the current DH test cross progenies was slightly higher than the average genetic variances reported previously for tropical germplasm (Bänziger et al., 1997). Beyene et al. (2013) also observed high genetic distances among tropical DH lines assessed by single nucleotide polymorphic (SNP) markers, indicating the effectiveness of the DH technique in creating genetic variability (Geiger and Gordillo, 2009).

Mean genetic variance for GY across the five populations was smaller under low N than optimum environments and it further diminished with an increase in severity of N stress during the off-season. Mean genetic variance for other secondary traits, however, was higher under low N than optimum conditions. Genetic variance is a prerequisite for the improvement of any trait of interest (Hoisington et al., 1999). Due to low genetic variance for GY under low N, gain from selection is low compared to optimum N environments where the genetic variance for GY is relatively high. Similarly to this study, several authors found low genetic variance under stress conditions and recommended the complementary use of secondary traits to select high yielding

genotypes under optimum and low N conditions (Lafitte and Edmeades, 1994; Bänziger et al., 1997; Beyene et al., 2013). Despite high genetic variance for GY under optimum conditions, mean broad sense heritability for optimum environments was on a par with mean heritability from low N environments. A high proportion of genotype by environment (GXE) interaction and error variances under optimum environments were the main impediments to the realization of high heritability corresponding to high genetic variance observed under optimum trials. Considering the range of optimum sites included in this study, high GXE interaction variance under optimum trials was not surprising (Ribaut et al., 1996). In addition, the use of standard protocols to develop managed low N fields in all sites and use of more replications in some trials may have contributed to lower GXE interaction and error variances in the low N trials. Appropriate field designs and data analysis methods could address the issue of higher GXE interaction and error variances observed in optimum trials.

3.5.2. Efficiency of indirect selection

Establishing two distinct breeding programmes for contrasting environments is an expensive approach. Such approach is justified only if simultaneous improvement or indirect selection through correlated response is not feasible (Atlin et al., 2000; Presterl et al., 2003). The efficiency of indirect selection for one environment/trait based on other environment/trait depends on the strength of the genetic correlation between two environments/traits. Despite positive correlation between high and low N sites for GY, the magnitude of the correlation coefficient in this study was small and non-significant in most cases. Similar results were reported by Bänziger et al. (1997) and Worku et al. (2007) in CIMMYT tropical germplasm. This is partly attributed to higher genotype by N level interaction (Presterl et al., 2003). Neutral or positive correlation between traits or environments imply the potential for simultaneous improvement of traits or the same trait in different environments (Ertiro et al., 2013).

Values equal to one for the ratio between indirect and direct selection implies equal efficiency of direct and indirect selection, while values less than one indicate lower efficiency of indirect selection compared to direct selection. In this study, indirect selection for low N through performances obtained from optimum conditions was found to be inefficient (IR/R<1). This result validates the need for evaluating genotypes under target environments for both tropical and temperate maize germplasm (Bänziger et

al., 1997; Presterl et al., 2003). Low efficiency of indirect selection is explained by low correlation between environments that resulted from a higher proportion of genotype x N variance than total variance (genotype + genotype x N) in combined analysis of low N and optimum environments from the same site. The correlation and the efficiency of indirect selection was further reduced when optimum and low N environments were in different growing seasons (increase in yield reduction). Bänziger et al. (1997) found equal efficiency between optimum and low N conditions when the relative yield reduction under low N was low (23%), and the efficiency of indirect select was significantly lower than one when the relative yield reduction was high (>43%). The efficiency of indirect selection declines further with increase in severity of N stress (and yield reduction under low N relative to optimum conditions) (Bänziger et al., 1997).

The poor correlation between low N and optimum environments and thereby poor efficiency of indirect selection, was partly explained by different mechanisms of NUE of crops under optimum and low N environments (Anbessa et al., 2009), N uptake efficiency under low N conditions and utilisation efficiency under optimum conditions (Hirel et al., 2001; Gallais and Hirel, 2004). Evaluating genotypes under both conditions would improve both components of NUE. Simultaneous improvement for contrasting environments have been reported to be feasible in different crops (Lorenzana and Bernardo, 2008; Hubner et al., 2013). Under high stress levels, however, the physiological mechanisms involved and genes responsible in control of traits would be different, resulting in low genetic correlation between environments (Bänziger et al., 1997). In such conditions, separate breeding programmes may be required to develop varieties adapted to specific conditions. Generally, cultivars with improved NUE possess a higher level of yield stability across a wide range of stress and non-stress environments (Presterl et al., 2003) and therefore can address productivity issues in SSA.

Selection for GY under stress environments is generally not as efficient compared to selection in optimum environments due to low heritability of GY under stress environments (Edmeades, 1999). Easy to measure, highly correlated and heritable secondary traits can be used to improve selection efficiency of GY under low N. However, use of only a single secondary trait for indirect selection for GY under low N is less efficient than direct selection. In the current study, the efficiency of indirect

selection for GY through most of the secondary traits was inefficient. To achieve genetic improvement of GY under low N, breeders usually use an index of secondary traits with high genetic variance under low N. Bänziger and Lafitte (1997) combined information from all secondary traits in a Smith-Hazel index and noticed a 14% improved selection efficiency on average over selection for GY alone. Among secondary traits such as ears per plant and leaf senescence discriminated high-yielding genotypes the best (Bänziger and Lafitte, 1997). Leaf chlorophyll concentration, and in some instances ASI, provided information on environmental variation within experiments. The authors concluded that secondary traits can increase the efficiency of selection for GY in maize breeding programmes targeting low N environments. Therefore, instead of solitary use of secondary traits, most important secondary traits should be included in a selection index to improve GY under low N stress environments.

3.6. Conclusions

Low N stress is a widespread abiotic factor limiting maize yield in small scale maize farms. Use of inorganic fertilizers or different cropping systems (such as crop rotation) can be a remedy to address the issue of low productivity resulting from low soil N. Economic reasons as well as dwindling landholding are the main setbacks to practice these approaches. A complementary approach is the use of NUE maize varieties, which are efficient in uptake and utilisation of available N in the soil. In this study, low correlation was found between optimum and low N environments for GY and low genetic variance under low N environments that decreased as the intensity of stress increased. Low efficiency of indirect selection for GY under low N was found for GY under optimum conditions. Therefore, it is important to understand the genetic basis of GY under contrasting N environments unveiling the position of genes or quantitative trait loci underlying GY. Such endeavor will help the identification and use of markers associated with GY under low N conditions and improve the efficiency of selection.

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CHAPTER 4

Manuscript 2: A genome-wide marker-trait association study for genetic dissection of nitrogen use efficiency in tropical maize

4.1. Abstract

Like other crops, economically important traits such as GY and PH of maize and other crops are controlled by many genes with minor effect. Association mapping can be used to dissect the genetic bases of complex traits by exploiting historical and evolutionary recombination events at the population level. The major objective of this study was to identify SNP marker loci significantly associated with GY and secondary traits under low N and optimum conditions. Other objectives included the assessment of genetic diversity of the association panel and investigation of the population structure among the inbred lines. Test cross progenies of 411 inbred lines used for this study were planted in two replicated under each optimum and low N conditions in several location in Africa and Latin America. In all locations, low N fields which were previously depleted over several seasons were used, and no N fertilizer was applied throughout the growing season. Phenotypic data was collected for GY, AD, ASI, PH, EH, EPO and leaf senescence under the two management conditions. All inbred lines were genotyped with genotyping by sequencing (GBS). Genotypic variance for GY, PH, EH and SEN was higher under optimum conditions while higher for AD and ASI under low N conditions. Grain yield had higher heritability (>0.5) under both optimum and low N conditions while secondary traits had medium to high heritability. About 99% of the pairwise comparison among 411 inbred lines had a kinship value of <0.5. Genome-wide LD decay at r^2=0.2 and r^2=0.34 were 0.24 Mbp and 0.19 Mbp, respectively. Chromosome specific LD decays ranged from 0.13 to 0.34 Mbps with an average of 0.22 Mbp at the critical r^2 = 0.20, and ranged from 0.04 to 0.18 Mbps with average of 0.10 Mbp. Out of the total 182, 252 SNPs; 38 and 45 significant SNPs were detected under optimum and low N conditions, respectively, at 5% Bonferroni significance level. Out of these 83 significant SNPs, three SNPs on chromosomes 1, 2 and 6 were associated either with different traits or the same trait under different management conditions, suggesting pleiotropic effects of genes associated with the significant markers. A total of 158 putative protein coding genes were associated with

the significant SNPs, of which seven SNPs were linked with four known genes. Markers associated with the putative and known genes can be used for marker assisted selection in NUE breeding.

Key words: Association mapping, NUE, marker assisted selection, LD,

4.2. Introduction

Low N stress is a widespread problem in maize production in sub-Sharan Africa where farmers can hardly afford the application of the right amount and kind of fertilizers required for the normal growth and development of plants mainly due to the high price of inorganic fertilizers (Lafitte and Edmeades, 1994). Through classical correlation studies, distinct genetic mechanisms have been reported for grain yield under optimum and under low N stressed conditions (Bänziger et al., 1997; Worku et al., 2007; 2008). However, most traits of agricultural importance are controlled by multiple QTL/genes and are difficult to investigate with conventional approaches per se.

The development of molecular markers for the detection and exploitation of DNA polymorphisms in plant systems is one of the most significant developments in the field of molecular biology and biotechnology (Soto-Cerda and Cloutier, 2010). Genetic mapping and molecular characterisation of functional loci facilitates genome-aided breeding for crop improvements including NUE and drought tolerance (Yu and Buckler, 2006a). Linkage analysis has been commonly used for dissecting the genetic basis of economically important complex traits in plants. Several such studies have been conducted to understand the genetic architecture of grain yield and secondary traits under different environmental conditions (Ribaut et al., 2007). Despite many linkage analysis studies conducted in various plant species to dissect the quantitative traits, only few QTL were cloned or tagged at the gene level (Moose and Mumm, 2008) because map-based cloning of QTL is a time consuming and expensive process in maize and other crop species (Yan et al., 2011). The mapping resolution is limited because only two alleles per locus and few recombination events are considered to estimate the genetic distances between marker loci and to identify the causative genomic regions for QTL (Soto-Cerda and Cloutier, 2010).

Association mapping has emerged as a tool to resolve complex trait variation down to the sequence level by exploiting historical and evolutionary recombination events at the population level (Nordborg and Tavare, 2002). This approach was initially started in human disease and then extended to plants, substantially increasing the mapping resolution over the traditional linkage mapping (Flint-Garcia et al., 2003). Association mapping detects the correlation between genotype and phenotype in unrelated individuals on the basis of linkage disequilibrium. It identifies QTL by examining the marker-trait associations that can be attributed to the strength of linkage disequilibrium between markers and functional polymorphisms across a set of diverse germplasm (Zhu et al., 2008). Association mapping has increased mapping resolution, reduced research time, and greater allele number (Flint-Garcia et al., 2003; Yu and Buckler, 2006a) compared to the traditional linkage analysis.

A number of association mapping studies have been conducted to investigate the causal variants associated with many important traits in maize, including flowering time (Wallace et al., 2016), maysin synthesis (Szalma et al., 2005), forage quality (Andersen et al., 2007), carotenoid content (Harjes et al., 2008), provitamin A (Azmach et al., 2013) and kernel size (Li et al., 2010). Despite the widespread use of association mapping for the dissection of complex traits, little was done for the dissection of the genetic architecture of NUE in maize. NUE is a complex trait that is a product of N uptake and N utilisation efficiency. In addition to limited work on NUE, genome-wide association studies (GWAS) are constrained by the power of statistical tools to identity true associations, calling for better computer software for data analysis.

The application of GWAS has been limited by the presence of false positives and false negatives. Significant results from different association studies have hardly been reproducible due to false positives resulting from population structure which is regarded as the major problem for association mapping (Zhu et al., 2008). Given the geographical origins, local adaptation, and breeding history of assembled genotypes in an association mapping panel, these dependent samples usually contain both population structure and familial relatedness (Yu and Buckler, 2006a). LD generated by population structure within the sample needs to be accounted for in the analysis to avoid spurious results. Population structure and kinship among individuals can be incorporated as covariates in a Mixed Linear Model (MLM) to control false positives. But the confounding problem between the covariates and test marker also weakens

the signals of QTNs (quantitative trait nucleotides), resulting in false negatives (Liu et al., 2016). Recently, a user-friendly R GWAS package known as FarmCPU (Fixed and random model Circulating Probability Unification), implemented a method to address the "confounding problem" and used several mathematical or programming strategies to increase the speed and save memory making FarmCPU adapted for big data sets (Liu et al., 2016). In this study "the state of the art" analytical package was used to identify marker-trait association in testcrosses of 411 tropical inbred lines evaluated under optimum and low N conditions.

The major objective of this study was to identify SNP marker loci significantly associated with grain yield and secondary traits under low N and optimum conditions. Other objectives included: (1) assessing the phenotypic and genetic diversity of the association panel and (2) investigating the population structure among the inbred lines

4.3. Materials and methods

4.3.1. Plant material

Four hundred and eleven inbred lines used for this study were derived from a panel of 424 diverse tropical maize inbred lines established by the Improved Maize for African Soils (IMAS) project to dissect the genetic basis of NUE and for marker discovery. All the inbred lines were CIMMYT maize lines developed by CIMMYT through conventional breeding methods. The list of the inbred lines, the source germplasm and the method employed for the development of the lines can be found at http://www.cimmyt. Single cross hybrids were generated for evaluation of the inbred lines by crossing with CML539, a broadly-adapted CIMMYT maize inbred line from heterotic group A.

4.3.2. Field experiments and statistical analysis

Testcross progenies obtained by crossing 411 inbred lines with an inbred tester were evaluated across nine optimum and 13 managed low N stressed sites in Africa and Latin America. The list of the trials, testing sites and the management practices employed for each trial are presented in Appendix Table 1. For managed low N sites, the number of years of depletion at each location varied from 2 to 6. Experiments were planted in one-row plots, with a final planting density of 6.67 plants/m^2 (Mexico) and 5.33 plants/m^2 (Kenya, Zambia, Zimbabwe and South Africa). At all locations, two

seeds per hill were sown, then thinned to one after emergence. An alpha-lattice design was used, with two replications. In optimal trials, the recommended amount of fertilizer was applied at planting as basal application and a second application was applied 3-4 weeks after sowing. In low N trials, all plots received P and/or K and recommended plant, weed, and insect control measures were followed. Data was collected for GY, AD, ASI, PH, EH, EPO, and SEN. GY was calculated from field weight by adjusting grain moisture to 12.5% and shelling percentage of 80%. AD is the number of days from planting to when 50% of plants in the plot started shedding pollen on the main axis of the tassel. ASI was calculated as the difference between the number of days when 50% of plants in a plot emerged 2-3 cm silk and pollen shedding. PH and EH were measured in centimeters as a distance from the base of a plant to the first branch of the tassel and the upper most ear from ten representative plants, respectively. EPO was calculated as the ratio between PH and EH. SEN was recorded by visual assessment using a 1 to 10 scale, where 1 indicates all leaves of all plants in a plot were green and 10 indicates that all leaves were dead. At harvest, edge plants were removed from all rows from trials planted under low N, to avoid border effects.

Analyses of variance within and across environments in each population under each management condition was determined by the restricted maximum likelihood (REML) method using the R program embedded in META-R software (Alvarado et al., 2015). Variance components were determined following the linear mixed model:

$$Y_{ijko} = \mu + g_i + l_j + r_{kj} + b_{ojk} + e_{ijko},$$

where Y_{ijko} was the phenotypic performance of the i_{th} genotype at the j^{th} environment in the k^{th} replication of the o^{th} incomplete block, μ was an intercept term, g_i was the genetic effect of the i^{th} genotype, l_j was the effect of the j^{th} environment, r_{kj} was the effect of the k^{th} replication at the j^{th} environment, b_{ojk} was the effect of the o^{th} incomplete block in the k^{th} replication at the j^{th} environment, and e_{ijko} was the residual.

The effects of environments and replications were treated as random effects and the other effects as fixed. Heritability on an entry-mean basis was estimated from the variance components as the ratio of genotypic to phenotypic variance. In addition, best

linear unbiased prediction (BLUP) of each inbred line across environments within each management were calculated for all the traits.

4.3.3. DNA extraction and genotyping

All the inbred lines in the IMAS assocation panel were genotyped by the project for dissection of the genetic basis of NUE. For all inbred lines, genomic DNA was extracted from young leaves collected in a bulk of 10 plants per entry, using a modified version of the CIMMYT high throughput mini-prep Cetyl Trimethyl Ammonium Bromide (CTAB) method (Semagn, 2014). DNA samples were genotyped at the Institute of Biotechnology at Cornell University (http://www.biotech.cornell.edu/brc/genomics-facility), USA using ApeKI as restriction enzyme and 96-plex multiplexing (Elshire et al., 2011). Row GBS data for a total of 955,120 SNP loci distributed across the ten maize chromosomes was received from the Institute of Genomic Diversity (IGD), Cornell University, USA. Different filtering criteria applied to the row data to get input data for linkage disequilibrium and GWAS. For linkage disequilibrium, the row data was filtered based on no missing data and 5% minor allele frequency (MAF). For GWAS, the genotype data was filtered with MAF of 5% and a minimum count of SNPs on 90% of the sample size using Trait analysis by association, evolution and linkage (TASSEL v.5.2.24) software (Bradbury et al., 2007).

4.3.4. Population structure, kinship and genetic distance

Checking the presence of populatin structure is one of the most important factors in marker-trait association studies. Population structure in the current assocation panel was investigated using classical multidimensional scaling (principal coordinate analysis) embeded in TASSEL v.5.2.24 software (Bradbury et al., 2007). The same software was also used for analysis of kinship and genetic distances (Identity by state – IBS).

4.3.5. Linkage disequilibrium

Genome wide and chromosome specific LD were estimated as a squared allele frequency correlation coefficient (r^2) between all possible pairs of SNPs using TASSEL v5.2.31 (Bradbury et al., 2007). For genome-wide LD, 4479 SNPs distributed across the ten chromosomes, filtered based on no missing data per marker and 10% minimum minor allele frequencies, were used. For chromosome specific LD

estimation, the SNPs were filtered with no missing data per marker and 1% MAF. Full matrix LD analysis was performed with no imputation for missing data, and setting heterozygous calls to missing. After analysis, all LD estimates with missing value for distance were removed and only LD estimates having P<0.001 were considered significant (Pasam et al., 2012) and used for further analysis. Rate of LD decays were estimated by plotting localized regression curves (LOESS) of the r^2 values versus the corresponding physical distances between the SNP pairs, followed by observation of the intersection point between the fitted LOESS curve and a critical r^2 values (Breseghello and Sorrells, 2006). Two background critical r^2 values for estimating LD decays within and across chromosomes were considered in the present study to offer comparison. The first baseline critical r^2 was determined by taking the parametric 95 percentile of distribution of r^2 values for unlinked SNPs, taking SNPs on different chromosomes and SNPs beyond 50 Mbp apart on the same chromosome as unlinked (Pasam et al., 2012). The second baseline $r^{2\,-}$ value was 0.2, an arbitrary value often used to describe LD decay (Flint-Garcia et al., 2003; Zhu et al., 2008). Scatter plots and fitted smooth curves for estimating LD decay were plotted using the LOESS function (R Core Team, 2016).

4.3.6. Genome wide association analysis

GWAS analysis was done with the R package “FarmCPU – Fixed and random model Circulating Probability Unification” (Liu et al., 2016). The minimum input data required to run FarmCPU are genotypic data (GD), phenotypic data (Y) and genotypic map (GM) data. It takes genotypic data in numerical format and the “.hmp” format was converted to numeric (0, 1, 2) with the “GAPIT” package (Lipka et al., 2012). The programme also takes principle components or Kinship (Q) matrix and other fixed effects as optional input to reduce the rate of discovery of false positives. In this study, the first three principal compoents (PC) obtained from TASSEL (Bradbury et al., 2007) were used as an input for GWAS in FarmCPU (Appendix Table 17). The kinship was calculated with the default kinship algorithm in FarmCPU. The analysis was performed with maxLoop of five, p threshold of 0.01, QTN threshold of 0.01 and MAF threshold of 0.05. The maxLoop refers to the total number of iterations used. The p threshold, QTN threshold and MAF threshold refers to *p* values selected into the model for the first iteration, the *p* value selected into the model from the second iteration and the minimum minor allele frequency of SNPs used in the analysis. For the p values

threshold, 0.01 refers to the Bonferroni threshold (0.01/number of the total markers used). In addition, Bonferroni test threshold (0.01/number of markers) was used to set a significant level in Manhattan plots. “FarmCPU” also uses the “GAPIT” function to produce results, such as the Manhattan plot, the quantile-quantile (QQ) plot, GWAS results table and effect table of user-provided covariates, principal compoent analysis (PCA) in this case. PCA was carried out with TASSEL v5.24 (Bradbury et al., 2007).

4.4. Results

4.4.1. Summary of SNP and inbred lines

The summary of 182252 SNPs used in this study is presented in Table 4.1. From 955,120 GBS SNPs used to genotype 411 inbred lines, only 19% (220,878 SNPs) was retained after filtering with the twin criteria of 5% MAF and 10% missing per marker. The number of markers retained ranged from 12,338 on chromosome 10 to 29,248 on chromosome 1. For all the retained markers, the minimum MAF ranged between 0.05 and 0.50. Alleles with a frequency below 50% were considered minor. The percentage of missing markers per individual varied from 0 to 10% and the overall average was 4.2%.

The proportion of heterozygosity of SNPs (number of taxa that are heterozygous for a given SNP divided by the total number of individuals) ranged from 0 to 0.77, with an overall average of 0.10. The minimum proportion of heterozygous SNPs were found on chromosome 2 and the highest on chromosome 1. The heterozygosity of inbred lines (number of heterozygous markers per inbred line divided by the total number of markers) ranged from 0.002 to 0.354 with an overall average of 0.103. About half of the inbred lines showed heterozygosity of less than 0.05, and 67% of the inbred lines had heterozygosity of less than 0.125.

Table 4.1 The distribution of SNP markers, percentage of missing markers, minor allele frequency and heterozygous markers across the ten maize chromosomes in diverse tropic maize inbred lines

Chr.	Raw data	Filtered	*Average distance	Missing (%)			MAF	Heterozygous		
				Ave	Min	Max	Ave	Min	Max	Ave
1	148752	29248	0.029	0.042	0.00	0.10	0.214	0.00	0.77	0.103
2	115173	22180	0.022	0.042	0.00	0.10	0.213	0.00	0.37	0.104
3	108224	20921	0.021	0.041	0.00	0.10	0.213	0.00	0.43	0.100
4	94726	17263	0.017	0.041	0.00	0.10	0.213	0.00	0.56	0.100
5	110328	21566	0.022	0.042	0.00	0.10	0.213	0.00	0.48	0.104
6	76475	14336	0.014	0.042	0.00	0.10	0.214	0.00	0.45	0.102
7	80517	15323	0.015	0.043	0.00	0.10	0.207	0.00	0.43	0.098
8	81431	15602	0.016	0.041	0.00	0.10	0.219	0.00	0.61	0.101
9	72368	13475	0.013	0.042	0.00	0.10	0.216	0.00	0.72	0.103
10	67126	12338	0.012	0.042	0.00	0.10	0.210	0.00	0.45	0.100
Total	955120	182252	0.018	0.042	0.00	0.10	0.213	0.00	0.53	0.101

*Average distance between adjacent markers in Mbp; Chr., chromosome; MAF, minor allele frequency; Ave, average; min, minimum; max, maximum. Average distance, missing, MAF and heterozygosity is reported for SNPs after filtering 10% missing and 5% MAF

4.4.2. Population structure, kinship and genetic distance

The 411 individuals in the current association panel formed a clear population structure (Figure 4. 1), which is one of the reasons for false positive results during marker trait association analysis. In FarmCPU, the first three PCs are recommended to be added in the GWAS model to minimize the risk of false positives (Liu et al., 2016) arising from population structure. Even though 79 PCs were required to explain 50% of the variance in the inbred lines, only three PCs (explaining only 10% of the variance) were included in the FarmCPU GWAS analysis. The FarmCPU method output includes the effects of user provided PCs, which turned out to be small for all the traits analysed.

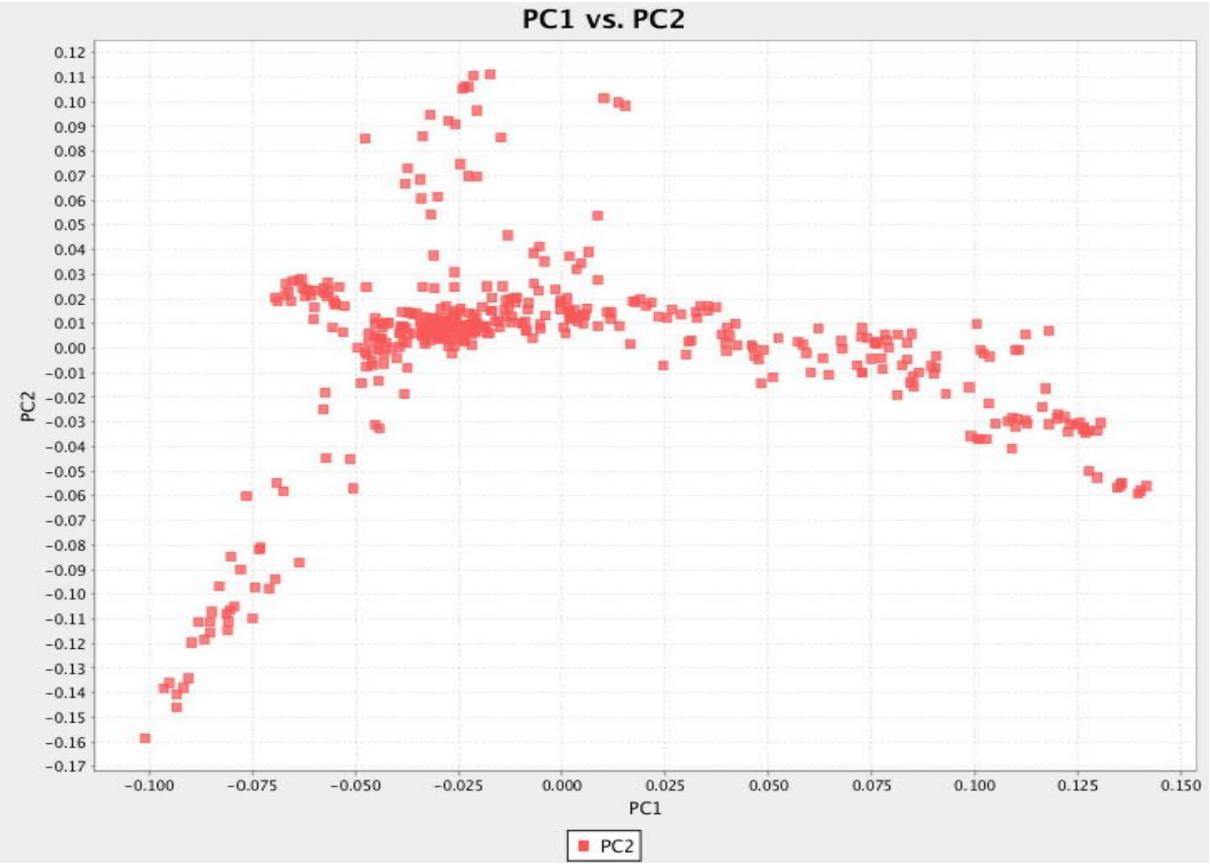

Figure 4.1 Principal coordinate analysis for 411 individuals with 182252 GBS SNP markers

Another important parameter that affects the GWAS is kinship among the tested inbred lines. About 99% of the pairwise kinship comparisons among 411 inbred lines had a kinship value of < 0.5, indicating the lack of relatedness among the inbred lines used for GWAS. The kinship heatmap (Figure 4.2) generated using the vanRanden algorithm in the "GAPIT" basic scenario also indicated low levels of relatedness among most pairs of inbred lines. In the heatmap, the count of the kinship values reached a maximum at the value of zero, further confirming low levels of relatedness among the tested inbred lines. In addition, genetic distance among 84255 pairwise comparisons ranged from 0.004 to 0.3390 with an average of 0.3115. The proportion of pairwise comparisons with values higher than 0.3 was 14.95% and with values higher than 0.2 was more than 99%, indicating the amount of genetic diversity in this association panel.

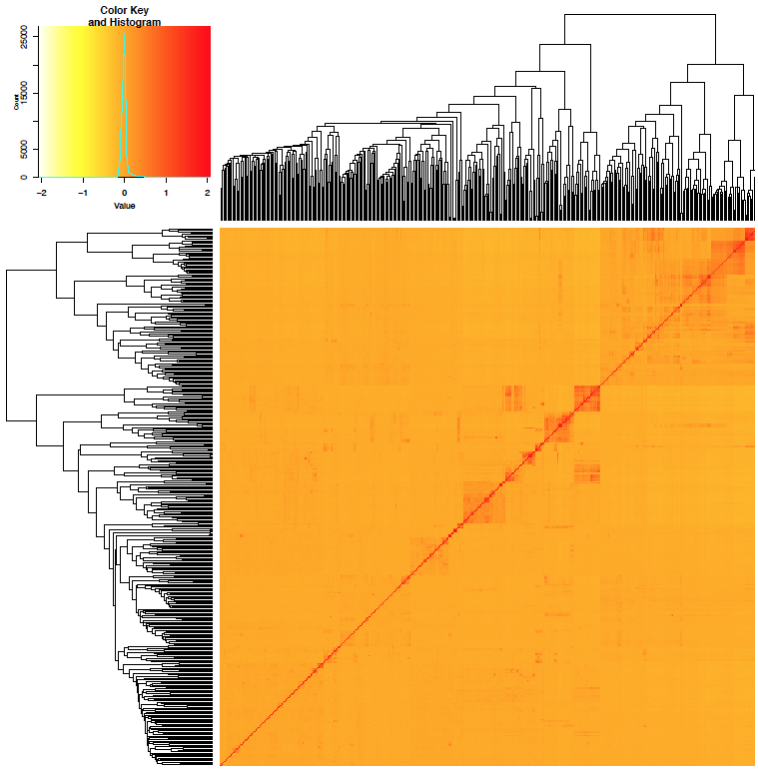

Figure 4.2 Kinship heatmap generated for 411 inbred lines from 182, 252 GBS SNP markers

4.4.3. Linkage disequilibrium

The distance over which LD persists will determine the number and density of markers, and experimental design needed to perform an association analysis (Flint-Garcia et al., 2003). The genome-wide and chromosome specific LD in this study were estimated at two critical r^2 levels (r^2=2.0 and r^2=0.34; Table 4.2). In the genome-wide LD analysis, r^2 values for only 6% of the total pairwise comparisons was significant ($p<0.001$). The proportion of significant r^2 values for the ten chromosomes were more or less similar (3-4%). Among the significant r^2 values, the proportion with $r^2>0.2$ was lowest for genome-wide LD (3%) compared to individual chromosomes (ranging from 5-8% with average of 6%).

Table 4.2 Genome-wide and chromosome wise LD decay at two critical r^2 values (0.2 and 0.34)

Chr.	No of SNPs	No. of pairwise comparisons	r^2			LD decay	
			% p<0.001	% >0.2	Avg	r^2=0.2	r^2=0.34
1	1261	794430	3%	5%	0.09	0.23	0.14
2	979	478731	3%	5%	0.09	0.20	0.09
3	1003	502503	4%	6%	0.09	0.17	0.11
4	781	304590	3%	7%	0.1	0.22	0.07
5	885	391170	3%	6%	0.09	0.20	0.12
6	618	190653	3%	5%	0.09	0.17	0.04
7	695	241165	4%	5%	0.09	0.25	0.10
8	717	256686	4%	6%	0.09	0.26	0.09
9	576	165600	4%	8%	0.11	0.34	0.18
10	659	216811	4%	8%	0.11	0.13	0.10
GW	4479	10028481	6%	3%	0.08	0.24	0.19

Chr., chromosome; GW, genome-wide; LD, linkage disequilibrium; Avg., average

Genome-wide LD decay at r^2=0.2 and R^2=0.34 were 0.24 Mbp and 0.19 Mbp, respectively (Figure 4.3). Chromosome specific LD decays ranged from 0.13 to 0.34 Mbps with an average of 0.22 Mbp at the critical r^2 value of 0.20, and ranged from 0.04 to 0.18 Mbps with an average of 0.10 Mbp. At r^2=0.2, LD decay was fastest for chromosome 10 (0.13 Mbp) and extended for chromosome 9 (0.34 Mbp). At r^2=34, the LD decayed fast for chromosome 6 (0.04 Mbp) and again delayed for chromosome 9 (0.18). The LD decay at the arbitrary r^2=0.2 was less variable than the LD decay at the calculated r^2=0.34.

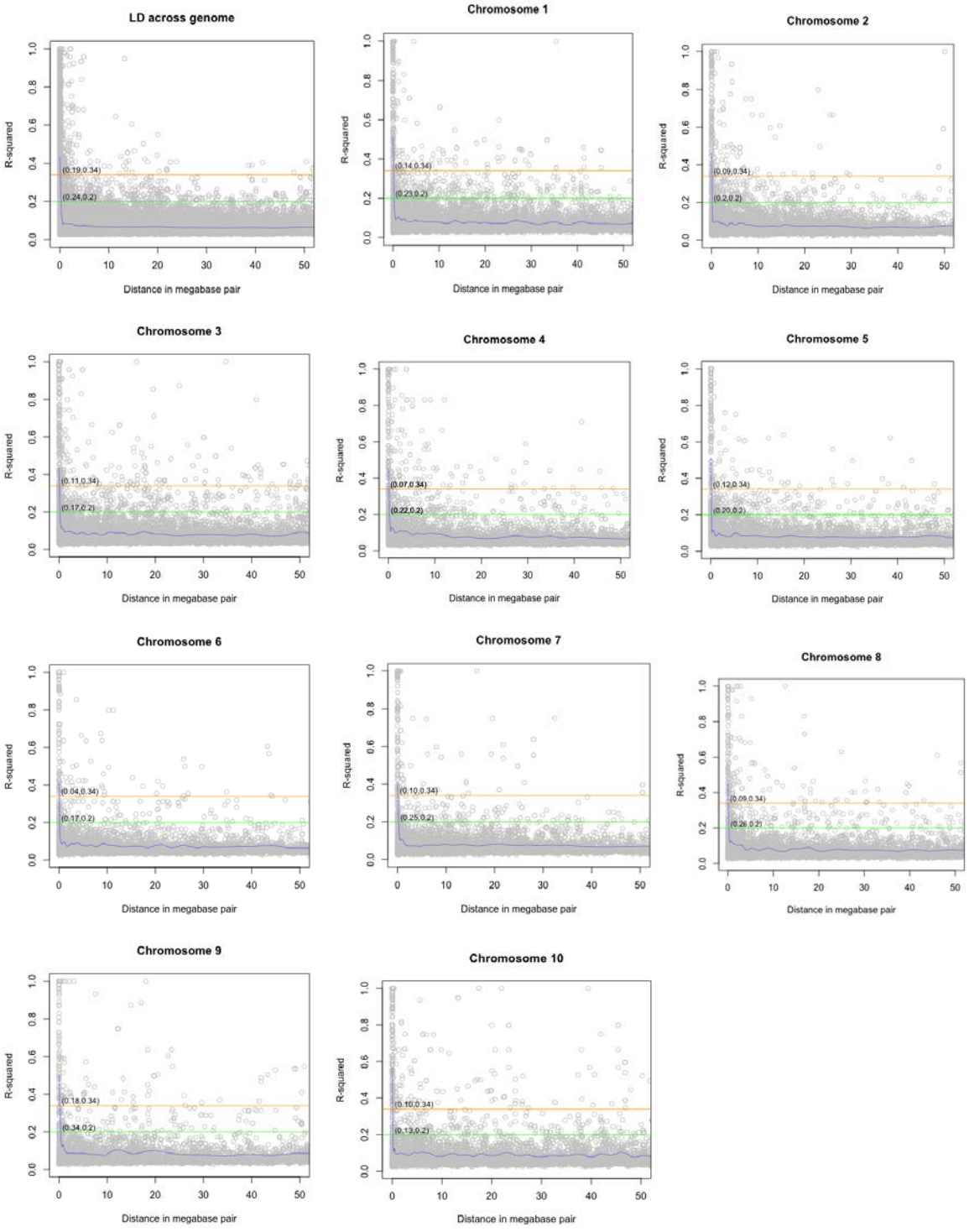

Figure 4.3 Genome-wide and chromosome specific LD decay plots at two cutoff points (green line, r^2=0.2 (arbitrary r^2 value) and orange line, r^2=0.34 (Calculated r^2 value))

4.4.4. Genome-wide marker traits association study

Genome-wide marker-trait associations between 182, 252 GBS SNP markers and eight traits were performed using the new R package for GWAS known as “FarmCPU” (Liu et al., 2016). This package uses a kinship matrix generated through “FarmCPU algorithm” embedded in the package itself to account for relatedness among individuals and the first three externally provided PCs (Appendix Table 17) to account for population structure. Out of the total 182, 252 SNP; 38 and 45 significant SNPs were detected under optimum and low N conditions, respectively at 5% Bonferroni significance level (Tables 4.3 and 4.4; Figure 4.4). The number of significant SNPs dropped to 33 under optimum and 27 under low N conditions when a stringent Bonferroni 1% significance level was used. The distribution of significant SNPs across chromosomes varied between 2 (chromosome 9) and 15 (chromosome 1) at Bonferroni 5% and ranged from 2 (chromosome 9) to 12 (chromosome 1) at Bonferroni 1%. The average number of significant SNPs per chromosome were 8.3 at Bonferroni 5% and 6.0 at Bonferroni 1%. At both Bonferroni 1% and 5%, the minimum MAF for significant SNPs was 22% ranging between 8% and 35% for Bonferroni 1% and between 13 and 35% at Bonferroni 5%.

Table 4.3 Number of markers significantly associated with grain yield and secondary traits at 5% and 1% Bonferroni threshold level

Trait	Bonferroni threshold (low N)		Bonferroni threshold (Optimum)		Total
	1%	5%	1%	5%	
GY	5	6	4	5	11
AD	7	8	4	6	14
ASI	4	7	10	11	18
EH	9	10	1	2	12
EPO	5	6	3	4	10
EPP	1	4	-	-	4
PH	2	4	2	5	9
SEN	-	-	3	5	5
Total	33	45	27	38	83

GY, grain yield; AD, anthesis date; ASI, anthesis silking interval; EH, ear height; EPO, ear position; EPP, ear per plant; PH, plant height; SEN, leaf senescence

Table 4.4 List of all SNPs significantly associated with grain yield and secondary traits under optimum and low N management conditions

SNP	Chr.	Position	P.value	MAF	effect	Bonferroni	Trait	Mgt
S1_283191977	1	283191977	2.36E-08	0.09	0.57	0.01	AD	Low N
S2_131348717	2	131348717	2.43E-10	0.20	-0.49	0.01	AD	Low N
S2_210662089	2	210662089	1.77E-08	0.15	0.45	0.01	AD	Low N
S4_170809248	4	170809248	3.78E-10	0.27	-0.49	0.01	AD	Low N
S6_150843360	6	150843360	4.26E-09	0.06	0.76	0.01	AD	Low N
S7_123828656	7	123828656	4.87E-08	0.45	-0.37	0.01	AD	Low N
S10_126930458	10	126930458	1.79E-11	0.08	-0.76	0.01	AD	Low N
S10_147898784	10	147898784	2.68E-07	0.19	-0.40	0.05	AD	Low N
S2_142865720	2	142865720	2.19E-07	0.32	-0.22	0.05	AD	Optimum
S2_210662089	2	210662089	4.87E-08	0.15	0.30	0.01	AD	Optimum
S3_149683417	3	149683417	1.75E-09	0.11	-0.40	0.01	AD	Optimum
S6_102939532	6	102939532	2.51E-07	0.05	0.46	0.05	AD	Optimum
S7_156476052	7	156476052	6.10E-14	0.41	0.35	0.01	AD	Optimum
S8_170275834	8	170275834	9.43E-13	0.41	0.31	0.01	AD	Optimum
S1_5810155	1	5810155	3.93E-08	0.19	0.17	0.01	ASI	Low N
S2_226325975	2	226325975	1.54E-07	0.29	-0.15	0.05	ASI	Low N
S3_32033690	3	32033690	1.58E-07	0.49	0.13	0.05	ASI	Low N
S3_147401613	3	147401613	1.06E-08	0.43	-0.15	0.01	ASI	Low N
S4_37297564	4	37297564	1.68E-07	0.22	-0.16	0.05	ASI	Low N
S6_164497574	6	164497574	9.27E-09	0.27	0.16	0.01	ASI	Low N
S10_4586049	10	4586049	6.45E-09	0.48	-0.16	0.01	ASI	Low N
S1_274946693	1	274946693	2.08E-07	0.08	-0.21	0.05	ASI	optimum
S2_6636633	2	6636633	4.99E-09	0.17	0.17	0.01	ASI	optimum
S2_54204647	2	54204647	2.82E-08	0.44	0.11	0.01	ASI	optimum
S3_128687310	3	128687310	8.68E-10	0.33	0.16	0.01	ASI	optimum
S4_235073935	4	235073935	4.22E-08	0.06	0.22	0.01	ASI	optimum
S5_195672028	5	195672028	4.59E-14	0.07	0.37	0.01	ASI	optimum
S7_24409023	7	24409023	3.88E-12	0.25	0.18	0.01	ASI	optimum
S7_155590511	7	155590511	8.19E-08	0.22	0.12	0.01	ASI	optimum
S8_136094451	8	136094451	5.01E-10	0.26	-0.14	0.01	ASI	optimum
S9_118046290	9	118046290	7.10E-08	0.22	0.13	0.01	ASI	optimum
S10_33353122	10	33353122	2.32E-09	0.16	-0.20	0.01	ASI	optimum
S1_16698847	1	16698847	2.94E-12	0.12	-2.55	0.01	EH	Low N
S1_199339693	1	199339693	1.60E-08	0.06	1.92	0.01	EH	Low N
S1_274946693	1	274946693	3.84E-09	0.08	-2.31	0.01	EH	Low N
S2_196870448	2	196870448	1.54E-08	0.11	1.63	0.01	EH	Low N
S3_217796834	3	217796834	7.05E-08	0.15	1.37	0.05	EH	Low N
S5_83133270	5	83133270	5.66E-10	0.06	2.60	0.01	EH	Low N
S6_7046560	6	7046560	4.63E-09	0.24	-1.26	0.01	EH	Low N
S7_40379325	7	40379325	4.01E-08	0.21	1.23	0.01	EH	Low N
S10_126687226	10	126687226	1.81E-10	0.07	2.24	0.01	EH	Low N
S10_145097517	10	145097517	2.10E-08	0.33	1.17	0.01	EH	Low N
S2_140662928	2	140662928	2.44E-07	0.27	-1.71	0.05	EH	Optimum
S8_158098622	8	158098622	7.18E-09	0.37	1.75	0.01	EH	Optimum
S1_207055175	1	207055175	1.37E-07	0.22	0.00	0.05	EPO	Low N
S1_274946693	1	274946693	3.01E-09	0.08	-0.01	0.01	EPO	Low N
S1_285229689	1	285229689	2.52E-10	0.42	-0.01	0.01	EPO	Low N
S2_33350339	2	33350339	8.02E-10	0.33	0.00	0.01	EPO	Low N
S6_97945994	6	97945994	4.75E-11	0.09	0.01	0.01	EPO	Low N
S10_123956017	10	123956017	6.75E-09	0.13	0.01	0.01	EPO	Low N
S1_71065792	1	71065792	1.76E-07	0.07	0.01	0.05	EPO	Optimum
S6_97945994	6	97945994	1.47E-08	0.09	0.01	0.01	EPO	Optimum
S8_72067641	8	72067641	2.88E-09	0.37	0.00	0.01	EPO	Optimum
S10_143712477	10	143712477	2.71E-08	0.08	0.01	0.01	EPO	Optimum
S1_122756821	1	122756821	2.54E-08	0.47	-0.01	0.01	EPP	Low N
S4_174009677	4	174009677	1.17E-07	0.07	-0.02	0.05	EPP	Low N
S5_188825516	5	188825516	1.01E-07	0.18	-0.01	0.05	EPP	Low N
S10_148304779	10	148304779	1.33E-07	0.31	0.01	0.05	EPP	Low N
S1_25425465	1	25425465	8.51E-08	0.14	-0.09	0.01	GY	Low N
S1_202550249	1	202550249	4.40E-08	0.40	-0.07	0.01	GY	Low N
S2_107767802	2	107767802	7.25E-10	0.15	0.11	0.01	GY	Low N
S5_152923661	5	152923661	3.33E-08	0.12	-0.10	0.01	GY	Low N
S5_214168220	5	214168220	2.35E-07	0.34	-0.07	0.05	GY	Low N
S7_128740455	7	128740455	1.57E-09	0.12	0.11	0.01	GY	Low N
S2_144477756	2	144477756	1.62E-07	0.09	0.23	0.05	GY	Optimum
S4_178469568	4	178469568	2.49E-08	0.37	-0.16	0.01	GY	Optimum
S5_183614607	5	183614607	7.31E-11	0.06	0.39	0.01	GY	Optimum
S8_75414416	8	75414416	2.29E-09	0.21	0.23	0.01	GY	Optimum
S10_147459915	10	147459915	1.78E-12	0.08	0.35	0.01	GY	Optimum
S1_17679579	1	17679579	4.65E-08	0.10	2.32	0.01	PH	Low N
S3_199254673	3	199254673	1.88E-13	0.21	-3.54	0.01	PH	Low N
S4_237693358	4	237693358	2.59E-07	0.12	2.50	0.05	PH	Low N
S8_25351243	8	25351243	1.05E-07	0.32	-1.48	0.05	PH	Low N
S3_64819581	3	64819581	1.62E-07	0.06	3.46	0.05	PH	Optimum
S4_46166070	4	46166070	7.98E-08	0.37	-1.69	0.05	PH	Optimum
S4_184955101	4	184955101	3.98E-08	0.25	2.10	0.01	PH	Optimum
S7_6297685	7	6297685	5.22E-12	0.26	-2.77	0.01	PH	Optimum
S10_143502717	10	143502717	1.61E-07	0.16	2.19	0.05	PH	Optimum
S1_220067760	1	220067760	3.46E-09	0.47	-0.08	0.01	SEN	Optimum
S4_177150249	4	177150249	1.53E-08	0.19	0.10	0.01	SEN	Optimum
S5_8351127	5	8351127	1.02E-07	0.45	-0.08	0.05	SEN	Optimum
S8_159648136	8	159648136	2.12E-07	0.24	-0.08	0.05	SEN	Optimum
S9_153449703	9	153449703	1.70E-08	0.47	-0.08	0.01	SEN	Optimum

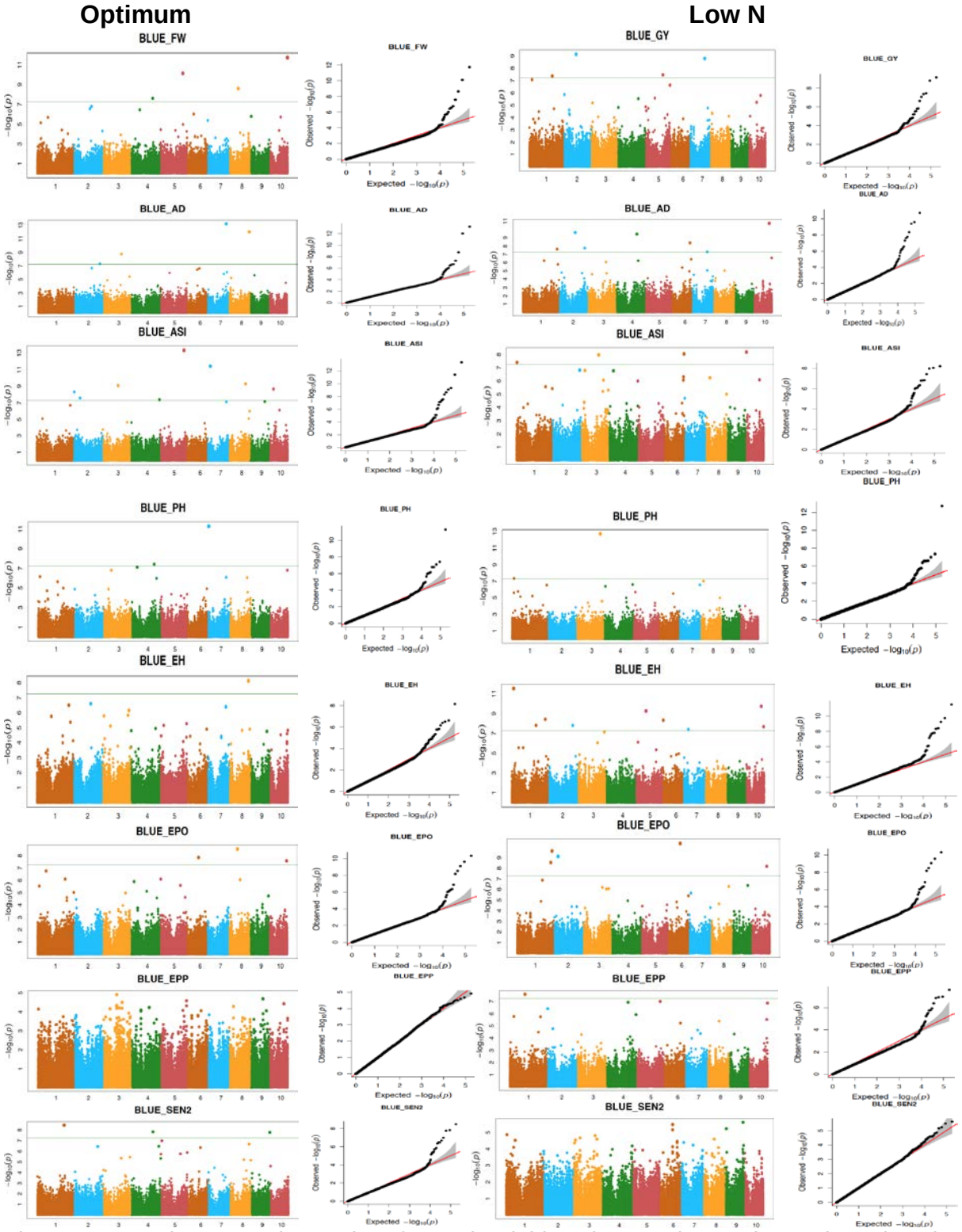

Figure 4.4 Manhattan and QQ-plots for grain yield and secondary traits evaluated under optimum and low N conditions. The horizontal lines at Manhattan plots show the threshold p value at Bonferroni cutoff point of 0.01. For GWAS analysis, best linear unbiased predictions (BLUES) were used for all traits

For GY, five significant SNPs under optimum and six under low N conditions were detected at Bonferroni 5% threshold. Of these significant SNPs, all survived the stringent significance threshold of Bonferroni 1% except one SNP under each optimum

and low N conditions. The significant SNPs for GY were found on chromosomes 1, 2, 4, 5, 7, 8 and 10 with the most significant one being located on chromosome 10 (p=1.78E-12). Chromosomes 4, 8 and 10 hosted SNPs identified only under optimum conditions while chromosomes 1 and 7 housed SNPs detected under low N conditions. Chromosomes 2 and 5 hosted SNPs identified under both optimum and low N conditions. At Bonferroni 5%, EPP had four significant SNPs, all under low N conditions. These SNPs were distributed across chromosomes 1, 4, 5 and 10. The number of significant SNPs dropped to just one when a stringent Bonferroni 1% threshold was used. The SNPs for EPP on chromosomes 4, 5 and 10 were situated near the SNPs identified for GY under optimum conditions.

The largest number of significant SNPs as well as the most significant SNPs in the current GWAS study were identified for ASI followed by AD. For ASI, 18 significant SNPs were detected at 5% Bonferroni, which dropped to 14 at 1% Bonferroni. These SNPs were distributed across all ten chromosomes. Chromosome 6 had a SNP detected under low N while chromosomes 5, 7, 8 and 9 had only SNPs for optimum conditions. All other chromosomes carried SNPs for both optimum and low N conditions. The SNP on chromosome 5 was the most significant in the current study (p=4.59E-14). For AD, a total of 13 significant SNPs; six under optimum and eight under low N, were detected across all chromosomes except 5 and 9 at 5% Bonferroni. At 1% Bonferroni, the number of SNPs decreased only by two. One SNP on chromosome 2 (S2_210662089; p=1.77E-08 under low N; p=4.87E-08 under optimum) was common between optimum and low N conditions. SNPs on chromosomes 2, 6 and 7 were detected under both optimum and low N conditions while all other SNPs were specific to either low N or optimum conditions. Two SNPs on chromosome 3 (S3_147401613 for ASI under low N and S3_149683417 for AD under optimum) and another two SNPs on chromosome 7 (S7_155590511 for ASI and S7_156476052 for AD; both under optimum) were located a few Mbps away from each other.

PH, EH and EPO are interrelated agronomic traits on maize. At 5% Bonferroni level; 9, 12 and 10 significant SNPs were detected for PH, EH and EPO, respectively. Out of the total SNPs, 4, 10 and 6 for PH, EH and EPO, respectively were detected under low N conditions. A SNP detected for EPO on chromosome 6 (S6_97945994; p=4.75E-11 under low N and p=1.47E-08 under optimum) was common between

optimum and low N conditions. Chromosomes 3 and 4 for PH; chromosome 2 for EH; and chromosome 1, 6 and 10 for EPO had significant SNPs both under optimum and low N conditions. Other chromosomes harboured SNPs detected only under one management condition. Unlike all other traits investigated in this GWAS, significant SNPs for SEN were detected only under optimum N conditions on chromosomes 1, 3, 4, 5, and 8. Availability of markers common for different traits is crucial for improving two or more traits simultaneous. In this study, a common SNP was identified for EPO and EH under low N conditions on chromosome 1 (S1_274946693; p=3.01E-09 for EPO and p=3.84E-09 for EH). In addition, there were closely linked SNPs on chromosome 1 that are underlying EPO and EH under low N conditions; and PH and EPO under optimum and EH under low N conditions. For SEN, five significant SNPs were detected on chromosomes 1, 4, 5, 8 and 9. All these SNPs were seen under optimum conditions. Surprisingly, no significant SNP was identified under low N conditions.

A total of 158 putative protein coding genes were associated with the significant SNPs (Table 4.5). Seven SNPs were linked with four known genes. Fertilization Independent Endosperm 1 (*FIE1*) and Teosinte Glume Architecture *(TGA1*) genes were in LD with S4_37297564 and S4_46166070, respectively on chromosome 4. *FIE1* was associated with ASI under low N and *TGA1* was associated with PH under optimum conditions.

Table 4.5 Putative protein coding genes in linkage disequilibrium with markers significantly associated with different traits under optimum and low N conditions

Gene stable ID	Gene description	Chr	Gene Start (bp)	Gene End (bp)	Strand	Gene name	Trait affected	Management
Zm00001d027453	Plant-specific domain TIGR01568 family protein	1	5589770	5590513	-1		ASI	Low N
Zm00001d027455	Non-specific serine/threonine protein kinase	1	5610415	5614949	-1		ASI	Low N
Zm00001d027456	Threonine endopeptidase	1	5661737	5663089	-1		ASI	Low N
Zm00001d027458	Cell growth defect factor 2	1	5701680	5704055	-1		ASI	Low N
Zm00001d027468	ADP,ATP carrier protein	1	5943755	5947624	1		ASI	Low N
Zm00001d027469	Cytochrome c oxidase assembly protein COX19	1	6020779	6023318	1		ASI	Low N
Zm00001d027885	YGGT family protein	1	16721226	16722692	1		EH	Low N
Zm00001d027919	40S ribosomal protein SA	1	17615562	17626036	-1		PH	Low N
Zm00001d027921	Protein DETOXIFICATION	1	17697601	17709046	1		PH	Low N
Zm00001d027922	Eukaryotic translation initiation factor 3 subunit K	1	17722776	17726155	-1		PH	Low N
Zm00001d027924	AP2-EREBP transcription factor	1	17775779	17777344	-1		PH	Low N
Zm00001d027925	AP2-EREBP transcription factor	1	17807124	17808643	-1		PH	Low N
Zm00001d027929	AP2-EREBP transcription factor	1	17839868	17840788	-1		PH	Low N
Zm00001d029448	TIFY6	1	71161670	71164215	-1		EPO	Optimum
Zm00001d031808	Short-chain dehydrogenase/reductase SDR	1	202464156	202467819	1		GY	Low N
Zm00001d031811	Patatin	1	202554373	202556086	-1		GY	Low N
Zm00001d031813	Patatin	1	202632511	202634549	-1		GY	Low N
Zm00001d031814	Patatin	1	202667670	202670842	-1		GY	Low N
Zm00001d031815	Patatin	1	202686992	202690272	-1		GY	Low N
Zm00001d031933	Signal peptide peptidase-like 3	1	207276632	207281619	1		EPO	Low N
Zm00001d033822	Histone H2A	1	274809375	274810292	1		**ASI**	**optimum**
Zm00001d033823	Putative uncharacterized protein	1	274819675	274820688	-1		**ASI**	**optimum**
Zm00001d033830	Adenylyl cyclase-associated protein	1	275160006	275165400	-1		**ASI**	**optimum**
Zm00001d033822	Histone H2A	1	274809375	274810292	1		AD	Low N
Zm00001d033823	Putative uncharacterized protein	1	274819675	274820688	-1		AD	Low N
Zm00001d033830	Adenylyl cyclase-associated protein	1	275160006	275165400	-1		AD	Low N
Zm00001d034143	GNAT transcription factor	1	285008208	285010916	1		EPO	Low N
Zm00001d034160	DNA binding protein	1	285335178	285337132	1		EPO	Low N
Zm00001d002149	Hexosyltransferase	2	6819751	6827540	-1		ASI	optimum
Zm00001d004689	Herbicide safener binding protein	2	131242393	131243911	-1		AD	Low N
Zm00001d004807	Coatomer subunit beta	2	140587723	140592773	1		EH	Optimum
Zm00001d004812	Calcium-dependent protein kinase ZmCPK11	2	140809252	140813969	1		EH	Optimum

Gene stable ID	Gene description	Chr	Gene Start (bp)	Gene End (bp)	Strand	Gene name	Trait affected	Management
Zm00001d006061	NADH dehydrogenase [ubiquinone] 1 alpha	2	196886906	196890424	-1		EH	Low N
Zm00001d006539	Beta-amylase	2	210700847	210703733	1		**AD**	**Low**
Zm00001d007267	Chlorophyll a-b binding protein, chloroplastic	2	226141607	226143698	-1		ASI	Low N
Zm00001d042019	Peroxidase	3	147549893	147551603	-1		ASI	Low N
Zm00001d042019	Peroxidase	3	147549893	147551603	-1		ASI	Low N
Zm00001d042013	Serine/threonine-protein kinase	3	147399992	147402361	1		ASI	Low N
Zm00001d043422	RING-H2 finger protein ATL2K	3	199163496	199164221	-1		PH	Low N
Zm00001d043420	BZIP transcription factor	3	199110559	199113757	-1		PH	Low N
Zm00001d043420	BZIP transcription factor	3	199110559	199113757	-1		PH	Low N
Zm00001d044054	GTP-binding nuclear protein	3	217928184	217932236	-1		EH	Low N
Zm00001d044054	GTP-binding nuclear protein	3	217928184	217932236	-1		EH	Low N
Zm00001d044054	GTP-binding nuclear protein	3	217928184	217932236	-1		EH	Low N
Zm00001d044045	Esterase	3	217679351	217682059	1		EH	Low N
Zm00001d049608	FIE1	4	37421922	37427789	-1	FIE1	ASI	Low N
Zm00001d049822	Teosinte glume architecture 1	4	46350597	46355118	1	TGA1	PH	Optimum
Zm00001d051891	Putative LOB domain-containing family protein	4	173818577	173819660	1		EPP	Low N
Zm00001d051892	Anthocyanin 5-aromatic acyltransferase	4	173824341	173825720	1		EPP	Low N
Zm00001d051894	Putative RING zinc finger domain superfamily protein	4	173865361	173866293	-1		EPP	Low N
Zm00001d051898	Serine/threonine-protein phosphatase	4	173925846	173928349	1		EPP	Low N
Zm00001d052034	Thioredoxin H-type 5	4	177028520	177029881	1		SEN	Optimum
Zm00001d052040	Cytochrome c oxidase copper chaperone	4	177078013	177079956	1		SEN	Optimum
Zm00001d052043	Auxin-responsive protein	4	177090545	177102253	-1		SEN	Optimum
Zm00001d052044	Putative RING zinc finger domain superfamily protein	4	177336257	177340312	1		SEN	Optimum
Zm00001d052069	Putative MYB DNA-binding domain superfamily protein	4	178364585	178366789	-1		GY	Optimum
Zm00001d052254	DNA binding protein	4	184820400	184821661	1		PH	Optimum
Zm00001d052260	Hexosyltransferase	4	185139277	185141193	-1		PH	Optimum
Zm00001d053580	Acetyltransferase component of pyruvate	4	235225983	235235156	-1		ASI	optimum
Zm00001d053632	40S ribosomal protein S8	4	237551123	237553636	-1	rps8	PH	Low N
Zm00001d053633	40S ribosomal protein S8	4	237652430	237654962	1	rps8	PH	Low N
Zm00001d053635	40S ribosomal protein S8	4	237827704	237830052	1	rps8	PH	Low N
Zm00001d013306	PRA1 family protein	5	8172127	8172948	-1		SEN	Optimum
Zm00001d013307	WRKY transcription factor	5	8180118	8184577	-1		SEN	Optimum
Zm00001d013309	Ribosomal protein S10	5	8293227	8293556	1		SEN	Optimum

Gene stable ID	Gene description	Chr	Gene Start (bp)	Gene End (bp)	Strand	Gene name	Trait affected	Management
Zm00001d015292	Cellulase	5	82970088	82974239	-1		EH	Low N
Zm00001d015300	Ribosomal protein L19	5	83323891	83326591	-1		EH	Low N
Zm00001d016248	Citrate synthase	5	152956416	152961046	-1		GY	Low N
Zm00001d017047	60S ribosomal protein L6	5	183747380	183749370	-1		GY	Optimum
Zm00001d017199	Temperature-induced lipocalin-1	5	188679379	188680432	-1		EPP	Low N
Zm00001d018089	VQ motif family protein	5	214041553	214042179	1		GY	Low N
Zm00001d018090	Annexin	5	214042874	214045954	-1		GY	Low N
Zm00001d018099	Adenosine 5'-phosphosulfate reductase 4	5	214157648	214160124	-1		GY	Low N
Zm00001d035143	Non-specific serine/threonine protein kinase	6	6884406	6891194	1		EH	Low N
Zm00001d036698	Chloroplast pentatricopeptide repeat protein 10	6	98069169	98071529	-1		**EPO**	**Low N**
Zm00001d036700	CASP-like protein	6	98074454	98078079	-1		**EPO**	**Low N**
Zm00001d038186	Peptide transporter PTR2	6	150861505	150866590	-1		AD	Low N
Zm00001d038784	Auxin-responsive protein	6	164332917	164336272	1		ASI	Low N
Zm00001d038792	Phosphotransferase	6	164389095	164395441	-1		ASI	Low N
Zm00001d038794	Pectinesterase	6	164493063	164495421	-1		ASI	Low N
Zm00001d018819	Viviparous-14	7	6342456	6344361	1		PH	Optimum
Zm00001d019262	Proteasome subunit beta type	7	24655394	24659033	-1		ASI	optimum
Zm00001d020580	Histone H2B	7	123664001	123664456	1		AD	Low N
Zm00001d020583	Hexosyltransferase	7	123675725	123676681	-1		AD	Low N
Zm00001d020584	Histone H4	7	123703834	123704145	1	H4C7	AD	Low N
Zm00001d020585	Histone H4	7	123712201	123712512	1	H4C7	AD	Low N
Zm00001d020586	Pectinesterase	7	123729032	123731282	-1		AD	Low N
Zm00001d020591	50 kDa gamma-zein	7	123954263	123955189	1		AD	Low N
Zm00001d020592	27 kDa gamma-zein	7	123982344	123983015	1		AD	Low N
Zm00001d020692	Hexosyltransferase	7	128747473	128750503	-1		GY	Low N
Zm00001d020693	Putative uncharacterized protein	7	128776562	128777569	1		GY	Low N
Zm00001d021544	PHI-1	7	155503583	155504602	-1		ASI	optimum
Zm00001d021546	Malic enzyme	7	155518816	155528949	-1		ASI	optimum
Zm00001d021554	ATP synthase delta chain	7	155779197	155787322	1		ASI	optimum
Zm00001d021569	Protein DETOXIFICATION	7	156294946	156300431	-1		AD	Optimum
Zm00001d021576	Glycosyltransferase	7	156641990	156643471	-1		AD	Optimum
Zm00001d021577	Glycosyltransferase	7	156646623	156648065	1		AD	Optimum
Zm00001d008914	Putative RING zinc finger domain superfamily protein	8	25177981	25178538	-1		PH	Low N

Gene stable ID	Gene description	Chr	Gene Start (bp)	Gene End (bp)	Strand	Gene name	Trait affected	Management
Zm00001d008916	Small nuclear ribonucleoprotein E	8	25186392	25205767	-1		PH	Low N
Zm00001d008918	SURF1-like protein	8	25221056	25222003	-1		PH	Low N
Zm00001d009589	Chlorophyll a-b binding protein, chloroplastic	8	71899226	71900023	-1		EPO	Optimum
Zm00001d009595	Putative WRKY DNA-binding domain superfamily	8	72169414	72173597	1		EPO	Optimum
Zm00001d009669	Sugar transport1 isoform 1	8	75146233	75152432	-1		GY	Optimum
Zm00001d010985	Organic anion transporter	8	135928640	135930519	1		ASI	optimum
Zm00001d010994	Structural constituent of ribosome	8	136140894	136141484	1		ASI	optimum
Zm00001d010998	Putative homeobox DNA-binding domain superfamily	8	136302778	136306209	1		ASI	optimum
Zm00001d011001	Membrane protein	8	136349224	136350587	1		ASI	optimum
Zm00001d011666	Putative calcium-dependent protein kinase family	8	158082120	158084527	1		EH	Optimum
Zm00001d011673	Farnesyl pyrophosphate synthetase	8	158269867	158274000	1		EH	Optimum
Zm00001d011708	Putative uncharacterized protein	8	159401583	159404367	1		SEN	Optimum
Zm00001d011710	Cytidine deaminase	8	159460166	159461104	-1		SEN	Optimum
Zm00001d011721	Putative leucine-rich repeat receptor-like protein kinase	8	159899428	159902796	-1		SEN	Optimum
Zm00001d012220	Putative ENTH/ANTH/VHS superfamily protein	8	170362957	170364624	1		AD	Optimum
Zm00001d012221	Acyl-desaturase	8	170366926	170368457	-1		AD	Optimum
Zm00001d012224	Hexosyltransferase	8	170419466	170424898	-1		AD	Optimum
Zm00001d012228	4,5-DOPA dioxygenase extradiol	8	170458719	170462003	1		AD	Optimum
Zm00001d047081	C2C2-GATA transcription factor	9	117752835	117754586	1		ASI	optimum
Zm00001d047087	Beta-expansin 1a	9	118041045	118043785	-1		ASI	optimum
Zm00001d047089	Beta-expansin 5	9	118067914	118069999	1		ASI	optimum
Zm00001d047090	Beta-expansin 1a	9	118161559	118163554	1		ASI	optimum
Zm00001d048252	Eukaryotic translation initiation factor 3 subunit K	9	153250495	153253737	1		SEN	Optimum
Zm00001d048253	40S ribosomal protein SA	9	153255360	153257954	-1		SEN	Optimum
Zm00001d048256	40S ribosomal protein SA	9	153315971	153318553	-1		SEN	Optimum
Zm00001d048263	Putative tify domain/CCT motif transcription factor	9	153418013	153418531	-1		SEN	Optimum
Zm00001d048268	Putative tify domain/CCT motif transcription factor	9	153485703	153486254	1		SEN	Optimum
Zm00001d048271	Proteasome subunit alpha type	9	153586355	153591283	1		SEN	Optimum
Zm00001d023395	Pop3 peptide	10	4672326	4673327	-1		ASI	Low N
Zm00001d023396	Putative RING zinc finger domain superfamily protein	10	4674375	4678089	-1		ASI	Low N
Zm00001d025613	CASP-like protein	10	123904076	123905406	1		EPO	Low N
Zm00001d025614	Putative IQ calmodulin-binding and BAG domain	10	123911243	123912298	-1		EPO	Low N
Zm00001d025616	Plastid-specific 30S ribosomal protein 3	10	124037257	124039095	1		EPO	Low N

Gene stable ID	Gene description	Chr	Gene Start (bp)	Gene End (bp)	Strand	Gene name	Trait affected	Management
Zm00001d025699	Nucleotide binding protein	10	126708804	126710162	-1		EH	Low N
Zm00001d025704	Wax synthase isoform 1	10	126794136	126795161	-1		EH	Low N
Zm00001d025706	CFM6	10	126880549	126888843	-1		AD	Low N
Zm00001d026321	Kelch motif family protein	10	143570399	143571745	1		PH	Optimum
Zm00001d026326	F-box domain containing protein	10	143599140	143600834	-1		PH	Optimum
Zm00001d026326	F-box domain containing protein	10	143599140	143600834	-1		EPO	Optimum
Zm00001d026335	Autophagy-related protein	10	143747463	143750718	-1		EPO	Optimum
Zm00001d026337	Starch synthase IIIb-1	10	143786832	143796162	-1		EPO	Optimum
Zm00001d026391	GNAT transcription factor	10	144991865	144994560	-1		EH	Low N
Zm00001d026394	Hexosyltransferase	10	145133662	145136374	1		EH	Low N
Zm00001d026397	RNA-binding protein AKIP1	10	145157330	145158895	-1		EH	Low N
Zm00001d026510	Putative HLH DNA-binding domain superfamily protein	10	147364599	147365225	-1		GY	Optimum
Zm00001d026514	Inositol 1,3,4,5,6-pentakisphosphate 2-kinase isoform 1	10	147437436	147441371	-1		GY	Optimum
Zm00001d026518	BSD domain containing protein	10	147474669	147476975	1		GY	Optimum
Zm00001d026521	Inner membrane protease subunit 1	10	147481845	147487342	-1		GY	Optimum
Zm00001d026537	Putative homeobox DNA-binding domain superfamily	10	147855536	147856873	-1		GY	Optimum
Zm00001d026537	Putative homeobox DNA-binding domain superfamily	10	147855536	147856873	-1		AD	Low N
Zm00001d026540	Auxin response factor	10	147919136	147925555	-1		AD	Low N
Zm00001d026542	G2-like transcription factor	10	147942412	147945498	-1		AD	Low N
Zm00001d026546	Dirigent protein	10	147981376	147981964	-1		AD	Low N
Zm00001d026547	Trafficking protein particle complex subunit 3	10	147985161	147988097	-1		AD	Low N
Zm00001d026569	Acyl-[acyl-carrier-protein] hydrolase	10	148186747	148188802	1		EPP	Low N
Zm00001d026575	Reticulon-like protein	10	148249658	148253209	1		EPP	Low N
Zm00001d026576	Leucine-rich repeat (LRR) family protein	10	148260149	148261444	1		EPP	Low N
Zm00001d026577	Cysteine protease 1	10	148261491	148265297	-1		EPP	Low N
Zm00001d026578	60S acidic ribosomal protein P2A	10	148267758	148269932	-1		EPP	Low N
Zm00001d026587	Zinc finger C-x8-C-x5-C-x3-H type family protein	10	148406795	148411190	-1		EPP	Low N

4.5 Discussion

A marker-trait association study was performed for eight agronomic traits (GY, AD, ASI, PH, EH, EPO, EPP and SEN), which were evaluated under optimum and managed low N stressed conditions. The result of GWAS is affected by many factors including, but not limited to, accuracy of phenotypic measurements and homogeneity of the phenotype, complexity of the genetic architecture of the phenotype, the extent of genetic diversity in the germplasm and LD relationships between causal variants and genotyped SNPs (Flint-Garcia et al., 2003; Zhu et al., 2008; Soto-Cerda and Cloutier, 2010; Scherer and Christensen, 2016). However, factors affecting the accuracy of GWAS could be improved through appropriate experimental designs and statistical packages.

Abundant differences at the phenotypic level and a high density of polymorphisms at the DNA sequence level are essential factors for high quality genetic mapping (Yan et al., 2009). Phenotypic data under low N conditions usually have low heritability due to the inherent variability in low N stressed fields. In this study, extensive genetic variance with medium to high heritability and high genetic variance were attained under both optimum and low N conditions. Relatively small experimental errors in this experiment were attributed to the use of many locations (nine optimum and 13 low N) with appropriate experimental designs (alpha lattice) which effectively estimated mainly genetic factors associated with the traits. As a result, the mean phenotypic data of most traits were normally distributed presenting an ideal condition for genome-wide marker-trait association study (data not shown).

The inbred lines used in this study were assembled from different tropical breeding programmes within the CIMMYT global maize programme and national agricultural research systems (NARS) in Africa and they were bred for tolerance to various biotic and abiotic stresses (Gowda et al., 2015). It is not unexpected to get high genetic distance and lower kinship among the inbred lines, hence stratification of the inbred lines into different groups based on the breeding goals and adaptation. Standard GWAS test statistics assume that all samples in the analysis are unrelated and selected from a uniform, random-mating population. Any departure from this assumption can cause unexpected results (Scherer and Christensen, 2016) leading

to spurious associations due to false positive associations. Use of appropriate statistical analysis that accounts for family relatedness and population structure is crucial in order to avoid the occurrence of false positives.

The extent of LD in a set of germplasm affects the mapping resolution and the number of markers required for association mapping studies (Yu and Buckler, 2006b). LD is further affected by the extent of genetic diversity captured by the population under study (Soto-Cerda and Cloutier, 2010). Genome-wide and chromosome specific LD decays in this study were extended over a few hundred kilobytes. Genome-wide LD decay was 230 kb at critical r^2=0.2 and at 190 at r^2=0.34. For individual chromosomes, this value was in the range of 130 to 340 kb at r^2=0.2 and 40 to 180 kb at r^2=0.34. Gowda et al. (2015) found similar results for a subset of inbred lines (385) used in this study. Previous studies on maize showed rapid LD decay (1 kb) in landraces, approximately 2 kb in diverse inbred lines and up to several hundred kb in commercial elite inbred lines (Jung et al., 2004). The relatively high LD in the current study is due to the use diverse elite inbred lines assembled from tropical breeding programmes within CIMMYT and from NARS in Africa (Gowda et al., 2015). Based on the observed LD, significant marker-trait associations can be identified using low to moderate marker numbers (Yan et al., 2011).

Taking family relatedness and population structure into consideration, FarmCPU identified several SNPs associated with the causative variant for each trait under optimum and low N conditions. Out of 83 SNP-trait associations declared significant at Bonferroni 5% threshold, three SNPs on chromosomes 1, 2 and 6 were associated either with different traits or different management conditions for the same trait, suggesting pleiotropic effects of genes associated with the significant markers. Common SNPs under optimum and low N conditions also suggested the expression some genes associated with the significant genes regardless of the N level. Such SNP markers would be useful for simultaneous improvement of traits and the same trait for different management conditions.

In addition to discovering SNPs significantly associated with markers, identifying putative genes in LD with significant SNPs, and study the function of the genes and the biological pathways in which the putative genes participate (Scherer and

Christensen, 2016) is crucial for using significant SNPs in breeding programmes. Putative genes were searched on the ensemble (http://plants.ensembl.org/biomart/martview/ce35c2dc12e78418361fb4cffa43bdbe) and maize gdb (http://www.maizegdb.org/) websites. The *FIE1* gene, likely to have acquired important novel functions for endosperm development and its maternal alleles, gets activated two days after pollination (Hermon et al., 2007) indicating its role in ASI. Narrow ASI is one of the desirable secondary traits that is positively correlated with high grain yield under stress conditions. The marker linked to the gene could be used for selecting genotypes having favourable alleles for narrow ASI.

teosinte glume architecture1(TGA1), on the other hand, is one of the key genes in the evolution of teosinte that exposed the kernel on the surface of the ear on modern maize such that it could be readily utilised as a food source by humans (Wang et al., 2006). The significant association of markers linked with PH indicates that this gene or other genes linked to this gene are directly involved in the control of plant height. By assaying the border effects of TGA1 in order to reduce or eliminate TGA1 gene expression using RNAi (ribonuclic acid interference) construct, maize lines expressing an RNAi construct targeting TGA1 displayed pleiotropic morphological effects on several branching and kernel traits (Wang et al., 2015). With regard to branching, these RNAi lines likely remove the repressive function of TGA1/neighbor of tag1 (NOT1), allowing the outgrowth of axillary branches. Both TGA1 and NOT1 belong to the SQUAMOSA promoter binding proteins (SBP) family of transcription factors. Members of this family have been shown to regulate meristem development, and manipulations of these regulators have produced both plant architecture and ear phenotypes (Chuck et al., 2009; 2014). The presence of the *TGA1/NOT1* duplication in maize may have facilitated the sub- functionalisation of *TGA1/NOT1* such that *TGA1* alone controls the fruitcase/cob in teosinte/maize while *TGA1* functions in a redundant manner with *NOT1* to regulate plant architecture traits. So, the SNP marker associated with *TGA1* can be used to improve plant height through MAS.

Another SNP (S4_237693358) on chromosome 4 that was associated with plant height under low N conditions, was linked with three gene models namely, Zm00001d053632, Zm00001d053633, and Zm00001d053635. These genes models were in LD with a known gene “RPS8”, ribosomal protein S8. RPS8 belongs to the

40S ribosomal protein S8 family. This gene was association with PH under low N conditions, which indicates tha rps8 genes might have a role in the control of plant height under stress conditions. Another SNP “S7_123828656” on chromosome 7 was associated with AD under low N conditions and was in LD with seven gene models, of which two (Zm00001d020584 and Zm00001d020585) were associated with a known gene histone H4 gene (*H4C7*). The gene belongs to the histone H4 protein family.

4.6. Conclusions

GWAS is a powerful method to detect marker trait association without a need to develop a mapping population that is distinct from the breeding population. In this study, a new and powerful statistical package known as FarmCPU was used to identify marker-trait associations for seven agronomic traits measured under low and optimum N conditions. Eighty three significant marker-trait associations were identified for all the traits under both optimum and low N conditions. No common markers were identified for grain yield between optimum and low N conditions, confirming different genetic mechanism for grain yield under optimum and low N conditions. The result further confirms higher efficiency of direct selection in target environments for the improvement of grain yield. For some secondary traits, common markers were obtained under optimum and low N conditions, suggesting the possibility of simultaneous improvement for two or more secondary traits using the same markers. The physical position of significant markers coincided with 158 putative protein coding genes. Four known genes were associated with traits under optimum and low N conditions. Some of these genes were previously reported to have association with the traits they are associated with in this study. Therefore, the markers associated with these putative and known genes could be used for implementation of marker assisted selection for the improvement of the traits associated with it.

4.7. References

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CHAPTER 5

Manuscript 3: Genetic dissection of grain yield and agronomic traits under optimum and low nitrogen stressed environments

5.1 Abstract

Optimum application of nitrogen (N) in maize is crucial to exploit yield potential of the crop. Yet, most small scale farmers in sub-Saharan Africa either use a very low amount or no N fertilizer for maize production, which severely reduces the yield of maize. Understanding the genetic basis of grain yield and other traits under N stressed environments could improve the efficiency of selection for low N stressed environments. In this study, five doubled haploid (DH) populations were evaluated in multiple environments under optimum (Opt) and N stressed conditions during the main rainy season (LNM) and off-season (LNO) in Kenya and Rwanda from 2014 to 2015. Identifying the genomic regions associated with grain yield (GY), anthesis date (AD), anthesis-silking interval (ASI), plant height (PH), ear height (EH), ear position (EPO), and leaf senescence (SEN) specifically under optimum, and N stressed environments could facilitate the use of MAS (MAS) to develop N use efficient (NUE) maize varieties. The main objectives of this study were to map and characterise the quantitative trait loci (QTL) for GY and some secondary traits under optimum and low N stressed conditions. All traits showed significant genotype and genotype x environment interaction variations and moderate to high heritability in each of the five populations. All DH lines were genotyped with genotyping by sequencing (GBS). A total of 13, 43, 13, 25, 30, 21 and 10 QTL were identified for GY, AD ASI, PH, EH, EPO, and SEN, respectively. For GY, PH, EH and SEN, the highest number of QTL were found under low N (LNO/LNM) stressed environments compared to optimum environments. No common QTL between optimum and low N stressed conditions were identified for GY and ASI. For secondary traits, though there were some common QTL for optimum and low N conditions, most QTL conferring tolerance to N stress were in a different chromosome position compared to the map position of the QTL detected under optimum conditions. Overall, the QTL detected with >10% of explained genotypic variance can be exploited in MAS programmes and are possible candidates for further genetic dissection.

5.2 Introduction

In sub-Saharan Africa, most maize is produced under N deficient conditions owing to limited availability of resources, low purchasing power of farmers, and low incentive from governments (Lafitte and Edmeades, 1994; Bänziger et al., 1997). In this scenario, developing cultivars tolerant to low N stressed environments are highly desired for sustainable production and ensuring food security in the region. Contrary to farmers' practice, most breeding programmes in the region develop new varieties under optimally managed on-station experimental plots. The genetic mechanism for grain yield under optimum and low N stressed conditions are different, and varieties developed for optimal environments often respond differently under N limiting environments (Bänziger et al., 1997; Worku et al., 2008). Understanding the genetic architecture of GY and traits correlated with it, would accelerate genetic improvement in maize yield.

GY is the most economically important trait in maize breeding programmes in developing countries. Other agronomically relevant traits including ASI, PH, EH, EPO, and SEN are often used by breeders to find desirable plant architecture and for indirect selection of high yielding maize varieties. The availability of reliable large effect QTL for GY and other traits under optimum as well as low N stressed conditions would accelerate the development and release of new maize varieties meeting yield demand under optimum and poor soil conditions, particularly for resource poor farmers. Unfortunately, not much is known about the genetic architecture of most of these traits under N stressed conditions and QTL with major effect have not yet been reported. Breeding for low N stressed conditions thus far focused on direct selection for grain yield and indirect selection for correlated secondary traits under N stressed conditions. Selection based of phenotypic traits is less accurate and expensive compared to marker based selection.

QTL analysis based on high density linkage maps will provide the basic understanding of the genetic architecture of quantitative traits, thereby relating specific genetic loci with the biological mechanisms associated with desirable phenotypes (Agrama, 2006). The identification and characterisation of QTL will help the breeders/geneticists to identify genomic regions associated with the expression of complex traits and their

precise genetic contribution at target loci. Several QTL studies have been undertaken in an effort to understand the genetic basis of abiotic stress tolerance in maize (Ribaut et al., 1996; Hirel et al., 2001; Malosetti et al., 2007; Almeida et al., 2013; 2014; Semagn et al., 2013; Fan et al., 2015). However, most studies focused on drought stress, and little research has been done on the dissection of the genetic basis of low N tolerance. The few studies conducted to understand the NUE and associated traits in maize have given good insight into the genetic basis of low N tolerance in maize (Agrama et al., 1999; Ribaut et al., 2007). One of the challenges in translating QTL identified into MAS, has been the environment-dependent and genotype specific nature of QTL identified (Collins et al., 2008). Most QTL reported under low and optimum N so far are mainly based on studies from one population in one/few optimum and low N environments. For example, Ribaut et al. (2007) used one mapping population with 240 $F_{2:3}$ families evaluated under one optimum and two low N sites in Mexico. Agrama (1999) also evaluated 214 F_3 families in one location over two seasons. Previous QTL mapping efforts for low N were conducted in single optimum or low N stressed sites using only one mapping population. Multi-location trial data from more than one mapping population would provide a clear picture about the stability of QTL across environments and genetic backgrounds. In this study, five doubled haploid (DH) populations were evaluated in three to five optimum environments, and one to three environments under managed low N stress in the wet (LNM) and off- (LNO) seasons. The main objectives of this study were 1) to identify the QTL associated with GY, and other related traits under optimum and low N stressed (LNM and LNO) conditions, and 2) to identify common genomic regions across management conditions, traits and genetic backgrounds. The identification of major QTL for GY and/or other traits that are common across different N conditions and genetic backgrounds would facilitate the application of MAS for the improvement of grain yield under low N stress conditions.

5.3 Materials and methods

5.3.1 Plant materials

Five DH populations from the Improved Maize for African Soil (IMAS) and the Water Efficient Maize for Africa (WEMA) projects of CIMMYT were used in this study. All five populations were developed through an *in vivo* DH technique as described by Geiger

and Gordillo (2009). Population 1 (CML494/CML550), population 2 (CML504/CML550) and population 3 (CML511/CML550) consisted of 108, 219 and 111 DH lines, respectively, developed from four inbred lines from CIMMYT heterotic group B. Population 4 (CML505/ LaPostaSeqC7-F64-2-6-2-2-B-B) and population 5 (CML536/LaPostaSeqC7-F64-2-6-2-2-B-B) consisted of 159 and 109 DH lines, respectively, and were developed from three inbred lines from CIMMYT heterotic group A. For population 4 and 5, genotypic data was avaialble for only the subset of the lines testcrossed and reported in chapter 3, section 3.3.1, and therefore only those DH lines with genotypic data were used for this study. CML550, LaPostaSeqC7-F64-2-6-2-2-B-B and CML494 are among the top 20 low N donor lines identified from a 412 panel of lines tested under low N in multiple environments, while CML504, CML505 and CML536 were sensitive to low N stress (data not shown). Consequently, one population used in this study represented low N tolerant x tolerant (CML494/CML550) while the remaining four populations represented tolerant x sensitive crosses. The DH lines from the five populations were testcrossed to a tester from the complementary heterotic group. DH lines from population 1 were test crossed to an inbred line tester CML312, population 2 and 3 were testcrossed to a single cross tester CML312/CML443, and population 4 and 5 were testcrossed to an inbred line tester, CML395. Testcross progenies from all five the populations were evaluated under optimum and managed low N conditions in the main and off-seasons of 2014 and 2015 in Kenya and Rwanda. The low N stress trials conducted during the off-season yielded significantly lower than the low N stressed trials conducted during the main rainy seasons, and therefore separate genetic analyses were performed for N stressed trials in the main rainy season (LNM) and off-season (LNO).

5.3.2 Field experiments and data analysis

Details on field experiment and data analysis are presented in Chapter 3 section 3.3.1. Briefly, the DH testcross progenies from all five populations were planted across 1-5 optimum, 1-3 LNM and 1-2 LNO environments in Kenya and Rwanda from 2014 to 2015 (Table 5.1). In each trial, 3-5 commercial varieties were included as standard checks. All optimum sites received the recommended amount of N fertilizer for the specific locality at planting and top-dressing one month after planting. All low N trials in all sites were planted in N-depleted fields, where maize has been planted for several

seasons without N fertilizer application and crop residues were removed after harvest every season. Data was collected for GY, AD, ASI, PH, EH, EPO, and SEN. GY was calculated from field weight by adjusting grain moisture to 12.5% and shelling percentage of 80%. AD is the number of days from planting to when 50% of plants in the plot started shedding pollen on the main axis of the tassel. ASI was calculated as the difference between the number of days when 50% of plants in a plot emerged 2-3 cm silk and pollen shedding. PH and EH were measured in centimeters as a distance from the base of a plant to the first branch of the tassel and the upper most ear from ten representative plants, respectively. EPO was calculated as the ratio between PH and EH. SEN was recorded by visual assessment using a 1-10 scale, where 1 indicates all leaves of all plants in a plot were green and 10 indicates that all leaves were dead. At harvest, edge plants were removed from all rows from trials planted under low N, to avoid border effects. Analyses of variance within and across environments in each population under each management condition was done with the restricted maximum likelihood method using the R program embedded in META-R software (Alvarado et al., 2015).

Table 5.1 Pedigree and size of populations used and number of optimum (OPT) and low nitrogen stress environments in the main season (LNM) and off season (LNO)

Population	Pedigree	Size	Tester	HG	No of environments			
					OPT	LNM	LNO	Total
1	CML494 x CML550	108	CML312	B	5	3	2	10
2	CML504 x CML550	219	CML312/CML443	B	3	3	2	8
3	CML511 x CML550	111	CML312/CML443	B	5	3	2	10
4	CML505 x LaPostaSeqC7-F64-2-6-2-2-B-B	159	CML395/CML444	A	1	1	1	3
5	CML536 x LaPostaSeqC7-F64-2-6-2-2-B-B	109	CML395/CML444	A	3	1	1	5

HG, heterotic group; OPT, optimum; LNM, low N during main season; LNO, low N during off-season

Variance components were determined following the linear mixed model:

$$Y_{ijko} = \mu + g_i + l_j + r_{kj} + b_{ojk} + e_{ijko},$$

where Y_{ijko} was the phenotypic performance of the i^{th} genotype at the j^{th} environment in the k^{th} replication of the o^{th} incomplete block, μ was an intercept term, g_i was the genetic effect of the i^{th} genotype, l_j was the effect of the j^{th} environment, r_{kj} was the effect of the k^{th} replication at the j^{th} environment, b_{ojk} was the effect of the o^{th} incomplete block in the k^{th} replication at the j^{th} environment, and e_{ijko} was the residual.

The effects of environments and replications were treated as random effects and the other effects as fixed. Heritability on an entry-mean basis was estimated from the variance components as the ratio of genotypic to phenotypic variance. In addition, best linear unbiased prediction (BLUP) of each DH line across environments within each management level was calculated for all the traits.

5.3.3 Genotyping, genetic maps and QTL analysis

DNA extraction and genotyping was done as described in Chapter 4, section 4.3.3. The genotype data was filtered with a minor allele frequency (MAF) of 0.05 and a minimum count of 95% of the sample size using TASSEL v.5.2.24 software (Bradbury et al., 2007). Then only marker loci homozygous for both parents and polymorphic between the two parents were retained in all populations. Finally, markers were selected based on distance (more than 250 Mb apart) to get the number of markers handled by the QTL analysis software and to ensure uniform distribution of markers on the genome.

Linkage maps for all five populations were constructed using QTL IciM mapping ver. 4.0.6.0. (http://www.isbreeding.net) software using a criterion of more than 3.0 logarithm of odds (LOD) (Li et al., 2007). Recombination frequencies between two linked loci were transformed into cM distances using Kosambi's mapping function (Kosambi, 1944). QTL analysis was performed using the across locations BLUPs for each population within each management condition. QTL associated with each trait were identified using an inclusive interval mapping (ICIM) method implemented in the software QTL IciM Mapping v.4.0.6.0 (Li et al., 2007). The walking step in QTL scanning was 1 cM and a LOD threshold of 3.0 was used to declare putative QTL (Ribaut et al., 1997). The sign of the additive effects of each QTL was used to identify the direction (the origin of the favourable allele) and effect size of each QTL.

5.4. Results

5.4.1 Trial mean, genetic variance and heritability of traits

Detailed results on the mean performance, genetic variance, heritability, and genetic and phenotypic correlations of all traits are presented in a submitted paper (Berhanu

et al., 2017, under review). Briefly, genotypic differences in all trials were highly significant for GY, AD, ASI, PH, EH, EPO and SEN under both optimum and low N management conditions. Increase in the intensity of N stress decreased trial mean for GY, PH, EH and EPO, and increased trial mean for ASI and SEN (Fig. 5.1). Average genetic variance in all populations was higher under optimum conditions for GY, PH, EH and EPO, but it was high under low N conditions for AD, SEN and ASI. Despite relatively higher genetic variance under optimum than low N conditions, broad sense heritability for GY and most secondary traits under low N and optimum conditions was on a par. Phenotypic and genetic correlations for GY was consistently positive and significant with PH, EH, EPO and AD.

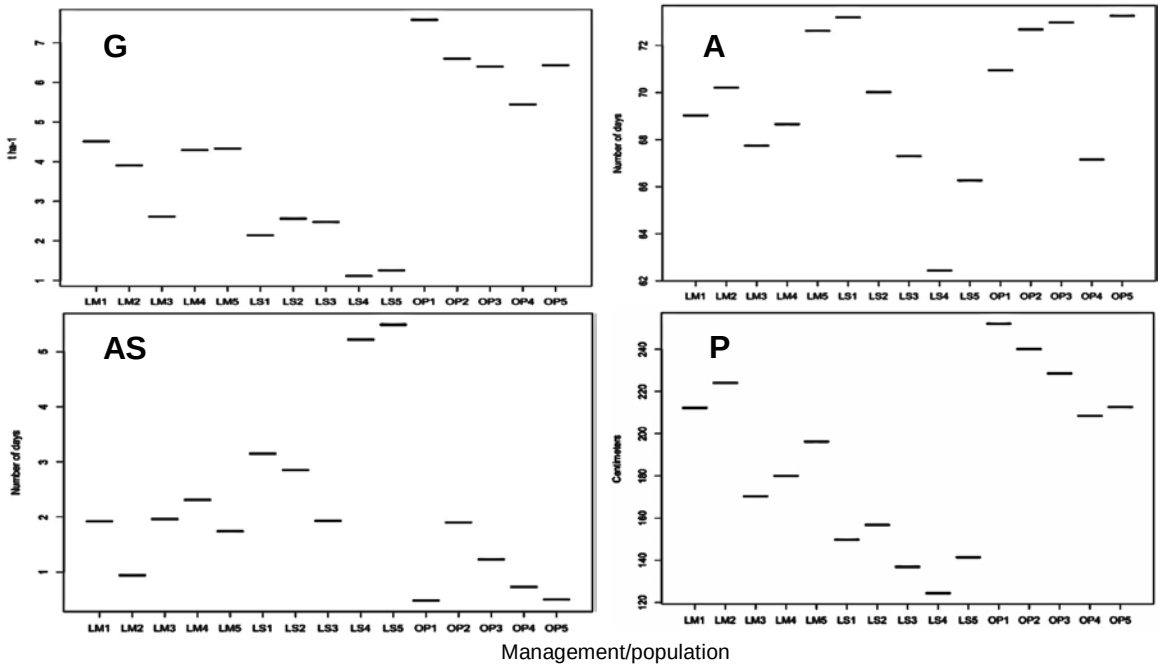

Figure 5.1 The mean of grain yield, anthesis date, anthesis silking interval, and plant height under optimum (OP), moderately low N stress (LM) and severely low N stressed (LS) conditions. The numbers after the management conditions on x-axis indicate populations 1 to 5

5.4.2 QTL mapping in five DH populations

A total map length of 3688.3, 4004.6, 3871.9, 7193.1 and 3426.4 cM were obtained from 2104, 2699, 1962, 1985 and 2086 SNP markers (Table 5.2) for populations 1, 2, 3, 4 and 5, respectively. The average distance between adjacent markers ranged from 1.48 cM for population 2 to 3.62 cM for population 4.

QTL analysis identified 155 significant QTL for GY, AD, ASI, PH, EH, EPO and SEN across ten maize chromosomes under optimum conditions (55), LNM (49) and LNO

(51) conditions (Table 5.3). Though slightly higher under optimum conditions, the total number of QTL identified for all N conditions, traits and populations were comparable. The total number of QTL identified for GY, AD, ASI, PH, EH, EPO and SEN were 13, 43, 13, 25, 30, 21 and 10, respectively. The distribution of QTL was variable among chromosomes, ranging between six (chromosomes 9) and 51 (chromosome 1) with an average of 15.5 QTL in each chromosome. The three chromosomes with the largest number of QTL were chromosome 1(51), chromosome 3 (26) and chromosome 8 (20). The distribution of the QTL across the five populations were 28, 84, 16, 13, and 14 for population 1, 2, 3, 4 and 5, respectively. The nature of the identified QTL varied from being unique for one trait, management and population to being common among management conditions, traits and populations.

For GY, 13 significant QTL were identified under optimum (3), LNM (2) and LNO (8) conditions across all chromosomes, except chromosomes 5, 6 and 9 (Table 5.4). Common QTL for optimum and low N stressed conditions were not identified in all five populations. QTL underlying GY under optimum, LNM and LNO conditions were identified on chromosomes 1, 2, 7 and 10 of population 2. About 62% of all QTL for GY individually contributed more than 10% of the observed phenotypic variance for GY. The proportion of phenotypic variance explained by each QTL varied between 6.05 to 17.55% with an average of 10.79%. The total phenotypic variance explained (TPVE) by all QTL under optimum conditions was 16.68% for population 1, 39.17% for population 2 and 9.32% for population 5. QTL for LNM were found only in population 2 and the TPVE was 11.50%.

Table 5.2 Number of markers and total map distance used in each population for QTL analysis

Chr.	CML494/CML550		CML504/CML550		CML511/CML550		CML505/LaPostaSeqC7-F64-2-6-2-2-B-B		CML536/LaPostaSeqC7-F64-2-6-2-2-B-B	
	SNPs	Distance (cM)	SNPs	Distance(cM)	SNPs	Distance(cM)	SNPs	Distance (cM)	SNPs	Distance(cM)
1	371	644.9	404	511.5	285	642.5	314	1068.4	311	512.5
2	242	432.4	310	460.6	237	428.5	216	841.4	256	270.9
3	233	417.7	286	538.8	197	434.7	259	961.6	237	489
4	238	359.3	325	336.7	244	470.7	254	796.0	277	381.5
5	211	502.7	283	539.4	193	568.4	118	581.7	211	311.1
6	138	249.6	195	320.3	180	225.2	172	808.5	177	312.4
7	172	420.8	228	375.4	153	318.4	151	671.1	152	432.7
8	195	379.9	250	441.6	177	322.5	170	506.0	176	247.9
9	162	103.9	221	294.7	139	199.5	162	543.7	140	236
10	142	177.1	197	185.6	157	261.5	169	414.7	149	232.4
Total	2104	3688.3	2699	4004.6	1962	3871.9	1985	7193.1	2086	3426.4

Table 5.3 Number of QTL detected for grain yield (GY), anthesis date (AD), anthesis-silking interval (ASI), plant height (PH), ear height (EH), ear position (EPO), and leaf senescence (SEN) under optimum, low nitrogen stress in main rainy season (LNM) and off-season (LNO), across the ten chromosomes

	LNM								LNO								OPT								
Chr.	**AD**	**ASI**	**EH**	**EPO**	**GY**	**PH**	**SEN**	**Total**	**AD**	**ASI**	**EH**	**EPO**	**GY**	**PH**	**SEN**	**Total**	**AD**	**ASI**	**EH**	**EPO**	**GY**	**PH**	**SEN**	**Total**	**Total**
1	5	1	4	1		4	1	16	7	1	3	4	2	3		20	3		5	2	2	3		15	51
2	2						1	3					1			1				1	1	1	1	4	8
3	1	1	3			2		7		3			3	2	2	10	3	2	1	1		1	1	9	26
4			1	2				3	1			1	1			3	3			2				5	11
5			2					2	1						1	2	3	1	1	1				6	10
6	1					1		2				1			1	2	1		1	1			1	4	8
7	1		2		1	1		5	1							1	1	1						2	8
8			2	1		2		5	1	1	2	2	1	2		9	4			1		1		6	20
9	1							1	1					1	1	3		1				1		2	6
10	2	1	1		1			5											2					2	7
Total	**13**	**3**	**15**	**4**	**2**	**10**	**2**	**49**	**12**	**5**	**5**	**8**	**8**	**8**	**5**	**51**	**18**	**5**	**10**	**9**	**3**	**7**	**3**	**55**	**155**

Table 5.4 Genetic characteristics of detected QTL for grain yield (GY) under optimum, low nitrogen stress in main season (LNM) and off-season (LNO) in DH lines derived from five bi-parental populations

Population	Mgt	Chr.	Pos (cM)	LeftMarker	RightMarker	LOD	PVE(%)	TPVE (%)	Add	Fav Allele
CML550 x CML494	OPT	2	318	S2_15120146	S2_15909091	4.48	17.23	16.68	0.12	CML550
CML550 x CML504	OPT	1	46	S1_283186611	S1_280222332	4.43	6.31	39.17	-0.12	CML504
	OPT	1	183	S1_219232023	S1_217114738	10.24	15.04		-0.18	CML504
	LNM	7	278	S7_106325823	S7_105221050	3.39	6.84	11.50	-0.04	CML504
	LNM	10	143	S10_11189892	S10_10138689	3.06	6.05		0.04	CML550
	LNO	2	173	S2_200745112	S2_202138908	3.10	6.66	11.55	-0.09	CML504
CML550 x CML511	LNO	3	228	S3_127100888	S3_128233351	4.69	17.55	23.34	-0.10	CML511
	LNO	4	177	S4_165623425	S4_162255436	3.71	13.56		-0.09	CML511
CML505/LaPostaSeqC7-F64-2-6-2-2-B-B	LNO	1	531	S1_177877619	S1_183811363	3.91	10.14	22.30	0.06	LP
	LNO	3	169	S3_204998702	S3_203869859	4.38	10.34		-0.05	CML505
CML536/LaPostaSeqC7-F64-2-6-2-2-B-B	LNO	1	439	S1_41220359	S1_39739703	3.64	9.90	38.54	0.08	LP
	LNO	3	345	S3_38439419	S3_31449087	3.68	10.10		0.08	LP
	LNO	8	157	S8_166372615	S8_168274395	3.77	10.54		0.08	LP

The TPVE under LNO was 11.50% for population 2, 23.34% for population 3, 22.30% for population 4 and 30.54% for population 5. The average QTL effect size under optimum (0.14 t ha^{-1}) conditions was the highest compared to LNM (0.04 t ha^{-1}) or LNO (0.08 t ha^{-1}) conditions. Interestingly, the favorable alleles of the QTL detected under all management conditions were contributed by both low N tolerant and susceptible parents.

Forty-three significant QTL were identified for AD under optimum and low N stressed conditions (Table 5.5) across all chromosomes and populations. The number of QTL identified were 18 under optimum, 13 under LNM and 12 under LNO conditions. The largest number of QTL were detected in population 2 (16) followed by population 1 (15). The phenotypic variance explained by each QTL ranged between 3.19% and 95.81% with an average of 17.40%. The total proportion of phenotypic variance explained by all QTL under optimum conditions was 71.31% for population 1, 46.88% for population 2, 29.02% for population 3 (only one QTL), and 13.36% for population 4 (only one QTL). Under LNM TPVE was 28.86% for population 1, 58.04% for population 2, 12.04% for population 3 (only one QTL), 8% for population 4 (only one QTL) and 37.71% for population 5. The TPVE under LNO was 47.27% for population 1, 33.58% for population 2, 46.11% for population 3, 25.45% for population 4 and 29.69% for population 5. The effect size of all QTL ranged from 0.11 to 3.56 days with an average of 0.54 days. Despite many (24) individual QTL explaining more than 10% phenotypic variance under different management conditions, only one QTL with high effect size under all management conditions was found. This QTL was identified on chromosome 1 (343 cM) from population 3. The effect size of this QTL was 2.99 days under optimum, 2.23 days under LNM conition and 3.56 days under LNO condition. ASI is another secondary trait related to flowering and indicates the tolerance of maize genotypes to low N stress. Only three QTL, one in each population 2, 4 and 5 explained greater than 10% phenotypic variation for ASI (Table 5.6) were identified. The total phenotypic variance explained by two QTL (39.41%) in population 5 was the highest attained in this study. The highest effect size for ASI was attained by these two QTL (0.53 and 0.32 days). Generally, the effect size for ASI varied between 0.05 and 0.53 days with an average of 0.14 days.

Table 5.5 Genetic characteristics of detected QTL for anthesis date (AD) under optimum, low nitrogen stress in main season (LNM) and off-season (LNO) in DH lines derived from five bi-parental populations

Population	Mgt	Chr	Pos	LeftMarker	RightMarker	LOD	PVE(%)	TPVE(%)	Add	Fav Allele
CML550/CML494	OPT	3	227	S3_152451120	S3_155619613	4.46	5.46	71.31	0.13	CML494
	OPT	3	355	S3_1289855	S3_171466703	3.22	3.88		-0.11	CML550
	OPT	4	100	S4_228926221	S4_228626317	13.47	20.21		0.26	CML494
	OPT	5	218	S5_196031436	S5_206019269	8.69	11.81		-0.20	CML550
	OPT	6	26	S6_162568586	S6_161010798	4.58	5.90		0.14	CML494
	OPT	7	152	S7_174157338	S7_173807263	9.21	12.27		-0.20	CML550
	OPT	8	245	S8_151911852	S8_152261359	10.64	14.96		0.22	CML494
	OPT	8	267	S8_142233374	S8_137468517	15.30	27.79		-0.30	CML550
	LNM	2	347	S2_31924520	S2_34925673	3.45	11.32		-0.24	CML550
	LNM	3	265	S3_45035564	S3_47832327	3.27	10.28		0.23	CML494
	LNO	1	426	S1_198541547	S1_198279139	3.11	7.25	47.27	-0.30	CML550
	LNO	1	519	S1_27140851	S1_26197963	4.42	10.51		-0.36	CML550
	LNO	4	131	S4_183134905	S4_181871673	3.71	8.68		0.33	CML494
	LNO	7	146	S7_175566913	S7_174157338	5.64	14.26		-0.41	CML550
CML550/CML504	OPT	1	43	S1_282409602	S1_283186611	3.73	4.47	46.88	-0.25	CML550
	OPT	1	292	S1_69865657	S1_69288842	5.40	6.44		-0.30	CML550
	OPT	3	67	S3_200876966	S3_201584853	4.18	4.90		0.27	CML504
	OPT	4	61	S4_224308438	S4_224048022	3.13	3.67		0.23	CML504
	OPT	5	457	S5_45438168	S5_44985543	30.55	50.60		-0.84	CML550
	OPT	5	466	S5_41538958	S5_40652438	18.05	26.80		0.62	CML504
	OPT	8	21	S8_168815355	S8_168493048	3.17	3.67		-0.23	CML550
	OPT	8	107	S8_135070884	S8_130930928	34.71	57.59		-1.05	CML550
	LNM	1	42	S1_284504632	S1_282409602	56.25	95.81	58.04	1.14	CML504
	LNM	1	383	S1_17383245	S1_14803778	10.23	10.49		-0.37	CML550
	LNM	2	229	S2_183919141	S2_184646201	7.38	7.05		-0.32	CML550
	LNM	6	240	S6_162558564	S6_168794605	5.44	5.07		0.26	CML504
	LNM	7	271	S7_112877861	S7_110568982	4.03	3.79		-0.22	CML550
	LNM	10	3	S10_150087021	S10_146943516	3.41	3.19		0.21	CML504
	LNO	1	351	S1_32079580	S1_33832111	34.66	81.62	33.58	1.00	CML504
	LNO	9	147	S9_104435623	S9_102698508	4.60	7.36		-0.30	CML550
CML550/CML511	OPT	1	343	S1_52345244	S1_230179861	8.16	29.42	29.02	-2.99	CML550
	LNM	1	343	S1_52345244	S1_230179861	3.18	12.75	12.04	-2.23	CML550
	LNO	1	343	S1_52345244	S1_230179861	12.52	39.42	46.11	-3.56	CML550
	LNO	1	521	S1_46413710	S1_42476919	4.60	12.06		-0.39	CML550
CML505/LaPostaSeqC7-F64-2-6-2-2-B-B	OPT	4	264	S4_69843767	S4_67493486	4.93	13.41	13.36	0.31	CML505
	LNM	1	771	S1_66013917	S1_60755570	3.21	9.11	8.00	0.29	CML505
	LNO	1	392	S1_220785207	S1_221241110	4.64	10.49	25.45	-0.39	LP
	LNO	5	445	S5_421	S5_141945888	5.31	15.99		0.40	CML505
CML536/LaPostaSeqC7-F64-2-6-2-2-B-B	LNM	9	141	S9_112940495	S9_111715623	8.42	26.35	37.71	0.44	CML536
	LNM	10	59	S10_89984330	S10_90815324	3.29	9.19		0.26	CML536
	LNO	1	415	S1_49415609	S1_48379091	4.27	13.51	29.69	-0.36	LP
	LNO	8	136	S8_147917080	S8_148274279	3.22	9.95		-0.30	LP

Table 5.6 Genetic characteristics of detected QTL for anthesis silking interval (ASI) under optimum, low nitrogen stress in main season (LNM) and off-season (LNO) in DH lines derived from five bi-parental populations

Population	Mgt	Chr.	Pos (cM)	Left Marker	Right Marker	LOD	PVE(%)	TPVE(%)	Add	Fav Allele
CML550/CML504	OPT	3	77	S3_204126924	S3_206481369	4.91	7.58	31.26	0.06	CML504
	OPT	5	376	S5_169668014	S5_163945834	3.43	5.32		-0.05	CML550
	OPT	7	263	S7_119597893	S7_113205468	3.70	5.59		-0.05	CML550
	OPT	9	122	S9_119779555	S9_119132452	3.08	4.67		-0.05	CML550
	LNM	1	300	S1_66387567	S1_65350627	4.12	7.04	24.10	-0.09	CML550
	LNM	3	49	S3_197718647	S3_196434589	6.13	11.54		0.12	CML504
	LNM	10	180	S10_3908652	S10_1148472	3.76	7.16		-0.09	CML550
	LNO	8	97	S8_139630981	S8_135070884	3.61	7.52	12.84	0.10	CML504
CML505/LaPostaSeqC7-F64-2-	OPT	3	200	S3_183867892	S3_199561708	4.57	12.94	11.70	0.17	CML505
	LNO	3	205	S3_193795900	S3_186485761	3.40	8.29	19.68	0.10	CML505
	LNO	3	357	S3_151334181	S3_149229159	4.04	9.79		-0.12	LP
CML536/LaPostaSeqC7-F64-2-	LNO	1	466	S1_27505154	S1_26435510	8.84	27.12	39.41	-0.53	LP
	LNO	3	163	S3_213298747	S3_211719240	3.66	9.98		-0.32	LP

From the total of 25 QTL identified for PH from all populations on all chromosomes except chromosomes 4, 5 and 10, seven were under optimum, 10 under LNM and eight under LNO conditions (Table 5.7). Of all five populations, only population 2 had QTL for all three management conditions. Thirteen QTL from the three conditions individually explained more than 10% phenotypic variance for PH. For the QTL in population 2, the TPVE was 59.82% under optimum, 61.72% under LNM and 49.52% under LNO conditions. For populations 1 and 3, the total phenotypic variance explained by all QTL under LNM were 20.40% (one QTL) and 44.52%, respectively. Three QTL together explained 26.45% of the phenotypic variation observed for PH in population 1. One QTL in each population 3, 4 and 5, explained 24.33%, 8.05% and 13.39% of the observed phenotypic variance for PH. The effect size of the individual QTL for PH ranged from 0.87 to 8.34 cm with an average of 2.19 cm. Like AD, a QTL on chromosome 1 (343 cM) of population 3 combined more than 10% phenotypic variance and the highest effect size for PH. Two other QTL on chromosomes 1 (194.94 Mbp to 195.75 Mbp) and 8 (92.20 Mbp to 94.58 Mbp) of population 2 explained high phenotypic variance (16.96 and 13.92%) and had high effect size (3.27 and 2.99 cm). Like PH, the largest number of QTL for EH and EPO (Tables 5.8 and 5.9) was identified from population 2. The QTL on chromosome 1 of population 3, which combines a higher proportion of phenotypic variance explained and high QTL effect for AD and PH, also had the same effect for EH.

Unlike other traits in this study, QTL for SEN were identified only from population 2 with the largest number being under LNO (Table 5.10). The total phenotypic variance explained under optimum, LNM and LNO conditions was 23.65%, 15.06%, and 45.87%, respectively. The highest amount of phenotypic variance and largest number of QTL under LNO indicates the genetic variability existing under LNO for SEN and the contrasting nature of the two parents that constituted population 2.

Table 5.7 Genetic characteristics of detected QTL for plant height (PH) under optimum, low nitrogen stress in main season (LNM) and off-season (LNO) in DH lines derived from five bi-parental populations

Population	Mgt	Chr	Pos (cM)	Left Marker	Right Marker	LOD	PVE(%)	TPVE(%)	Add	Fav Allele
CML550/CML494	LNM	3	224	S3_46511540	S3_153262861	3.70	14.00	20.40	2.04	CML550
	LNO	3	179	S3_172906641	S3_168838491	3.83	12.85	26.45	1.08	CML494
	LNO	9	62	S9_151147419	S9_150224858	4.71	17.13		1.23	CML494
CML550/CML504	OPT	1	46	S1_283186611	S1_280222332	8.32	8.04	59.82	-1.74	CML550
	OPT	1	107	S1_237562292	S1_236572842	3.80	3.39		-1.13	CML550
	OPT	1	206	S1_195754378	S1_194942819	15.72	15.89		-2.44	CML550
	OPT	2	141	S2_219659850	S2_218462880	3.64	3.20		1.09	CML504
	OPT	3	53	S3_197718647	S3_196434589	4.18	3.80		1.22	CML504
	OPT	8	341	S8_75951924	S8_77725407	10.78	10.40		1.98	CML504
	OPT	9	224	S9_21192733	S9_19527579	3.75	3.33		-1.26	CML550
	LNM	1	46	S1_283186611	S1_280222332	7.69	7.23	61.72	-2.14	CML550
	LNM	1	206	S1_195754378	S1_194942819	17.30	16.96		-3.27	CML550
	LNM	1	271	S1_86945521	S1_83434475	6.44	5.60		-1.88	CML550
	LNM	3	82	S3_206195841	S3_208333232	3.77	3.19		1.46	CML504
	LNM	7	231	S7_126750234	S7_125835674	4.30	3.66		-1.52	CML550
	LNM	8	334	S8_92199584	S8_94575375	14.50	13.92		2.99	CML504
	LNO	1	48	S1_283186611	S1_280222332	8.08	10.25	49.52	-1.27	CML550
	LNO	1	206	S1_195754378	S1_194942819	12.49	16.56		-1.62	CML550
	LNO	8	301	S8_136912486	S8_137757371	3.94	4.71		0.87	CML504
CML550/CML511	LNM	1	343	S1_52345244	S1_230179861	5.52	14.26	44.52	-8.12	CML550
	LNM	6	127	S6_96152977	S6_94700288	3.37	8.30		-1.20	CML550
	LNM	8	65	S8_145140385	S8_137303469	4.53	11.71		1.44	CML550
	LNO	1	343	S1_52345244	S1_230179861	3.78	13.77	24.33	-8.34	CML550
CML505/LaPostaSeqC7-F64-2-	LNO	3	190	S3_194824065	S3_195397829	3.04	8.48	8.05	-1.54	LP
CML536/LaPostaSeqC7-F64-2-	LNO	8	156	S8_162185699	S8_166372615	3.58	14.03	13.39	1.79	LP

Table 5.8 Genetic characteristics of detected QTL for ear height (EH) under optimum, low nitrogen stress in main season (LNM) and off-season (LNO) in DH lines derived from five bi-parental populations

Population	Mgt	Chr	Pos (cM)	LeftMarker	RightMarker	LOD	PVE(%)	TPVE(%)	Add	Fav Allele
CML550/CML494	LNM	3	84	S3_207741357	S3_206481439	3.05	6.16	53.16	-1.02	CML550
	LNM	3	219	S3_46177572	S3_46511540	8.26	19.32		1.83	CML494
	LNM	5	177	S5_188667809	S5_191088426	5.69	12.14		-1.64	CML550
	LNM	7	300	S7_17479195	S7_17180908	5.91	12.87		-1.53	CML550
	LNM	8	267	S8_142233374	S8_137468517	7.89	20.00		-1.84	CML550
	LNO	8	268	S8_130729873	S8_123153386	4.40	15.68	24.29	-1.12	CML550
CML550/CML504	OPT	1	25	S1_292891535	S1_291386678	5.45	5.50	56.64	-1.07	CML550
	OPT	1	183	S1_219232023	S1_217114738	11.92	12.43		-1.61	CML550
	OPT	1	292	S1_69865657	S1_69288842	10.46	10.67		-1.49	CML550
	OPT	3	66	S3_200596344	S3_200876966	4.16	4.07		0.94	CML504
	OPT	6	222	S6_153666432	S6_154619899	3.60	3.42		0.85	CML504
	OPT	10	9	S10_146943516	S10_144137339	6.44	7.00		1.22	CML504
	OPT	10	157	S10_6373041	S10_5482369	3.76	3.65		-0.87	CML550
	LNM	1	136	S1_230879834	S1_230186447	3.80	3.98	52.26	-1.14	CML550
	LNM	1	206	S1_195754378	S1_194942819	4.12	4.34		-1.17	CML550
	LNM	3	82	S3_206195841	S3_208333232	3.61	3.79		1.12	CML504
	LNM	4	200	S4_71535960	S4_62855411	7.57	10.05		-1.79	CML550
	LNM	8	329	S8_101755334	S8_95745623	4.66	5.08		1.27	CML504
	LNM	10	110	S10_73624067	S10_34023384	3.03	3.15		-1.00	CML550
	LNO	1	46	S1_283186611	S1_280222332	3.48	6.27	27.42	-0.55	CML550
	LNO	1	206	S1_195754378	S1_194942819	6.86	12.59		-0.78	CML550
	LNO	1	392	S1_15735866	S1_14031653	5.27	9.40		-0.67	CML550
CML550/CML511	OPT	1	343	S1_52345244	S1_230179861	4.65	16.71	22.87	-12.84	CML550
	OPT	1	514	S1_50951450	S1_49392612	3.15	11.15		-2.06	CML550
	LNM	1	343	S1_52345244	S1_230179861	4.72	16.79	23.70	-7.82	CML550
	LNM	1	513	S1_51752437	S1_50951450	3.35	11.51		-1.28	CML550
CML505/LaPostaSeqC7-F64-2-6-2-2-B-B	OPT	5	168	S5_179858396	S5_177665119	3.72	9.94	14.79	0.89	CML505
	LNM	5	397	S5_11374553	S5_9616695	3.59	9.20		0.68	CML505
CML536/LaPostaSeqC7-F64-2-6-2-2-B-B	LNM	7	32	S7_166270114	S7_163512547	3.71	13.01	23.10	-1.38	LP
	LNO	8	156	S8_162185699	S8_166372615	3.05	12.11	11.40	0.93	CML536

Table 5.9 Genetic characteristics of detected QTL for ear position (EPO) under optimum, low nitrogen stress in main season (LNM) and off-season (LNO) in DH lines derived from five bi-parental populations

Population	Mgt.	Chr.	Pos (cM)	LeftMarker	RightMarker	LOD	PVE(%)	TPVE(%)	Add	Fav Allele
CML550/CML494	OPT	6	10	S6_166674013	S6_163629442	3.04	13.14	10.53	0.00	CML494
	LNM	8	262	S8_148261703	S8_148007217	5.25	18.69	23.30	-0.01	CML550
	LNO	8	263	S8_147097779	S8_146474892	8.04	28.90	28.75	-0.01	CML550
CML550/CML504	OPT	1	311	S1_58363752	S1_57288598	8.90	12.81	38.35	0.00	CML504
	OPT	3	110	S3_221158754	S3_221901376	4.23	5.77		0.00	CML504
	OPT	4	84	S4_191754231	S4_191538363	3.85	5.32		0.00	CML504
	OPT	4	246	S4_36724590	S4_33663643	6.86	9.80		0.00	CML504
	OPT	8	72	S8_152113114	S8_151194215	6.04	9.61		0.00	CML504
	LNM	1	311	S1_58363752	S1_57288598	7.78	12.03	33.54	0.00	CML504
	LNM	4	57	S4_227110696	S4_226284987	3.62	5.36		0.00	CML504
	LNM	4	248	S4_34335173	S4_34552572	7.30	11.25		0.00	CML504
	LNO	1	298	S1_67649374	S1_66013609	3.75	5.50	38.34	0.00	CML504
	LNO	1	392	S1_15735866	S1_14031653	3.26	4.70		0.00	CML504
	LNO	4	82	S4_193745467	S4_193147210	3.60	5.22		0.00	CML504
	LNO	6	158	S6_112817455	S6_114440133	3.13	4.47		0.00	CML504
	LNO	8	80	S8_151194215	S8_149801553	5.13	7.54		0.00	CML504
CML550/CML511	OPT	1	621	S1_10362814	S1_6174894	4.36	15.14	28.05	-0.01	CML550
	OPT	2	310	S2_15909091	S2_13288169	3.94	12.85		0.01	CML511
CML505/LaPostaSeqC7-F64-2-6-2-2-B-B	OPT	5	124	S5_186676927	S5_185412709	3.87	9.94	15.30	0.00	CML505
CML536/LaPostaSeqC7-F64-2-6-2-2-B-B	LNO	1	51	S1_271764010	S1_273696896	4.15	12.86	31.04	0.01	CML536
	LNO	1	400	S1_55503537	S1_53413566	5.21	16.55		0.01	CML536

Table 5.10 Genetic characteristics of detected QTL for ear position (EPO) under optimum, low nitrogen stress in main season (LNM) and off-season (LNO) in DH lines derived from five bi-parental populations

Population	MGT	Chr.	Pos (cM)	LeftMarker	RightMarker	LOD	PVE(%)	TPVE(%)	Add	Fav allele
CML550/CML504	OPT	1	168	S2_203937216	S2_204466878	4.42	7.62	23.65	0.03	CML504
	OPT	3	404	S3_134682595	S3_137089605	3.66	6.34		0.02	CML504
	OPT	6	249	S6_162558564	S6_168794605	3.35	6.15		-0.02	CML550
	LNM	1	204	S1_198219595	S1_197055941	4.21	8.12	15.06	-0.01	CML550
	LNM	2	174	S2_202138908	S2_201146790	3.44	6.51		0.01	CML504
	LNO	3	68	S3_200876966	S3_201584853	6.38	8.57	45.87	-0.06	CML550
	LNO	3	537	S3_181558923	S3_177129159	3.66	4.88		0.04	CML504
	LNO	5	309	S5_201939197	S5_85666905	4.65	8.77		0.08	CML504
	LNO	6	187	S6_131027488	S6_131760613	22.66	35.66		0.11	CML504
	LNO	9	37	S9_153413533	S9_149896475	4.83	6.14		0.05	CML504

5.4.3 QTL overlapping among management conditions for each trait

Discovering common QTL between different N conditions facilitates the identification of markers commonly used for optimum and low N stress breeding environments for a target trait. In this study, several common QTL between optimum and N stress conditions were identified for different traits, mainly in population 2. For AD, one QTL under optimum conditions (282.41 to 283.19 Mbp) was overlapping with two QTL under LNM conditions (280.22 to 283.19 and 282.41 to 284.50 Mbp) on chromosome 1. Two common QTL were identified between optimum, LNM and LNO for PH on chromosome 1 (from 194.94 to 195.75 Mbp and from 280.22 to 283.19 Mbp). In addition, one QTL correspondence was detected between LNM and LNO conditions on chromosome 1 (52.35 to 230.18 Mbp) of population 3 for PH. For EH, a common QTL was found between LNM and LNO conditions on chromosome 1 (194.94 to 195.75) in population 2. On chromosome 1 of population 3, two additional QTL (49.39 to 51.75 Mbp and 52.35 to 230.18 Mbp) were observed for EH between optimum and LNM conditions. One QTL overlapping between optimum and LNM conditions for EPO was also found on chromosome 1 (57.29 to 58.36 Mbp) of population 2. In addition, closely linked QTL were detected between optimum (191.54 to 191.75 Mbp) and LNO (193.15 to 193.75 Mbp) conditions, and overlapping QTL were detected between optimum (33.66 to 36.72 Mbp) and LNM (34.34 to 34.55 Mbp) conditions on chromosome 4 of population 2. Other QTL correspondences under LNM and LNO conditions was found on chromosome 8 of population 1, and optimum and LNO conditions on chromosome 8 of population 2. For SEN, one QTL under optimum (203.94 to 204.47 Mbp) conditions was closely linked to a QTL (201.15 to 2012.14 Mbp) identified under LNM on chromosome 2 of population 2. No QTL correspondence was found among different management conditions for GY and ASI.

5.4.4 QTL for multiple traits in one/different population

Markers associated with common QTL among different traits and genetic backgrounds would facilitate the use of MAS to achieve yield improvement under low N stressed conditions. Several multi-trait QTL were identified from all populations, except population 4. Because of large numbers of such QTL, only QTL with high PVE ($\geq$10%) and relatively high effect size were reported here (Tables 5.3-5.10). In population 1, a QTL on chromosome 1 (45.04 to 47.83 Mbp) was involved in the control of AD, EH

and PH under LNM conditions. Another QTL in this population was found on chromosome 8 from 137.47 to 142.23 Mbp underlying AD under optimum and EH under LNM conditions. In population 2, four QTL on chromosome 1 were involved in the control of multiple traits: from 57.29 to 58.36 Mbp for EH under LNM and EPO under optimum and LNM; from 194.94 to 195.75 Mbp for EH under LNO and PH under optimum, LNM and LNO; from 217.11 to 219.23 Mbp for GY and EH under optimum and from 280.22 to 283.19 Mbp for PH under LNO and AD under LNM conditions. A QTL stretch identified from population 3 on chromosome 1 (343 cM) spanning from 52.35 to 230.18 Mbp was associated with AD under all the three N conditions and for PH under low N conditions (LNM and LNO). A QTL on chromosome 8 (162.19 to 168.27 Mbp) of population 5 was involved in the control of GY, PH and EH under LNO conditions. This is an adaptive QTL responsible for the control of GY, PH and EH only under severe low N stress conditions.

Some QTL common between different genetic backgrounds were found for AD (chromosome 8 in population 1 and 2), ASI chromosome 3 of population 2 and 4. Both the upstream and downstream of the multi-trait QTL (57.84-69.87 Mbp) identified from population 3 also integrated QTL identified for GY and other secondary traits from other populations. This indicates that this region could be common among multiple genetic backgrounds and needs further research to fine-tune the position of the QTL responsible in the control of multiple traits.

5.5 Discussion

5.5.1 Yield reduction, variances and heritability

Yield reduction under low N stress conditions is an indication of the role of N in growth and development of maize. In this study, mean grain yield was reduced by 71% under severe N stress (LNO) and by 39% under moderate N stress (LNM) conditions. The yield reduction under moderate stress in this study was similar to yield reduction reported earlier (Gallais and Hirel, 2004) under low N stress conditions. Under severe N stress, the yield reduction observed was similar to yield reduction reported in an earlier study (Ribaut et al., 2007) under severe low N stress conditions during the wet season in Mexico. Many authors attributed yield reduction under low N stress to reduction in number of kernels as a result of increased abortion (Bänziger et al., 1997;

Agrama et al., 1999; Gallais and Hirel, 2004; Ribaut et al., 2007). A big gap between silk emergence and pollen shed (ASI) under low N stress conditions is one of the causes for kernel abortion. The large difference in yield reduction under low N during main and off-seasons showed the seasonal variation of low N environments during the rainy and dry seasons.

According to Bänziger et al. (2000), if grain yield under low N stress is below 50% of the yield obtained under optimum N conditions, the yield reduction is related to mechanisms that impart tolerance to low N stress. In this study, grain yield obtained under severe stress conditions was only 29% of yield under optimum conditions and thus suitable for studying QTL underlying GY and secondary traits under low N stressed conditions. However, since high levels of stress affect genetic variance and hence detection of QTL (Ribaut et al., 1996), moderately N stressed environments were also included to capture QTL under all N stress levels. In addition to GY, higher genetic variance was observed for some secondary traits under low N conditions compared to optimum conditions, indicating the stress adaptive nature of these traits (Almeida et al., 2013) and therefore increases the power of QTL detection under low N (Agrama et al., 1999).

5.5.2 QTL for GY and secondary traits under optimum and low N conditions

QTL underlying GY and secondary traits under optimum and low N stress conditions could accelerate the development of NUE varieties. QTL analysis in this study identified 155 significant QTL in five populations for seven traits under optimum, LNM and LNO conditions. Some of these QTL were specific to only one trait, management condition and population while others were found across traits, management conditions and populations. The distribution of the QTL also varied across the ten chromosomes of maize. The total number of QTL identified under the three conditions were comparable, indicating the existence of genetic variability under all three conditions. The highest number of QTL were detected for AD among traits, and in population 2 among populations. The result was consistent with the highest genetic variance observed for AD under all management conditions. The highest number of QTL in population 2 indicates the contrasting nature of the constituting parents for most traits under all management conditions. Chromosomes 1, 3 and 8 had the

highest number of QTL and could be the targeted for further QTL studies for grain yield and related secondary traits under both optimum and low N stressed conditions.

For any target trait, identifying common QTL among management conditions, traits and populations is desirable for successful implementation of MAS schemes. GY is the primary trait of interest in most breeding programmes in sub-Saharan Africa where maize is a staple food. Most breeding programmes in the sub-continent often develop new varieties under optimally managed experimental fields and the resulting new varieties are commonly grown under N limiting small scale maize farms. The correlation between low and high N environments for grain yield have been reported to be low in both tropical (Bänziger et al., 1997; Worku et al., 2007), and temperate (Presterl et al., 2003) environments. Lack of common QTL between low and optimum N conditions for GY in this study agrees with classical correlation studies, and shows distinct genetic mechanisms for GY under low and optimum N conditions. N-uptake efficiency under optimum and both uptake and utilisation efficiencies under low N conditions play a role in GY (Gallais and Hirel, 2004; Gallais and Coque, 2005). As such, GY improvement for low N stressed environments should be through direct selection in target environments as previously suggested (Bänziger et al., 1997). More QTL detected for GY under low N than optimum conditions in this study indicates high genetic variability for GY under low N stressed conditions. Most of these QTL explained more than 10% of phenotypic variance, suggesting that the markers associated with these QTL could be nominated for MAS to improve GY under low N stressed conditions (Agrama et al., 1999). Marker assisted selection approach reduces the cost of extensive field testing and cuts the time required to develop NUE inbred lines and varieties (Agrama et al., 1999) through a conventional plant breeding approach. Previous QTL reports for GY under low and optimum N were highly variable (Agrama et al., 1999; Almeida et al., 2013). Like the current study, Agrama et al. (1999) did not find any common QTL between optimum and low N conditions for GY. Ribaut et al. (2007) on the other hand, reported QTL correspondence between optimum and low N conditions on chromosomes 1 and 3. Differences in number of markers, locations and populations used in different studies could attribute to the different results.

Successful use of QTL for improving complex traits have apparently been hampered by their small effect size, lack of consistency across different genetic backgrounds (Almeida et al., 2013) and locations. Identifying major effect QTL underlying single or multiple traits in various populations determine the successful use of QTL in MAS (Almeida et al., 2013). Since the advent of molecular markers, many QTL have been identified for GY and secondary traits under optimum and various stress environments mainly based on individual or few mapping populations (Ribaut et al., 1996; 2007; Agrama et al., 1999). In this study, QTL were identified that were common between optimum and low N stressed conditions (LNO or LNM) for all secondary traits except ASI. The QTL correspondence between optimum and low N stressed conditions (LNM and LNO) for secondary traits were in agreement with high genetic and phenotypic correlation reported between optimum and low N environments for each trait (Bänziger et al., 1997; Presterl et al., 2003; Worku et al., 2007). Common QTL for secondary traits justify the higher magnitude of correlation between optimum and low N stress environments. The common QTL could be used to simultaneously improve each secondary trait for both optimum and low N stressed conditions through markers associated with QTL identified under both optimum and low N stressed conditions. However, QTL correspondences identified between optimum and low N conditions for most secondary traits were not similar across populations, indicating the genetic background specific nature of QTL. In addition to common QTL for single traits between management conditions, multi-trait QTL would facilitate simultaneous improvement of traits or used in indirect selection for complex traits like GY through highly heritable and easily measurable traits. Populations 1, 2, 3 and 5 hosted QTL controlling multiple traits under different management conditions. The QTL identified in population 3 (chromosome 1: 52.35 -230.18 Mbp; 343 cM) particularly, was remarkable as it was common for both AD, PH, EH and integrated many QTL from other populations for AD, PH, GY and ASI under optimum and low N stressed conditions. QTL common between GY and easy to measure secondary traits can be used for indirect selection under low N stress environments.

Recurrent selection with markers associated with GY QTL under both optimum and low N stressed conditions can help accumulate favourable alleles for GY. Finding major QTL for complex traits like GY is challenging and needs to consider other alternatives. One alternative approach is the use of indirect selection through QTL

common between GY and secondary traits that are easy to measure, highly heritable and controlled by a few genes compared to GY. An adaptive QTL to low N stressed conditions identified on chromosome 8 (162.19 to 168.27 Mbp) in this study is promising for indirect selection for GY through selection for PH and EH. This QTL was identified from population 4 and was involved in the control of GY, PH and EH under LNO conditions. The markers associated with this QTL can be used for simultaneous improvement of GY, PH and EH. Ribaut et al. (2007) reported high correlation between GY and PH due to co-localised QTL for both traits, and suggested inclusion of PH in selection indices as important trait for improving GY under low N conditions. Pleiotropic QTL for GY with EPP and PH under low N was also reported by Agrama et al. (1999), but on a different chromosome than seen in this study, indicating the possibility of identifying such QTL in different genomic regions across different genetic backgrounds.

5.6 Conclusions

This study identified QTL underlying GY, AD, ASI, PH, EH, EPO and SEN under optimum and low N stressed conditions and SNP markers associated with each QTL. Some of the QTL identified were important to explain the genetic basis of correlation between optimum and low N environments for GY and secondary traits. The genetic mechanism under optimum and low conditions seem distinct for GY as there were no common QTL found under both conditions. Generally, the cost of phenotypic evaluation under low N environments is higher than under optimum conditions due to the need for establishment and management of managed low N stressed sites across locations. MAS through genomic regions associated with GY or indirectly through secondary traits correlated with GY under low N environments would help to reduce the cost of breeding for stress environments. QTL explaining more than 10% phenotypic variance and relatively higher effect size can be used for fine mapping and/or marker assisted breeding for rapid GY improvement under optimum and low N stressed conditions.

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CHAPTER 6

Manuscript 4: Effectiveness of genomic prediction for grain yield and secondary traits under optimum and managed low nitrogen stressed environments

6.1 Abstract

Rapid improvement of genotypes for abiotic stresses could be achieved through new methods such as genome-wide selection. The objective of this study was to evaluate the accuracy of genomic selection for grain yield and other secondary traits under optimum and low N stressed environments. Specific objectives included: 1) comparing the accuracy of genome-wide predictions under optimum and low N conditions for grain yield and some secondary traits, 2) assessing the effectiveness of genome-wide prediction for low N stress conditions through performance under optimum conditions and 3) comparing the response to selection based on genome-wide selection and phenotypic selection. Five DH populations were evaluated across optimum and managed low N stress sites in 2014 and 2015 for grain yield (GY), anthesis date (AD), anthesis silking interval (ASI), plant height (PH) and ear height (EH) along with two to five commercial checks in each trial. All the DH lines from the five populations were genotyped with genotyping by sequencing (GBS) single nucleotide polymorphic (SNP) markers. In the A/B_{within} model, the magnitudes of both genome-wide and phenotypic predictions were negatively affected by low N stress, and phenotypic prediction ability was always higher than genome-wide prediction ability for all traits under all N conditions. Low N stress had a larger effect on the prediction accuracy for grain yield than other secondary traits. The proportion of genome-wide prediction to phenotypic prediction abilities under each nitrogen condition was variable across traits. It decreased with an increase in N stress for GY, increased with increase in N stress for both PH and EH and was nearly equal for AD and ASI across the three N conditions. The average genome-wide prediction accuracy for GY was 61%, 17% and 35% of the corresponding phenotypic prediction accuracy under optimum, low N during main season (LNM) and low N during off-season (LNO) conditions, respectively.

The highest proportion of r_{MP} to r_P for PH and EH was observed under LNM conditions (82%) compared to optimum and LNO.

Key words: Genomic selection, low N, prediction accuracy, genotyping by sequencing

6.2 Introduction

Low soil N is among the major abiotic stresses causing low on-farm maize yield in most African countries south of the Sahara desert. Farmers in this region are characterised by low income and have limited access to inorganic fertilizers due to its high cost. Development of nitrogen use efficient (NUE) varieties is a cost effective and environmentally friendly approach to address the issue of low maize productivity resulting from sub-optimal N application (Bänziger et al., 1997; Presterl et al., 2003). Direct selection under managed low N stressed conditions have been used as a standard approach for the development of NUE (high grain yield per N available) (Gallais and Hirel, 2004) maize varieties (Bänziger et al., 1997; Presterl et al., 2003). This strategy allowed CIMMYT and other partners in sub-Saharan Africa to develop and release varieties that are high yielding under both optimum and N limiting environments. However, testing under low N environments is resource demanding in terms of high cost of development and management of low N stress sites across many locations (multi-location trials). Besides, low genetic variability and heritability for complex traits like grain yield is challenging to achieve progress through selection. Selection for such complex traits under stress environments could benefit from the incorporation of new tools and techniques such as molecular markers that would increase the efficiency of selection and thereby genetic gain.

Molecular markers have emerged as an alternative approach for improving the efficiency of selection for abiotic stress tolerance (Lande and Thompson, 1990). QTL mapping and characterization by Agrama et al. (1999) was among the pioneering studies to identify molecular markers associated with NUE in maize. Their study identified several genomic regions corresponding to agronomic traits measured under N limiting environments, suggesting the presence of QTL for NUE. Ribaut et al. (2007) also studied QTL for grain

yield and correlated secondary traits under optimum and low N stress environments and found QTL associated with grain yield and correlated traits under both optimum and low N conditions. Despite the identification of QTL for grain yield and secondary traits, none of the markers associated with grain yield have been used to improve selection under low N stress environments, as anticipated. In Chapter 5, QTL analysis was conducted in five bi-parental populations across optimum and low N sites to study the stability of QTL for grain yield and secondary traits across locations and genetic backgrounds. The study identified several QTL under optimum and low N stressed conditions for grain yield and six secondary traits. QTL overlapping between traits, management conditions and genetic backgrounds were identified for grain yield and some secondary traits with the objective of identifying QTL common across management conditions and genetic backgrounds. Though some QTL common between optimum and low N stressed conditions were identified for some traits in some populations, no QTL common across all genetic backgrounds were identified for any of the studied traits. In another study, genome-wide association was conducted to identify marker-trait association between SNP markers and grain yield and secondary traits under low N and optimum conditions. Significant marker trait association and putative and known protein coding genes were identified. Like the bi-parental QTL analysis, no common markers were identified for grain yield under optimum and low N conditions. Several factors play against the successful use of QTL for increasing selection efficiency under stress environments (Collins et al., 2008). These include the cost of developing mapping populations different from breeding populations (Heffner et al., 2009), individual QTL explaining only a small proportion of the total phenotypic variation, that estimated effect of QTL are usually not consistent for quantitative traits (Bernardo, 2008) and that many QTL are genetic background and location (management condition) specific (Semagn et al., 2013; Beyene et al., 2015a).

An alternative to selection based on markers with significant effects is genome-wide selection (Meuwissen et al., 2001) that uses many random markers to predict the performance of quantitative traits. In genomic selection, unlike MAS, testing the significance and identifying a subset of markers associated with a trait of interest, is not required. The availability of cheap and abundant molecular markers facilitates routine use

of a large number of molecular markers in plant breeding programmes (Bernardo and Yu, 2007; Eathington et al., 2007). Simulation (Bernardo and Yu, 2007) and empirical data (Massman et al., 2013) on maize have shown 14 to 50% higher gains with genome-wide selection than with QTL-based selection (marker assisted recurrent selection). Genome wide selection studies on maize (Dawson et al., 2013; Jacobson et al., 2014; Krchov et al., 2015), wheat (Dawson et al., 2013) and rice showed relatively higher prediction accuracy of genome-wide selection for grain yield and secondary traits of economic importance. Most of the studies reported, however, were conducted under optimally managed experimental conditions. A study conducted under drought stress conditions, found higher genetic gain through genome-wide selection for grain yield after two cycles of genome-wide selection under drought stress environments (Beyene et al., 2015). To our knowledge, no study has been conducted to assess the effectiveness of genomic selection under low N stress environments. Therefore, this study was conducted with the objectives of 1) comparing the accuracy of genome-wide predictions under optimum and low N conditions for grain yield and some secondary traits, 2) assessing the effectiveness of genome-wide prediction for low N stress conditions based on performance under optimum conditions and 3) to compare the response to selection based on genome-wide and phenotypic selection.

6.3 Materials and methods

6.3.1 Plant materials and phenotyping

In this study, five DH populations formed between seven inbred lines from heterotic groups A and B were used. Two bi-parental populations were formed among three elite inbred lines from heterotic group A (CML504, CML536 and LaPostaSeqC7-F64-2-6-2-2-B-B), and three populations were formed among four elite maize inbred lines from heterotic group B (CML494, CML504, CML511 and CML550) (Table 6.1). All DH lines derived from each population were testcrossed to a tester from the complimentary heterotic group. The details on the type of testers used, the number of locations under each management condition, the number of individuals in each population, the total number of markers used for genotyping of each population and years of the trials are

presented in Table 6.1. The different population-tester combinations resulted in seven trials consisting of 59 to 211 DH inbred lines. The trials were evaluated in 2014 and 2015 for GY, AD, ASI, PH, and EH across optimum and low N environments along with two to five commercial checks. Unlike for QTL analysis in Chapter 5, EPO was excluded because of its high correlation with PH and EH. Trials in each location were planted in one row plots using an α-lattice incomplete block design with two replications. Low N trials were planted under managed low N stress conditions during the main (LNM) and off-seasons (LNO) following standard procedures for low N sites (Bänziger et al., 1997; Worku et al., 2007). Low N trials during the main and off-season were treated as different environments due to difference in trial mean yields during the main and off-seasons. The same agronomic practices were applied for all optimum and low N trials except N fertilization that was not applied during planting and no top-dressing was done.

Table 6.1 The number of markers and genotypes used in three different methods of genome-wide prediction methods

No	Pedigree	Tester	Year	No of lines	Markers	Loc	Mgt	Heritability				
								GY	AD	ASI	PH	EH
						5	Optimum	0.65	0.77	0.47	0.81	0.79
	CML550/	CML312/				2	LNM	0.46	0.78	0.43	0.78	0.68
1	CML504	CML443	2014	211	14122	3	LNO	0.63	0.79	0.35	0.79	0.75
						5	Optimum	0.51	0.75	0.54	0.85	0.87
	CML550/	CML312/				3	LNM	0.45	0.75	0.38	0.58	0.56
2	CML511	CML443	2014	102	13821	2	LNO	0.63	0.85	0.49	0.67	0.68
						3	Optimum	0.38	0.64	0.18	0.61	0.77
	CML550/					3	LNM	0.29	0.59	0.18	0.61	0.68
3	CML494	CML312	2015	106	9294	2	LNO	0.17	0.67	0.00	0.39	0.53
	CML550/					3	Optimum	0.55	0.73	0.22	0.69	0.73
4	CML504	CML312	2014	114	14122	2	LNM	0.40	0.72	0.47	0.60	0.64
	CML550/					2	Optimum	0.53	0.66	0.50	0.78	0.71
5	CML511	CML312	2014	59	13821	3	LNM	0.17	0.70	0.23	0.71	0.64
						1	Optimum	0.31	0.63	0.66	0.63	0.77
	CML505/					1	LNM	0.25	0.58	0.24	0.42	0.38
6	LPFS64	CML395	2015	157	15660	1	LNO	0.51	0.60	0.24	0.51	0.40
						1	Optimum	0.56	0.78	0.76	0.53	0.66
	CML536/					1	LNM	0.43	0.53	0.35	0.50	0.51
7	LPFS64	CML395	2015	105	15271	1	LNO	0.56	0.69	0.55	0.49	0.47
							Optimum	**0.50**	**0.71**	**0.48**	**0.70**	**0.76**
							LNM	**0.35**	**0.66**	**0.33**	**0.60**	**0.59**
							LNO	**0.50**	**0.72**	**0.33**	**0.57**	**0.56**

Loc, location; GY, grain yield; AD, anthesis date; ASI, anthesis sillking interval; PH, plant height; EH, ear height

Analyses of variance within and across environments for each population under each management condition was done by the restricted maximum likelihood method using the R package (Bates et al., 2015; R Core Team, 2016) embedded in META-R software (Alvarado et al., 2015). Variance components were determined by following a linear mixed model: $Y_{ijko} = \mu + g_i + l_j + r_{kj} + b_{ojk} + e_{ijko}$, where Y_{ijko} was the phenotypic performance of the i^{th} genotype at the j^{th} environment in the k^{th} replication of the o^{th} incomplete block, μ was an intercept term, g_i was the genetic effect of the i^{th} genotype, l_j was the effect of the j^{th} environment, r_{kj} was the effect of the k^{th} replication at the j^{th} environment, b_{ojk} was the effect of the o^{th} incomplete block in the k^{th} replication at the j^{th} environment, and e_{ijko} was the residual. The effects of environments and replications were treated as random effects and the other effects as fixed. Heritability on an entry-mean basis was estimated from the variance components as the ratio of genotypic to phenotypic variance.

6.3.2. DNA extraction and genotyping

DNA extraction and genotyping was done as described in Chapter 4, section 4.3.3. For each genotype, imputed data of 955 690 SNP markers were received. Out of the total, 570 markers were not anchored to any of the ten maize chromosomes and were excluded from further analysis. The genotype data was filtered using a minor allele frequency (MAF) of 0.05 and a minimum count of 80% of the sample size using TASSEL v.5.2.24 software (Bradbury et al., 2007). Then SNP loci heterozygous for each parent and monomorphic between the two parents were excluded. To ensure uniform distribution of markers across the genome, the marker data was further filtered using a physical distance of 10 Kb between adjacent markers. The final number of markers ranged between 9294 and 15660 (Table 6.1).

6.3.3. Genome-wide prediction

Depending on the structure of the training and validation sets, three types of genomic prediction models were used. In the first case, hereafter A/B_{within}, predictions within populations (Table 6.1) were made under each of optimum, LNM and LNO conditions with cross validation for location as described by Jacobson et al. (2014) for the A/B model in their case. In A/B_{within}, only three heterotic group B populations phenotyped in more

than two locations were used. In the second case, hereafter $A/B_{opt/LN}$, phenotypic data from optimum environments were used as training set to predict the performance of the same genotypes under both LNM and LNO conditions. In the third case, hereafter $A/B_{pooled,}$ testcrosses of DH lines derived from three bi-parental populations connected by one parent and testcrossed to the same tester, were used. Pooled marker effect from two populations were used to predict the performance of the third population under each management condition. In all the three cases, the R (R Core Team, 2016) package ridged regression of best linear unbiased prediction (rrBLUP) version 4 (Endelman, 2011) was used for estimating marker effects with a delete-one procedure (Jacobson et al., 2014).

In A/B_{within}, training and validation sets were derived from the same population. Phenotypic and marker data of 80% (4/5N) of individuals within each population were considered as a training set, while phenotypic and marker data of the remaining 20% (1/5N) were considered as a test set, where N is the total number of genotypes in an A/B cross. The performance of the first individual was predicted as $y_P = \mu + xg$, where y_P was the predicted performance of the individual; μ was the estimated mean of individuals used as the training population except for the individual predictions; x was a $1 \times N_M$ (number of markers) row vector of genotype indicators; and g was $N_M \times 1$ marker effects obtained from the rrBLUP analysis. The elements of x were 1 if the test individual was homozygous for the SNP allele from parent A, –1 if the test individual was homozygous for the SNP allele from parent B, and 0 if the test individual was heterozygous. For genomic selection, r_{MP} was calculated as a Pearson correlation between the observed and marker predicted performance of the test set. The phenotypic selection, r_P was the Pearson correlation between the observed performance of individuals in the test set and observed performance of test individuals in the test set.

In the $A/B_{opt/LN}$ model, a set of individuals under optimum conditions were used to predict the performance of the same individuals under low N conditions. Phenotypic and marker data for 80% (4/5N) of individuals under optimum conditions were considered as a training set while phenotypic and marker data from 20% (1/5N) under both LNM and LNO were considered as a validation set. The performance of each individual was predicted using

the linear model $y = \mu 1_n + X\beta + e$, where y was an n x 1 vector of testcross phenotypic means of the DH lines, 1_n was an n x 1 vector with all elements equal to 1, μ was the overall testcross mean of the DH lines, X was an n x N_m design matrix with elements equal to 1, if the DH line was homozygous for the marker allele from the first parental inbred line, 0 if the DH line was heterozygous and -1 if the DH line was homozygous for the marker allele from the second parental inbred line, ß was an N_M x 1 vector of marker effects, and e was an n x 1 vector of residual effect. For genomic prediction, genomewide prediction (r_{MP}) was calculated as a Pearson correlation between marker predicted performance of test individuals under optimum conditions and observed performance of test individuals under both LNM and LNO. For phenotypic selection, r_P was the Pearson correlation between the observed performance of test individuals under optimum and observed performance of test individuals under both LNM and LNO conditions.

For the $A/B_{opt/LN}$ model, the value of response to selection (Rg) was calculated for high GY, low DA, low ASI, low PH and EH as outlined in Jacobson et al. (2014). Under optimum environments, the 20% of individuals with the best y_p values for each trait were identified. The mean observed performance of these individuals under LNM and LNO were obtained and denoted as $y_{0.20}$ under each condition. The R for each trait under LNM and LNO was calculated separately as $y_{0.20}$-μ, where μ is the overall mean of a trait under each LNM and LNO. For comparison, response to selection based on only phenotypic data (Rp) was also included. In this method, 20% of individuals with best performance for each trait were identified under optimum conditions. Then, the mean observed performance of these individuals under LNM and LNO were obtained and used to calculate the phenotypic response of selection under LNM and LNO conditions.

In the pooling approach, combined data by management from three heterotic group B populations evaluated in 2015 were used (Table 6.1). Training sets were constituted by pooling two of the three populations at a time. Each time, the third population was used as a prediction set. For each trait, each of the three crosses were analysed separately to obtain rrBLUP marker effects (M) within each cross. For a given trait, the performance of all N individuals in the A/B test population was predicted as $y = \mu 1 + Xm$, where y was an

N × 1 vector of predicted performance; μ was the estimated overall mean; 1 was an N × 1 vector with elements equal to 1; X was an N × N_M matrix of genotype indicators with elements of 1, –1, and 0 (the same as for x); and m was an N_M ×1 vector of rrBLUP marker effects averaged across any two populations forming the training set.

6.4 Results

6.4.1 Phenotypic data

Broad-sense heritability for GY and ASI was low to moderate under the three N conditions. Across the seven trials, heritability for GY ranged from 0.31 to 0.65 with a mean value of 0.50 under optimum; and from 0.17 to 0.46 with a mean of 0.35 under LNM and from 0.17 to 0.63 with a mean of 0.50 under LNO. For ASI, it ranged from 0.18 to 0.76 with an average of 0.48 under optimum, from 0.18 to 0.47 with average of 0.33 under LNM and from 0.00 to 0.55 with average of 0.33 under LNO. Heritability for AD, PH and EH were moderate to high under all three N conditions. The mean heritability was 0.71, 0.66 and 0.72 for AD, 0.70, 0.60 and 0.57 for PH and 0.76, 0.59 and 0.56 for EH under optimum, LNM and LNO, respectively (Table 6.1).

6.4.2 Genome-wide prediction accuracy within (A/B_{within}) populations

The genome-wide and phenotypic prediction accuracies were estimated for five agronomic traits (GY, AD, ASI, PH, and EH) in three populations under three soil N conditions (optimum, LNM and LNO) using the A/B_{within} model with cross validation for environments. For all the traits, average phenotypic prediction (r_P) accuracy was consistently higher than the average genome-wide prediction (r_{MP}) accuracy under all N conditions (Table 6.2, Figure 6.1). Both genome-wide and phenotypic predictions for GY was affected the most with an increase in N stress level while predications were least affected for PH and EH. For grain yield, r_{MP} in three populations ranged from 0.13 to 0.41 with an average of 0.23 under optimum, from -0.07 to 0.12 with average of 0.03 under LNM condition, and from 0.09 to 0.18 with average of 0.08 under LNO conditions (Table 6.2). The r_P for grain yield ranged from 0.33 to 0.44 with an average of 0.39 under optimum, from 0.09 to 0.25 with average of 0.19 under LNM conditions, from 0.05 to 0.35 with average of 0.23 under LNO conditions (Table 6.1).

Table 6.2 The genome-wide and phenotypic prediction accuracy for grain yield and secondary traits in three DH populations evaluated in 2014 and 2015

	Optimum		LNM		LNO	
Trait	rMP	rp	rMP	rp	rMP	rp
			CML550/CML504*			
GY	0.41	0.44	0.12	0.24	0.18	0.35
AD	0.30	0.59	0.36	0.59	0.29	0.47
ASI	0.17	0.22	0.21	0.25	0.22	0.17
PH	0.58	0.60	0.54	0.58	0.51	0.55
EH	0.52	0.58	0.43	0.49	0.42	0.47
			CML550/CML511			
GY	0.16	0.33	0.05	0.25	0.16	0.30
AD	0.05	0.55	-0.12	0.51	0.06	0.66
ASI	0.13	0.37	-0.08	0.25	0.08	0.32
PH	0.29	0.74	0.24	0.28	0.03	0.41
EH	0.36	0.71	0.31	0.35	0.21	0.36
			CML494/CML550			
GY	0.13	0.39	-0.07	0.09	-0.09	0.05
AD	0.40	0.54	0.22	0.31	0.41	0.42
ASI	-0.03	0.32	0.06	0.11	-0.14	-0.16
PH	0.17	0.51	0.25	0.38	0.20	0.22
EH	0.21	0.54	0.29	0.41	0.28	0.42
			Average			
GY	0.23	0.39	0.03	0.19	0.08	0.23
AD	0.25	0.56	0.15	0.47	0.25	0.52
ASI	0.09	0.30	0.06	0.20	0.05	0.11
PH	0.35	0.61	0.34	0.42	0.24	0.40
EH	0.36	0.61	0.34	0.42	0.30	0.42

*DH lines from CML550/CML504 and CML550/CML511 were test crossed to CML312/CML443 and evaluated in 2014. DH lines from CML550/CML494 were testcrossed to CML312 and evaluated in 2015

The proportion of r_{MP} to r_p was 61%, 17% and 35% under optimum, LNM and LNO conditions, respectively. In addition, both r_{MP} and r_p predication accuracy was reduced by 86% and 65% due to moderate N stress and by 50% and 40% due severe low N stress compared to the respective values under optimum conditions.

Low N stress also affected the r_{MP} and r_P for secondary traits in comparison to optimum conditions. The reduction in r_{MP} and r_P was relatively smaller for AD, PH and EH than the corresponding reductions observed for GY (Table 6.2). The relative efficiency of r_{MP} to r_P was below 50% under the three management conditions for AD and ASI (Figure 6.2).

However, the efficiency was relatively constant across the three N conditions. For PH and EH, the relative efficiencies of r_{MP} to r_P were 56% and 60% respectively, under optimum, 82% and 82% under LNM and 62% and 73% under LNO conditions.

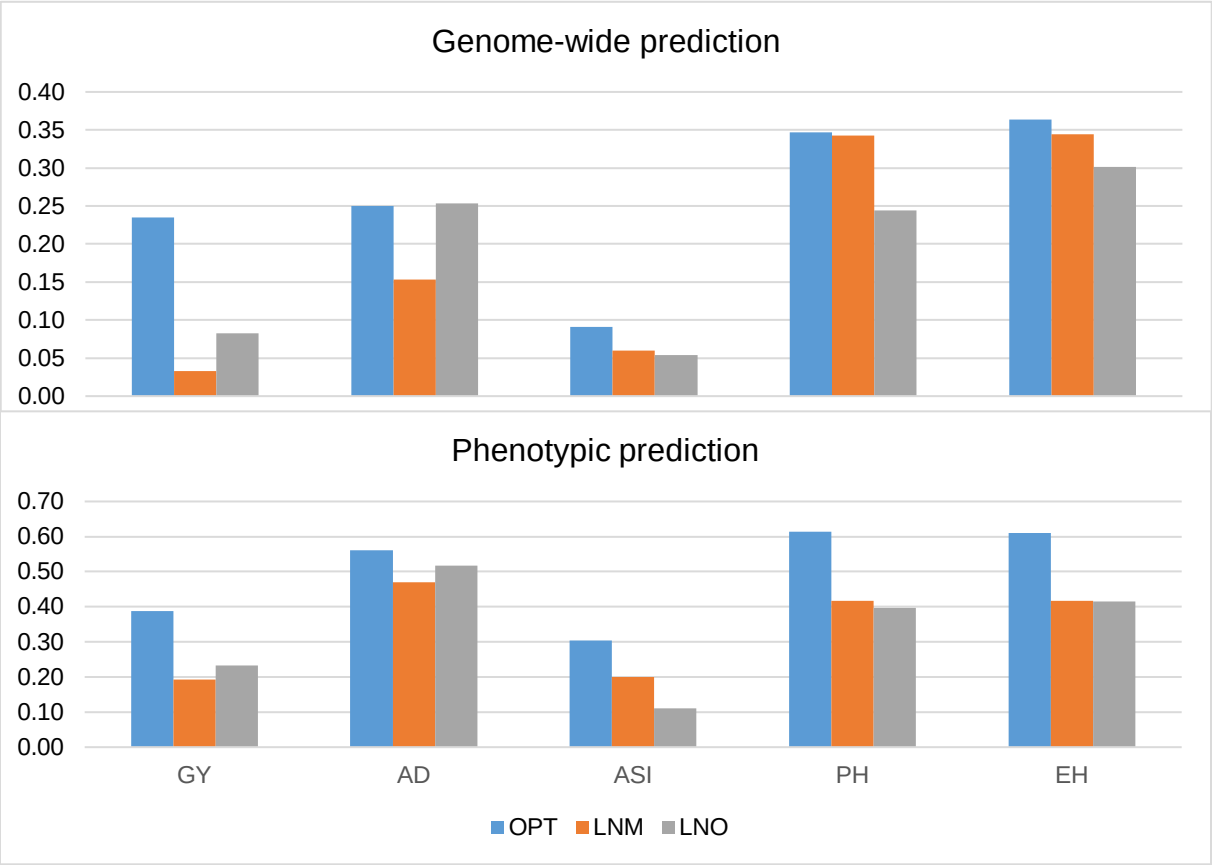

Figure 6.1 Comparison of genome-wide and phenotypic predictions for grain yield and some secondary traits

In the case of pooled data from two populations to predict the performance of the third population under each optimum and low N conditions, values for r_{MP} and r_P did not show a clear enough pattern for all traits under both optimum and LNM conditions to make sound conclusions.

6.4.3 Genomic prediction accuracy and response to selection for low N environments

Genomic and phenotypic prediction accuracy was assessed across seven (for LNM) and five (for LNO) population by tester combinations. The average r_{MP} and r_P under LNM was 0.12 and 0.29 for GY, 0.36 and 0.62 for AD, 0.16 and 0.34 for ASI, 0.37 and 0.56 for PH, 0.48 and 0.64 for EH (Table 6.3). Under LNO, the average r_{MP} and r_P was 0.11 and 0.25 for GY, 0.31 and 0.56 for AD, 0.19 and 27 for ASI, 0.26 and 0.45 for PH, and 0.38 and 0.49 for EH. The magnitude of r_P was always higher under both moderately and severely stress conditions than the corresponding values for r_{MP} for all traits. For GY, r_{MP} was 43% of r_P under both LMN and LNO conditions. The proportion of r_{MP} to r_P was 58% and 55% for AD, 48% and 68% for ASI, 66% and 57% for PH, 75% and 77% for EH under LNM and LNO conditions, respectively.

Table 6.3 Phenotypic and genome-wide predictions for low N conditions from performance of the same genotypes under optimum conditions

Population	Year	Mgt	GY		AD		ASI		PH		EH	
			rMP	rP	rMP	rP	rMP	rP	rMP	rP	rMP	rP
CML504/CML550	2014	LNM	0.20	0.31	0.49	0.69	0.39	0.44	0.66	0.68	0.58	0.67
		LNO	0.10	0.27	0.36	0.63	0.29	0.26	0.61	0.72	0.53	0.66
CML511/CML550	2014	LNM	0.20	0.55	-0.01	0.67	0.10	0.40	0.43	0.65	0.45	0.64
		LNO	0.11	0.34	0.01	0.70	0.08	0.42	0.13	0.61	0.26	0.65
CML504/CML550	2015	LNM	0.08	0.47	0.60	0.71	0.16	0.28	0.53	0.59	0.49	0.58
CML511/CML550	2015	LNM	0.10	0.29	0.23	0.66	0.12	0.51	0.32	0.79	0.53	0.75
CML494/CML550	2015	LNM	0.05	0.20	0.47	0.64	0.13	0.09	0.39	0.60	0.50	0.73
		LNO	0.06	0.30	0.53	0.60	0.05	0.05	0.25	0.42	0.45	0.52
CML505/LP	2015	LNM	0.17	0.11	0.38	0.43	0.10	0.28	0.38	0.52	0.45	0.54
		LNO	0.09	0.11	0.37	0.39	0.17	0.20	0.25	0.32	0.34	0.34
CML536/LP	2015	LNM	0.07	0.09	0.35	0.54	-0.05	0.11	0.08	0.37	0.34	0.55
		LNO	0.18	0.24	0.28	0.47	0.35	0.45	0.05	0.19	0.29	0.27
Average		LNM	0.12	0.29	0.36	0.62	0.16	0.34	0.37	0.56	0.48	0.64
		LNO	0.11	0.25	0.31	0.56	0.19	0.27	0.26	0.45	0.38	0.49

Mgt, management; GY, grain yield; AD, anthesis date; ASI, anthesis silking interval; PH, plant height; EH ear height; rMP, genomic prediction accuracy; rP, phenotypic prediction accuracy; LNM, low N stress during main season; LNO, low N stress during off season.

Response to selection based on the phenotypic method (Rp) was slightly higher than response to selection based on the genome-wide method (Rg) for all traits under LNM and LNO, except PH under LNO conditions (Table 6.4). Mean Rg and Rp under LNM

conditions were 0.02 and 0.15 t/ha for GY, -1.30 and -1.40 days for AD, -0.21 and -0.50 days for ASI, -3.58 and -6.64 cm for PH and -5.01 and -6.51 cm for EH. The mean Rg and Rp under LNM were also 0.10 and 0.18 t/ha for GY, -0.63 and -1.11 days for AD, -0.28 and -0.50 days for ASI, -2.90 and -1.74 cm for PH and -1.49 and -1.91 cm for EH. Except for GY under LNM, Rg was more than 50% of Rp for each trait.

Table 6.4 Comparison of response to selection based on phenotypic and genome-wide methods for grain yield and secondary traits

Population	Year	Mgt	GY		AD		ASI		PH		EH	
			Rg	Rp	Rg	Rp	Rg	Rp	Rg	Rp	Rg	Rp
CML504/CML550	2014	LNM	0.29	0.35	-2.21	-2.54	0.16	-0.50	-11.31	-3.81	-7.85	-8.11
		LNO	0.35	0.37	-1.36	-1.90	-0.46	-0.43	-7.53	-3.95	-1.28	-2.79
CML511/CML550	2014	LNM	0.30	0.63	-1.11	-0.35	-0.34	-0.72	-6.36	-5.04	-3.19	-4.37
		LNO	0.20	0.00	0.54	-0.75	0.11	-0.06	-1.85	-2.99	-0.13	-2.10
CML504/CML550	2015	LNM	0.15	0.28	-2.03	-2.63	-0.17	-0.34	-9.18	-11.50	-4.84	-4.79
CML511/CML550	2015	LNM	-0.17	0.11	-0.90	-1.71	-0.61	-0.48	-1.41	-9.06	-1.47	-3.39
CML494/CML550	2015	LNM	-0.43	-0.05	-0.41	-1.19	-0.23	-0.16	-3.22	-9.47	-3.32	-8.28
		LNO	0.16	0.14	-1.13	-1.57	0.04	0.52	-2.73	-7.59	-0.44	-3.15
CML505/LP	2015	LNM	0.12	-0.03	-0.65	-1.14	0.23	-0.71	2.35	-10.13	-7.04	-7.44
		LNO	-0.10	0.07	0.08	-0.21	-0.07	-0.92	-3.36	0.18	-1.95	1.24
CML536/LP	2015	LNM	-0.12	-0.22	-1.81	-0.22	-0.45	-0.40	4.05	2.49	-7.34	-9.20
		LNO	-0.11	0.29	-1.26	-1.13	-1.02	-1.60	0.97	5.66	-3.67	-2.77
Average		LNM	0.02	0.15	-1.30	-1.40	-0.21	-0.50	-3.58	-6.64	-5.01	-6.51
		LNO	0.10	0.18	-0.63	-1.11	-0.28	-0.50	-2.90	-1.74	-1.49	-1.91

Mgt, management; GY, grain yield; AD, anthesis date; ASI, anthesis silking interval; PH, plant height; EH ear height; Rg, response to selection based on genomic method; Rp, response to selection based on phenotypic method; LNM, low N stress during main season; LNO, low N stress during off season.

6.5. Discussion

Breeding crops for NUE is a challenging task due to the need for establishment and management of multi-location low N stressed phenotyping sites, and the inherent low variability and heritability of complex traits like grain yield under stress conditions. Indirect selection for high grain yield under low N environments through both grain yield under optimum or through secondary traits under low N conditions were found to be less efficient than direct selection for grain yield itself under low N conditions (Lafitte and Edmeades, 1994; Bänziger et al., 1997). MAS is advocated as an efficient method to enhance gain from selection under stress conditions (Lande and Thompson, 1990). However, the application of MAS in crop improvement in general and stress breeding in particular has

been limited due to scarcity of major effect QTL that are consistent across environments and genetic backgrounds (Collins et al., 2008). Genomic selection that uses information from all available markers is believed to increase the efficiency of selection for complex and simple traits with low heritability. In this study, genome-wide and phenotypic prediction accuracies were estimated and compared under optimum, LNM and LNO conditions. In addition, genome-wide and phenotypic predictions were made for low N stressed environments based on the performance of genotypes under optimum conditions and their response to selection was estimated and compared.

In the A/B_{within} model, the magnitude of both genome-wide and phenotypic predictions were negatively affected by low N stress, and phenotypic prediction ability was always higher than genome-wide prediction ability for all traits under all N conditions. Genome-wide prediction ability under LNM was between 14% (GY) and 99% (PH), and genome-wide prediction ability under LNO was between 35% (GY) and 100% (AD) of the corresponding genome-wide prediction ability under optimum conditions. Likewise, phenotypic prediction ability under LNM ranged from 50% (GY) to 84% (AD), and genome-wide prediction under LNO ranged from 37% (ASI) to 92% (AD) of the corresponding phenotypic prediction ability under optimum conditions. Low N stress had more effect on the prediction ability of grain yield than other secondary traits. The proportion of genome-wide to phenotypic prediction ability under each N condition was variable across traits. It decreased with an increase in N stress for GY, increased with increase in N stress for both PH and EH and was nearly equal for AD and ASI across the three N conditions. The average genome-wide prediction accuracy for GY was 61%, 17% and 35% of the corresponding phenotypic prediction under optimum, LNM and LNO conditions, respectively. The highest proportion of r_{MP} to r_P for PH and EH was observed under LNM conditions (82%) compared to optimum and LNO conditions. The results from this study showing higher accuracy of phenotypic prediction than genome-wide prediction and the effect of stress on the prediction ability, agrees with previous reports for grain yield and other agronomic traits under optimum N conditions (Jacobson et al., 2014; Krchov et al., 2015). In four DH populations evaluated in the US corn belt, phenotypic selection was as effective as, or more effective than, genome-wide selection for grain

yield and moisture (Krchov et al., 2015). In 30 test populations, Jacobson et al. (2014) showed that response to genomic selection with a GCA model was 68 to 76% of the corresponding response to phenotypic selection for grain yield, moisture and test weight. A study on CIMMYT germplasm demonstrated that genomic selection is more effective than pedigree-based conventional phenotypic selection for increasing genetic gains in grain yield under drought stress in tropical maize (Beyene et al., 2015). Comparing drought stressed environments and well-watered environments, Zhang et al. (2014) obtained consistently lower and more variable prediction accuracy (r_{MG}) under stress conditions than under well-watered conditions for all the target traits.

Correlation between optimum and low N stress environments is generally low to moderate for grain yield and moderate to high for secondary traits such as AD and PH (Lafitte and Edmeades, 1994; Bänziger et al., 1997). Because of this, indirect selection for grain yield under low N environments through grain yield selection under optimum conditions have been reported to be less efficient. In this study, both genome-wide and phenotypic predictions for LNM or LNO based on performances under optimum conditions was low for grain yield compared to secondary traits. Predictions for both LNM and LNO were similar. Phenotypic prediction accuracy was almost double that of genome-wide prediction ability for grain yield under both LMN and LNO conditions. The proportion of genome-wider prediction was more than 50% of the corresponding phenotypic predictions for AD, ASI, PH, EH under LNM and LNO. From these results, two cycles of indirect genome-wide selection for low N conditions will yield similar results with one cycle of indirect phenotypic selection for low N conditions. For secondary traits, two cycles of indirect selection with the genomic method surpasses one cycle of indirect selection with the phenotypic method. For example, for EH, two cycles of indirect selection with genome-wide selection will be equivalent to 1.5 cycles of indirect selection with the phenotypic method. Therefore, prediction ability of indirect selection for low N environments from performance under low N environments is higher with the phenotypic method compared to the genome-wide method. However, prediction with both genome-wide and phenotypic methods are comparable for secondary traits. Response to selection is crucial in practical plant breeding. On average, response to indirect selection for grain

yield and ASI under low N was only 36 and 49% of the corresponding response to indirect selection with the phenotypic method. These values were higher than 75% for PH, EH and AD, indicating the higher effectiveness of indirect selection for secondary traits than grain yield with genomic methods. However, the response to selection with the genome-wide method was still lower than with the phenotypic method.

The advantage of genome-wide selection mainly comes from the ability to run more than one cycle of selection in a year and the lower cost of genotyping than phenotyping (Bernardo and Yu, 2007). Therefore, the comparison should be based on unit of time and cost. These factors are particularly important under low N stressed conditions where cost of phenotyping is more expensive than under optimum conditions due to high cost of establishing and managing a network of uniform low N stress sites (multi-location trials). In addition, genetic variance and heritability of traits are affected by high levels of stress, thereby undermining gain from selection. Given the non-significant differences between r_{MP} and r_P for most traits, the low cost of genotyping and the feasibility of up to three cycles of selection with genome-wide selection (Bernardo and Yu, 2007), more genetic gain can be achieved through genome-wide selection than phenotypic selection under low soil N conditions. Beyene et al. (2015) demonstrated the effectiveness of genomic selection over conventional phenotypic selection for increasing genetic gains in grain yield under drought stress environments. Prediction accuracy for some secondary traits like PH and EH were less affected by increased N stress, creating an opportunity to use a selection index by incorporating marker information for grain yield and secondary traits. Heritability and genetic architecture of target traits affected prediction performance. Prediction accuracy of complex traits (grain yield) were consistently lower than those of simple traits (anthesis date and plant height) and prediction accuracy under stress conditions was consistently lower and more variable than under well-watered conditions for all the target traits because of their poor heritability under stress conditions (Zhang et al., 2014). In plants, the importance of generation time varies between crops, but the goal of reducing cycle time remains important. In maize, where breeding is done using DH and off-season nurseries, test cross performance selection still requires at least 2 years (Bernardo and

Yu, 2007), providing an opportunity for GS to reduce unit time per selection cycle by reducing the need for progeny test data in every cycle (Heffner et al., 2009).

6.6. Conclusions

Phenotyping under low N stress environments is a highly demanding task due to high cost for establishment and maintenance of managed low N stressed sites, the inherent low variability of traits under low N stress and low heritability of complex traits like grain yield under stress conditions. Indirect selection for grain yield under low N conditions from grain yield under optimum conditions was investigated as an alternative selection approach but found to be inefficient. Despite initial promises of MAS in crop improvement, little success stories are reported for improvement of quantitative traits. MAS has limited application in stress breeding due to scarcity of major effect QTL that are consistent across environments and genetic background. Genomic selection that uses information from all available markers is believed to increase the efficiency of selection for complex traits and simple traits with low heritability. In this study, genome-wide and phenotypic prediction were compared and higher efficiency of phenotypic prediction than genome-wide prediction was seen. In addition, prediction for performance under low N based on performance under optimum conditions was generally higher with the phenotypic method. However, the advantage of genomic prediction is mainly due to time and cost. Up to three cycles of selection can be done in a year with genomic prediction and the cost of genotyping is much cheaper than phenotyping. Therefore, incorporation of genomic selection in breeding for NUE can increase genetic gain from selection.

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CHAPTER 7

General conclusions and recommendations

Low N stress is a widespread abiotic factor limiting maize productivity under small holder subsistence farming systems in sub-Saharan Africa. Farmers in this sub-continent cannot afford to purchase sufficient inorganic fertilizers because of high cost of fertilizers and low income. Development of nitrogen use efficient maize varieties, which are efficient in uptake and utilisation of available N in the soil, are economically feasible for use by small scale farmers.

This study was conducted to investigate the efficiency of different selection methods for improvement of grain yield under low N conditions, and to identify quantitative trait loci and molecular markers associated with grain yield, days to anthesis, anthesis silking interval, plant height, ear height, ear position, and leaf senescence. The overall goal of the study was to identify the most efficient and cost-effective selection method for grain yield improvement under low N conditions. The specific objectives were to estimate the efficiency of indirect selection for grain yield under low N through grain yield under optimum conditions and through secondary traits under low N conditions, to identify QTL underlying grain yield under low and optimum N conditions using traditional linkage analysis, to identify marker trait associations for grain yield and secondary traits under optimum and low N conditions through genome-wide scans, and to estimate the efficiency of genomic selection for grain yield and secondary traits under optimum and low N conditions.

The results showed that the efficiency of indirect selection for grain yield under low N conditions through grain yield under optimum and through secondary traits under low N conditions, were generally low. This is mainly due to low genetic correlation between optimum and low N environments for grain yield. The results from the traditional bi-parental QTL analysis and genome-wide association study showed lack of common QTL/markers between optimum and low N conditions for grain yield, confirming different

genetic mechanisms under optimum and low N conditions. For some secondary traits, however, common QTL and marker-trait association were found between optimum and low N conditions. The results generally explained the genetic basis underlying genetic correlation between optimum and low N environments for grain yield. The results reconfirmed higher efficiency of direct selection in target environments for the improvement of grain yield reported previously. For some secondary traits, the results suggested the possibility of simultaneous improvement under optimum and low N environments. Even though markers associated with QTL/putative and known genes could be used for implementation of MAS for the improvement of grain yield under optimum or low N conditions, simultaneous improvement for both optimum and low N environments appears to be unfeasible.

Indirect selection for grain yield under low N from grain yield under optimum conditions as well as MAS for simultaneous improvement of grain yield under optimum and low N conditions are impractical due to lack of common QTL or marker trait associations. This necessitates selection of genotypes under the target low N stress sites. The cost of phenotypic evaluation under low N environments is high due to the need for establishment and management of multiple low N sites and high field variability requiring more replications to achieve higher precision. Genomic selection that uses information from all available markers is believed to increase the efficiency of selection for complex traits and simple traits with low heritability. Results from the genomic prediction study generally showed higher efficiency of phenotypic prediction than genome-wide prediction. In addition, prediction for performance under low N based on performance under optimum conditions was generally higher with the phenotypic prediction method. However, the advantages of genomic prediction are mainly due to saving of time and cost. Up to three cycles of selection can be done in a year with genomic prediction and the cost of genotyping is increasingly less expensive than phenotyping. Therefore, incorporation of the doubled haploid method and genomic selection in breeding for NUE can have the potential to increase genetic gain from selection per unit of time and cost.

In conclusion, direct selection for grain yield under target environments is the most efficient method and breeders should evaluate trials under both optimum and low N

environments to identify varieties that perform well under both conditions. Secondary traits could be used for indirect selection, but an index of secondary traits should be incorporated to increase the efficiency of selection for grain yield under low N. MAS has a promise for improvement of grain yield under specific environments. However, genomic selection holds the biggest promise for improving selection efficiency per unit time and cost under optimal and low N conditions as well as for indirect selection for grain yield under low N conditions. Generally, breeding strategy that incorporates germplasm with tolerance to low N (high NUE), appropriate selection methods that includes conventional methods (secondary traits with relatively high heritability and easy to measure) are important to develop new NUE varieties. New tools and technologies (marker assisted selection, doubled haploid, genomic selection) coupled with precise phenotyping under target environment

APPENDIX

Appendix Table 1. Information on trials used for the genome wide marker traits association study (Chapter 4)

Country	Location	Coordinates	Elevation (masl)	Management	Years/seasons
Kenya	Kiboko	-2.250, 37.730	990	Optimum	2011A, 2012A
				Low N	2011A, 2011B, 2012A
	Embu	-0.500, 37.450	1492	Low N	2011A, 2011B, 2012A
	Kibos	-0.070, 34.820	1184	Optimum	2012A
	Kitale	1.010, 35.000	1859	Optimum	2011A, 2011A
	Kakamega	0.270, 34.740	1526	Optimum	2011A, 2012B
				Low N	2011A
Mexico	Agua Fria	20.530, -97.430	90	Optimum	2012A
				Low N	2011A
	Tlatizapan	18.680, -99.130	940	Low N	2010A
South Africa	Cedara	-29.530, 30.280	1100	Optimum	2011B
				Low N	2011B
Zambia	Golden Valley Agricultural Research Trust (GART)	-14.170, 28.370	1173	Low N	2011B
Zimbabwe	Harare	-17.800, 31.050	1498	Low N	2010B, 2011B

A, main season; B, off-season; masl, meter above sea level; Low N, managed low N stress site (Source: Das et al. submitted to Crop Science)

Appendix Table 2. Mean GY, AD, ASI, PH, EH, EPO and EPP of 108 doubled haploid test cross progenies (CML494/CML550) and two commercial checks evaluated in three low N stress sites (Kiboko, Kitale and Mtwapa) in Kenya during the main growing season of 2015.

Genotype	GY	AD	ASI	PH	EH	EPO	EPP
(CML494/CML550)DH1	4.53	69.28	1.62	208.99	84.43	0.40	0.94
(CML494/CML550)DH4	4.61	68.94	1.86	214.45	87.23	0.40	0.93
(CML494/CML550)DH6	4.52	68.96	2.08	208.64	85.92	0.40	0.95
(CML494/CML550)DH7	4.63	67.62	1.88	209.38	83.38	0.40	0.93
(CML494/CML550)DH9	4.28	68.83	1.91	209.35	79.53	0.38	0.92
(CML494/CML550)DH11	4.46	68.75	2.00	214.75	85.87	0.40	0.94
(CML494/CML550)DH12	4.63	68.57	1.78	222.43	88.75	0.39	0.95
(CML494/CML550)DH15	4.56	68.42	2.03	220.69	89.40	0.40	0.94
(CML494/CML550)DH19	4.49	67.69	1.85	218.13	78.01	0.35	0.93
(CML494/CML550)DH20	4.43	70.02	1.79	211.34	83.56	0.39	0.94
(CML494/CML550)DH21	4.60	67.30	1.78	197.67	74.30	0.37	0.94
(CML494/CML550)DH24	4.37	69.59	2.00	211.49	80.00	0.37	0.94
(CML494/CML550)DH27	4.38	68.29	1.91	204.89	75.50	0.36	0.93
(CML494/CML550)DH28	4.47	68.59	1.92	212.61	83.77	0.39	0.94
(CML494/CML550)DH29	4.59	68.15	1.83	206.57	82.30	0.40	0.94
(CML494/CML550)DH30	4.44	68.15	1.71	205.77	81.69	0.39	0.93
(CML494/CML550)DH33	4.35	69.78	2.00	213.81	88.55	0.41	0.94
(CML494/CML550)DH34	4.47	69.65	1.68	210.48	79.78	0.37	0.95
(CML494/CML550)DH36	4.47	68.19	1.80	210.89	85.69	0.40	0.94
(CML494/CML550)DH37	4.75	67.90	1.89	222.06	83.63	0.38	0.94
(CML494/CML550)DH38	4.52	68.97	1.75	216.98	84.03	0.38	0.92
(CML494/CML550)DH39	4.57	68.90	1.95	215.51	84.04	0.39	0.94
(CML494/CML550)DH40	4.58	68.89	2.01	214.29	83.07	0.38	0.93
(CML494/CML550)DH41	4.67	68.46	1.73	211.78	89.16	0.42	0.94
(CML494/CML550)DH42	4.64	68.26	2.05	206.48	77.52	0.37	0.93
(CML494/CML550)DH43	4.45	68.20	1.87	211.10	79.90	0.38	0.96
(CML494/CML550)DH44	4.47	70.47	1.86	212.99	82.00	0.38	0.94
(CML494/CML550)DH45	4.73	68.49	1.83	201.86	79.37	0.38	0.93
(CML494/CML550)DH46	4.62	69.82	1.60	220.39	89.13	0.40	0.93
(CML494/CML550)DH47	4.55	68.64	2.12	210.32	82.16	0.39	0.93
(CML494/CML550)DH48	4.53	69.52	1.82	216.95	90.08	0.41	0.94
(CML494/CML550)DH50	4.67	68.80	1.91	208.74	77.42	0.37	0.95
(CML494/CML550)DH52	4.52	68.40	2.16	216.49	82.80	0.38	0.93
(CML494/CML550)DH54	4.65	68.64	1.90	213.48	82.52	0.38	0.96
(CML494/CML550)DH55	4.57	69.76	1.68	211.60	87.42	0.41	0.94
(CML494/CML550)DH56	4.49	68.82	2.05	213.71	81.82	0.38	0.96
(CML494/CML550)DH58	4.53	68.52	1.81	224.23	89.32	0.39	0.93
(CML494/CML550)DH61	4.52	68.91	1.94	214.29	81.75	0.38	0.95
(CML494/CML550)DH68	4.58	70.71	2.13	213.23	86.69	0.40	0.95
(CML494/CML550)DH70	4.41	68.79	1.86	207.47	83.87	0.40	0.93
(CML494/CML550)DH73	4.53	68.89	1.99	211.93	81.69	0.38	0.93
(CML494/CML550)DH75	4.63	69.14	1.72	209.17	85.62	0.41	0.94
(CML494/CML550)DH76	4.42	69.56	1.87	208.22	80.93	0.38	0.93
(CML494/CML550)DH80	4.33	69.96	2.01	212.13	80.11	0.38	0.93
(CML494/CML550)DH81	4.28	68.50	1.88	201.32	76.42	0.38	0.95
(CML494/CML550)DH82	4.41	68.90	1.84	211.85	84.49	0.39	0.94
(CML494/CML550)DH86	4.26	69.08	1.93	217.72	82.43	0.37	0.94
(CML494/CML550)DH89	4.45	68.90	2.01	208.64	78.53	0.37	0.93
(CML494/CML550)DH99	4.42	69.73	2.01	204.89	80.28	0.39	0.94
(CML494/CML550)DH110	4.34	68.76	1.69	206.77	83.46	0.40	0.93
(CML494/CML550)DH113	4.75	68.92	1.75	207.31	79.32	0.38	0.94
(CML494/CML550)DH114	4.84	68.61	1.77	210.80	81.00	0.38	0.94
(CML494/CML550)DH116	4.65	68.67	1.90	210.65	82.95	0.39	0.93
(CML494/CML550)DH118	4.31	69.95	1.69	215.39	84.87	0.39	0.93
(CML494/CML550)DH119	4.30	69.68	1.82	209.54	81.89	0.38	0.95
(CML494/CML550)DH120	4.50	70.21	2.08	208.28	87.72	0.41	0.95
(CML494/CML550)DH121	4.48	70.94	1.99	222.80	85.31	0.38	0.94
(CML494/CML550)DH122	4.87	70.45	1.90	224.29	91.07	0.40	0.95
(CML494/CML550)DH124	4.60	68.82	2.21	212.48	89.91	0.42	0.94
(CML494/CML550)DH125	4.50	68.91	1.87	210.55	83.69	0.40	0.94
(CML494/CML550)DH126	4.48	69.74	1.83	212.93	84.12	0.39	0.93
(CML494/CML550)DH127	4.65	68.28	1.97	213.06	85.70	0.40	0.93
(CML494/CML550)DH129	4.40	68.75	2.07	212.52	85.52	0.40	0.94
(CML494/CML550)DH130	4.58	68.14	1.92	203.21	74.17	0.36	0.94
(CML494/CML550)DH131	4.52	69.41	1.88	205.87	84.04	0.40	0.94

Genotype	GY	AD	ASI	PH	EH	EPO	EPP
(CML494/CML550)DH132	4.59	69.49	2.00	209.69	85.41	0.39	0.93
(CML494/CML550)DH136	4.43	69.15	1.89	216.52	91.54	0.42	0.93
(CML494/CML550)DH142	4.65	68.81	1.84	209.70	80.33	0.38	0.94
(CML494/CML550)DH144	4.44	70.29	1.78	222.51	83.95	0.38	0.93
(CML494/CML550)DH148	4.62	67.89	1.97	202.76	75.22	0.37	0.94
(CML494/CML550)DH149	4.67	67.75	1.94	210.24	82.97	0.39	0.94
(CML494/CML550)DH153	4.32	70.06	2.07	217.12	86.73	0.39	0.95
(CML494/CML550)DH154	4.38	69.25	1.99	205.82	80.81	0.39	0.95
(CML494/CML550)DH158	4.44	68.85	2.05	209.12	82.59	0.39	0.93
(CML494/CML550)DH163	4.46	69.34	1.89	213.76	84.30	0.39	0.94
(CML494/CML550)DH169	4.67	70.20	1.92	215.73	95.55	0.43	0.94
(CML494/CML550)DH170	4.60	68.96	1.94	215.86	87.03	0.39	0.95
(CML494/CML550)DH171	4.26	69.48	2.11	214.20	80.33	0.37	0.94
(CML494/CML550)DH174	4.56	68.94	1.79	210.75	78.75	0.37	0.94
(CML494/CML550)DH175	4.64	70.07	2.08	215.30	89.39	0.41	0.94
(CML494/CML550)DH179	4.40	69.90	2.08	215.80	83.88	0.38	0.93
(CML494/CML550)DH190	4.42	68.20	1.93	206.09	81.87	0.39	0.92
(CML494/CML550)DH192	4.44	68.44	1.94	205.57	79.01	0.38	0.94
(CML494/CML550)DH193	4.40	69.02	1.98	208.06	83.69	0.40	0.95
(CML494/CML550)DH194	4.19	68.72	2.11	203.56	77.22	0.37	0.92
(CML494/CML550)DH197	4.51	69.52	1.73	209.44	81.61	0.38	0.95
(CML494/CML550)DH198	4.61	68.94	1.90	211.87	85.05	0.40	0.95
(CML494/CML550)DH199	4.39	69.29	2.02	219.46	83.55	0.38	0.93
(CML494/CML550)DH200	4.51	69.08	2.00	213.70	89.30	0.41	0.95
(CML494/CML550)DH201	4.69	68.60	1.98	220.74	86.59	0.39	0.95
(CML494/CML550)DH206	4.31	68.73	2.15	206.58	80.80	0.38	0.94
(CML494/CML550)DH207	4.49	69.84	2.09	223.40	93.90	0.41	0.95
(CML494/CML550)DH208	4.38	69.06	2.07	210.19	85.06	0.40	0.94
(CML494/CML550)DH209	4.50	68.67	1.90	218.51	88.32	0.39	0.94
(CML494/CML550)DH211	4.25	69.90	1.98	209.30	85.85	0.41	0.93
(CML494/CML550)DH212	4.56	68.42	2.11	224.01	92.27	0.41	0.94
(CML494/CML550)DH215	4.54	69.97	2.02	207.87	84.87	0.40	0.95
(CML494/CML550)DH216	4.63	68.92	1.87	214.40	81.92	0.37	0.96
(CML494/CML550)DH219	4.57	69.44	2.02	211.36	83.41	0.39	0.94
(CML494/CML550)DH220	4.33	69.67	1.76	208.66	81.55	0.39	0.93
(CML494/CML550)DH221	4.40	68.69	1.91	210.50	77.11	0.36	0.93
(CML494/CML550)DH223	4.88	68.41	1.92	215.50	87.15	0.40	0.93
(CML494/CML550)DH224	4.42	70.14	1.80	214.42	84.58	0.39	0.94
(CML494/CML550)DH225	4.60	68.99	1.85	219.57	86.17	0.39	0.95
(CML494/CML550)DH226	4.49	69.58	1.82	208.30	84.45	0.40	0.95
(CML494/CML550)DH228	4.68	68.88	2.02	205.38	81.90	0.39	0.93
(CML494/CML550)DH232	4.54	68.08	1.91	216.76	82.93	0.38	0.94
(CML494/CML550)DH235	4.68	69.16	2.03	219.43	89.00	0.40	0.94
CML312/CML494	4.57	69.45	1.86	223.31	89.66	0.39	0.94
CML312/CML550	4.40	68.20	2.14	199.30	72.78	0.36	0.93
Locations	**3**	**3**	**3**	**3**	**3**	**3**	**3**
Replications	**2**	**2**	**2**	**2**	**2**	**2**	**2**
Error Variance	**0.73**	**3.04**	**1.66**	**177.07**	**63.37**	**0.00**	**0.02**
Genotypic Variance	**0.07**	**0.91**	**0.09**	**55.58**	**27.96**	**0.00**	**0.00**
GenxEnv Variance	**0.15**	**0.36**	**0.42**	**18.77**	**7.55**	**0.00**	**0.00**
Location Variance	**0.62**	**214.68**	**0.65**	**3324.34**	**920.10**	**0.00**	**0.02**
Heritability	**0.29**	**0.59**	**0.18**	**0.61**	**0.68**	**0.66**	**0.14**
Grand Mean	**4.51**	**69.03**	**1.92**	**212.17**	**83.64**	**0.39**	**0.94**
LSD	**1.68**	**3.42**	**2.52**	**26.08**	**15.60**	**0.06**	**0.24**
CV	**18.96**	**2.53**	**67.04**	**6.27**	**9.52**	**8.18**	**13.17**

GY, Grain yield; AD, Anthesis date; ASI, Anthesis silking interval; PH, Plant height; EH, ear height; EPO, ear position; EPP, ears per plant; LSD, least significant difference; CV, coefficient of variation; GenxEnv; genotype by environment interaction

Appendix Table 3. Mean GY, AD, ASI, PH, EH, EPO and EPP of 108 doubled haploid test cross progenies (CML494/CML550) and two commercial checks evaluated in two low N stress sites (Kiboko and Embu) in Kenya during off season of 2015

Genotype	GYF	AD	ASI	PH	EH	EPO	EPP
(CML494/CML550)DH1	2.09	72.56	3.16	149.19	55.18	0.37	0.76
(CML494/CML550)DH4	2.23	72.38	3.16	151.20	58.84	0.39	0.76
(CML494/CML550)DH6	2.10	73.15	3.16	145.22	56.06	0.40	0.76
(CML494/CML550)DH7	2.22	71.51	3.16	151.05	58.03	0.38	0.76
(CML494/CML550)DH9	2.18	72.32	3.16	148.36	55.70	0.38	0.76
(CML494/CML550)DH11	2.21	73.30	3.16	151.88	59.98	0.39	0.76
(CML494/CML550)DH12	2.21	72.75	3.16	152.32	54.98	0.35	0.76
(CML494/CML550)DH15	2.09	73.83	3.16	148.77	56.02	0.38	0.76
(CML494/CML550)DH19	2.13	74.64	3.16	152.78	55.16	0.35	0.76
(CML494/CML550)DH20	2.00	75.44	3.16	145.07	53.39	0.37	0.76
(CML494/CML550)DH21	2.06	70.90	3.16	141.54	48.78	0.34	0.76
(CML494/CML550)DH24	2.18	73.32	3.16	151.08	54.58	0.36	0.76
(CML494/CML550)DH27	2.01	71.78	3.16	147.71	51.98	0.35	0.76
(CML494/CML550)DH28	2.14	73.55	3.16	149.69	52.77	0.35	0.76
(CML494/CML550)DH29	2.14	71.71	3.16	146.14	54.57	0.38	0.76
(CML494/CML550)DH30	2.15	71.02	3.16	145.73	53.74	0.36	0.76
(CML494/CML550)DH33	2.07	75.05	3.16	148.16	55.41	0.38	0.76
(CML494/CML550)DH34	2.11	72.93	3.16	151.39	54.11	0.35	0.76
(CML494/CML550)DH36	2.11	72.48	3.16	146.91	55.53	0.38	0.76
(CML494/CML550)DH37	2.10	71.76	3.16	149.98	52.92	0.35	0.76
(CML494/CML550)DH38	2.12	73.11	3.16	150.60	54.49	0.35	0.76
(CML494/CML550)DH39	2.15	72.54	3.16	151.79	58.27	0.38	0.76
(CML494/CML550)DH40	2.18	73.34	3.16	151.60	57.19	0.38	0.76
(CML494/CML550)DH41	2.13	74.48	3.16	148.55	54.38	0.37	0.76
(CML494/CML550)DH42	2.22	71.72	3.16	149.63	53.34	0.35	0.76
(CML494/CML550)DH43	2.17	72.45	3.16	152.32	54.05	0.35	0.76
(CML494/CML550)DH44	2.04	72.85	3.16	151.62	55.10	0.36	0.76
(CML494/CML550)DH45	2.09	74.70	3.16	146.34	51.18	0.35	0.76
(CML494/CML550)DH46	2.17	74.15	3.16	151.55	58.19	0.39	0.76
(CML494/CML550)DH47	2.13	73.76	3.16	150.65	54.23	0.36	0.76
(CML494/CML550)DH48	2.19	73.33	3.16	149.78	57.49	0.38	0.76
(CML494/CML550)DH50	2.18	72.00	3.16	153.23	55.41	0.36	0.76
(CML494/CML550)DH52	2.09	73.43	3.16	151.89	52.70	0.34	0.76
(CML494/CML550)DH54	2.10	71.83	3.16	151.69	55.82	0.36	0.76
(CML494/CML550)DH55	2.20	72.08	3.16	151.71	58.55	0.38	0.76
(CML494/CML550)DH56	2.16	73.40	3.16	153.06	58.14	0.38	0.76
(CML494/CML550)DH58	2.22	71.51	3.16	154.72	56.06	0.36	0.76
(CML494/CML550)DH61	2.25	71.98	3.16	155.53	58.16	0.37	0.76
(CML494/CML550)DH68	2.26	74.70	3.16	156.42	59.32	0.37	0.76
(CML494/CML550)DH70	2.13	72.17	3.16	148.47	52.87	0.35	0.76
(CML494/CML550)DH73	2.18	73.12	3.16	151.98	57.22	0.38	0.76
(CML494/CML550)DH75	2.15	72.16	3.16	148.66	56.47	0.38	0.76
(CML494/CML550)DH76	2.15	72.30	3.16	147.31	54.30	0.37	0.76
(CML494/CML550)DH80	2.08	73.54	3.16	149.02	53.45	0.36	0.76
(CML494/CML550)DH81	2.14	72.84	3.16	148.70	55.28	0.37	0.76
(CML494/CML550)DH82	2.15	73.35	3.16	147.29	52.95	0.35	0.76
(CML494/CML550)DH86	2.07	73.52	3.16	150.91	54.75	0.36	0.76
(CML494/CML550)DH89	2.06	73.29	3.16	146.53	53.45	0.36	0.76
(CML494/CML550)DH99	2.09	73.62	3.16	145.55	53.73	0.37	0.76
(CML494/CML550)DH110	2.14	72.83	3.16	147.42	52.86	0.36	0.76
(CML494/CML550)DH113	2.18	70.91	3.16	150.87	55.53	0.36	0.76
(CML494/CML550)DH114	2.14	72.52	3.16	150.86	54.33	0.36	0.76
(CML494/CML550)DH116	2.16	72.41	3.16	154.21	58.64	0.38	0.76
(CML494/CML550)DH118	2.01	74.25	3.16	143.39	53.81	0.38	0.76
(CML494/CML550)DH119	2.05	74.05	3.16	150.32	54.31	0.36	0.76
(CML494/CML550)DH120	2.09	74.60	3.16	147.46	52.63	0.36	0.76
(CML494/CML550)DH121	2.12	75.45	3.16	151.19	55.90	0.37	0.76
(CML494/CML550)DH122	2.21	74.13	3.16	157.20	62.87	0.40	0.76
(CML494/CML550)DH124	2.00	73.66	3.16	148.08	58.00	0.40	0.76
(CML494/CML550)DH125	2.14	73.57	3.16	149.97	55.02	0.36	0.76
(CML494/CML550)DH126	2.06	73.77	3.16	147.66	55.14	0.38	0.76

Genotype	GYF	AD	ASI	PH	EH	EPO	EPP
(CML494/CML550)DH127	2.25	73.41	3.16	151.31	57.42	0.38	0.76
(CML494/CML550)DH129	2.18	73.07	3.16	152.47	57.79	0.38	0.76
(CML494/CML550)DH130	2.11	72.10	3.16	147.95	51.61	0.34	0.76
(CML494/CML550)DH131	2.17	73.01	3.16	150.73	56.23	0.37	0.76
(CML494/CML550)DH132	2.08	74.28	3.16	148.78	54.92	0.37	0.76
(CML494/CML550)DH136	2.16	74.69	3.16	151.92	59.57	0.39	0.76
(CML494/CML550)DH142	2.12	72.66	3.16	145.48	51.18	0.35	0.76
(CML494/CML550)DH144	2.12	74.79	3.16	151.57	56.55	0.37	0.76
(CML494/CML550)DH148	2.14	72.73	3.16	146.75	51.81	0.35	0.76
(CML494/CML550)DH149	2.12	72.61	3.16	146.03	51.82	0.36	0.76
(CML494/CML550)DH153	2.03	76.22	3.16	147.76	53.44	0.36	0.76
(CML494/CML550)DH154	2.24	72.27	3.16	147.73	53.80	0.37	0.76
(CML494/CML550)DH158	2.09	73.02	3.16	151.85	57.33	0.38	0.76
(CML494/CML550)DH163	2.14	72.38	3.16	150.54	53.71	0.35	0.76
(CML494/CML550)DH169	2.17	74.58	3.16	153.66	63.34	0.42	0.76
(CML494/CML550)DH170	2.15	73.42	3.16	155.23	58.49	0.37	0.76
(CML494/CML550)DH171	2.09	71.76	3.16	149.59	53.06	0.36	0.76
(CML494/CML550)DH174	2.06	73.29	3.16	149.23	52.61	0.35	0.76
(CML494/CML550)DH175	2.10	75.80	3.16	147.66	56.34	0.38	0.76
(CML494/CML550)DH179	1.98	76.07	3.16	146.46	51.10	0.35	0.76
(CML494/CML550)DH190	2.10	72.76	3.16	146.64	51.71	0.35	0.76
(CML494/CML550)DH192	2.12	72.87	3.16	147.83	51.79	0.34	0.76
(CML494/CML550)DH193	2.09	72.94	3.16	148.94	57.23	0.39	0.76
(CML494/CML550)DH194	2.15	72.78	3.16	143.10	51.39	0.37	0.76
(CML494/CML550)DH197	2.17	72.92	3.16	149.30	52.57	0.35	0.76
(CML494/CML550)DH198	2.19	73.64	3.16	153.21	58.73	0.38	0.76
(CML494/CML550)DH199	2.07	75.23	3.16	148.11	50.33	0.32	0.76
(CML494/CML550)DH200	2.13	74.06	3.16	150.00	57.14	0.38	0.76
(CML494/CML550)DH201	2.22	72.26	3.16	151.87	56.86	0.37	0.76
(CML494/CML550)DH206	2.08	72.38	3.16	145.80	52.59	0.36	0.76
(CML494/CML550)DH207	2.27	73.41	3.16	153.67	61.73	0.40	0.76
(CML494/CML550)DH208	2.16	73.46	3.16	151.30	57.52	0.38	0.76
(CML494/CML550)DH209	2.18	73.76	3.16	151.07	58.77	0.39	0.76
(CML494/CML550)DH211	2.18	71.69	3.16	150.49	57.62	0.38	0.76
(CML494/CML550)DH212	2.17	73.08	3.16	155.56	59.58	0.38	0.76
(CML494/CML550)DH215	2.15	73.91	3.16	149.90	59.30	0.40	0.76
(CML494/CML550)DH216	2.19	73.03	3.16	149.84	52.37	0.34	0.76
(CML494/CML550)DH219	2.14	73.34	3.16	147.95	54.27	0.36	0.76
(CML494/CML550)DH220	1.97	74.37	3.16	146.92	52.74	0.36	0.76
(CML494/CML550)DH221	2.06	72.63	3.16	150.00	53.91	0.36	0.76
(CML494/CML550)DH223	2.05	74.19	3.16	151.35	56.06	0.37	0.76
(CML494/CML550)DH224	2.19	75.08	3.16	147.26	53.20	0.36	0.76
(CML494/CML550)DH225	2.12	73.20	3.16	153.04	58.00	0.37	0.76
(CML494/CML550)DH226	2.13	72.92	3.16	153.23	59.25	0.38	0.76
(CML494/CML550)DH228	2.21	72.52	3.16	149.02	53.35	0.36	0.76
(CML494/CML550)DH232	2.12	72.66	3.16	147.81	54.90	0.37	0.76
(CML494/CML550)DH235	2.17	73.88	3.16	155.31	62.62	0.40	0.76
CML312/CML494	2.22	74.56	3.16	149.35	54.55	0.37	0.76
CML312/CML550	2.20	71.07	3.16	145.56	51.79	0.36	0.76
Locations	**2**	**2**	**2**	**2**	**2**	**2**	**2**
Replications	**2**	**2**	**2**	**2**	**2**	**2**	**2**
Error Variance	**0.33**	**3.58**	**2.52**	**158.10**	**59.83**	**0.00**	**0.02**
Genotypic Variance	**0.03**	**1.99**	**0.00**	**25.11**	**16.64**	**0.00**	**0.00**
GenxEnv Variance	**0.09**	**0.16**	**0.32**	**0.00**	**0.00**	**0.00**	**0.00**
Location Variance	**0.00**	**273.96**	**3.59**	**27.50**	**4.42**	**0.00**	**0.01**
Heritability	**0.17**	**0.67**	**0.00**	**0.39**	**0.53**	**0.63**	**0.00**
Grand Mean	**2.14**	**73.20**	**3.15**	**149.78**	**55.34**	**0.37**	**0.76**
LSD	**1.13**	**3.71**	**3.11**	**24.64**	**15.16**	**0.06**	**0.24**
CV	**26.89**	**2.59**	**50.29**	**8.39**	**13.98**	**8.92**	**16.24**

GY, Grain yield; AD, Anthesis date; ASI, Anthesis silking interval; PH, Plant height; EH, ear height; EPO, ear position; EPP, ears per plant; LSD, least significant difference; CV, coefficient of variation; GenxEnv; genotype by environment interaction

Appendix Table 4. Mean GY, AD, ASI, PH, EH, EPO and EPP of 108 doubled haploid test cross progenies (CML494/CML550) and two commercial checks evaluated in three optimum sites in Kenya (Kiboko and Kakamega) and Rwanda during the main season of 2015.

Genotype	GYF	AD	ASI	PH	EH	EPO	EPP
(CML494/CML550)DH1	8.01	70.21	0.59	254.05	128.81	0.51	1.02
(CML494/CML550)DH4	7.23	71.45	0.61	248.50	128.07	0.52	1.02
(CML494/CML550)DH6	7.61	70.94	0.64	256.03	127.53	0.50	1.02
(CML494/CML550)DH7	7.49	69.87	0.66	252.66	125.18	0.50	1.02
(CML494/CML550)DH9	7.59	70.12	0.57	244.07	120.13	0.51	1.02
(CML494/CML550)DH11	8.11	71.17	0.60	257.68	134.26	0.52	1.02
(CML494/CML550)DH12	8.04	70.95	0.66	256.74	131.56	0.51	1.02
(CML494/CML550)DH15	7.40	71.13	0.73	249.51	124.09	0.50	1.02
(CML494/CML550)DH19	7.70	70.81	0.69	253.75	122.96	0.49	1.02
(CML494/CML550)DH20	7.67	71.36	0.59	248.97	129.00	0.52	1.02
(CML494/CML550)DH21	7.46	69.93	0.60	246.10	112.22	0.47	1.02
(CML494/CML550)DH24	7.86	71.33	0.60	252.45	124.05	0.50	1.02
(CML494/CML550)DH27	7.70	69.15	0.61	242.25	111.35	0.48	1.02
(CML494/CML550)DH28	7.62	70.79	0.63	254.76	132.35	0.52	1.02
(CML494/CML550)DH29	7.29	70.27	0.60	249.08	127.63	0.51	1.02
(CML494/CML550)DH30	7.58	70.07	0.64	244.28	119.09	0.49	1.02
(CML494/CML550)DH33	7.67	71.66	0.70	257.12	137.53	0.53	1.02
(CML494/CML550)DH34	7.44	70.86	0.54	249.38	124.14	0.50	1.02
(CML494/CML550)DH36	7.18	70.55	0.69	257.54	123.54	0.49	1.02
(CML494/CML550)DH37	7.49	70.14	0.68	252.50	131.68	0.52	1.02
(CML494/CML550)DH38	7.46	71.46	0.60	252.44	132.30	0.52	1.02
(CML494/CML550)DH39	7.61	70.81	0.79	248.27	125.94	0.52	1.02
(CML494/CML550)DH40	7.44	71.22	0.66	251.56	126.12	0.51	1.02
(CML494/CML550)DH41	7.39	71.61	0.70	251.81	130.58	0.52	1.02
(CML494/CML550)DH42	8.00	70.34	0.50	251.13	120.31	0.49	1.02
(CML494/CML550)DH43	7.31	69.86	0.81	254.54	119.14	0.48	1.02
(CML494/CML550)DH44	6.65	71.37	0.63	252.14	124.95	0.50	1.02
(CML494/CML550)DH45	7.64	70.61	0.91	247.65	124.65	0.51	1.02
(CML494/CML550)DH46	7.71	71.22	0.68	259.92	131.92	0.51	1.02
(CML494/CML550)DH47	7.77	70.40	0.65	256.48	129.94	0.51	1.02
(CML494/CML550)DH48	7.62	71.37	0.66	254.38	131.29	0.51	1.02
(CML494/CML550)DH50	7.38	71.05	0.71	249.28	119.05	0.49	1.02
(CML494/CML550)DH52	7.94	70.16	0.64	248.29	117.91	0.49	1.02
(CML494/CML550)DH54	7.57	70.48	0.62	247.24	123.49	0.50	1.02
(CML494/CML550)DH55	8.03	70.81	0.64	250.90	135.59	0.54	1.02
(CML494/CML550)DH56	7.62	71.03	0.60	251.21	122.02	0.49	1.02
(CML494/CML550)DH58	7.45	70.84	0.61	257.46	130.00	0.51	1.02
(CML494/CML550)DH61	7.90	70.06	0.67	252.92	122.22	0.49	1.02
(CML494/CML550)DH68	7.56	72.20	0.74	257.66	130.25	0.50	1.02
(CML494/CML550)DH70	7.48	70.70	0.65	246.22	124.21	0.51	1.02
(CML494/CML550)DH73	7.48	71.39	0.69	252.77	128.58	0.51	1.02
(CML494/CML550)DH75	7.85	71.13	0.62	249.11	125.60	0.51	1.02
(CML494/CML550)DH76	7.59	70.22	0.73	248.60	126.73	0.51	1.02
(CML494/CML550)DH80	7.13	70.89	0.54	251.99	123.56	0.50	1.02
(CML494/CML550)DH81	7.34	70.12	0.61	245.47	122.42	0.50	1.02
(CML494/CML550)DH82	7.41	70.13	0.68	253.24	129.10	0.51	1.02
(CML494/CML550)DH86	7.57	71.03	0.62	252.14	130.13	0.51	1.02
(CML494/CML550)DH89	7.69	70.78	0.58	253.30	126.32	0.50	1.02
(CML494/CML550)DH99	7.50	70.78	0.65	245.28	124.26	0.51	1.02
(CML494/CML550)DH110	7.30	70.61	0.54	249.79	124.45	0.50	1.02
(CML494/CML550)DH113	7.41	70.66	0.61	250.05	122.93	0.50	1.02
(CML494/CML550)DH114	7.87	70.64	0.66	252.26	123.57	0.50	1.02
(CML494/CML550)DH116	8.14	71.14	0.61	255.06	128.33	0.51	1.02
(CML494/CML550)DH118	6.77	71.34	0.78	252.43	123.71	0.50	1.02
(CML494/CML550)DH119	7.28	71.38	0.58	250.41	126.47	0.51	1.02
(CML494/CML550)DH120	7.43	71.32	0.77	249.05	128.43	0.52	1.02
(CML494/CML550)DH121	7.63	72.64	0.66	257.80	133.00	0.51	1.02
(CML494/CML550)DH122	7.43	72.05	0.82	255.29	134.67	0.53	1.02
(CML494/CML550)DH124	7.04	71.08	0.66	249.45	129.96	0.52	1.02
(CML494/CML550)DH125	7.22	71.56	0.60	252.78	130.31	0.51	1.02
(CML494/CML550)DH126	7.99	71.61	0.79	252.93	130.39	0.52	1.02
(CML494/CML550)DH127	7.94	71.01	0.67	252.90	127.02	0.51	1.02
(CML494/CML550)DH129	8.02	70.54	0.68	250.25	123.48	0.50	1.02
(CML494/CML550)DH130	7.66	70.39	0.67	250.08	124.46	0.50	1.02
(CML494/CML550)DH131	7.48	70.79	0.64	245.63	128.00	0.52	1.02

Genotype	GYF	AD	ASI	PH	EH	EPO	EPP
(CML494/CML550)DH132	7.63	70.92	0.75	251.07	123.08	0.50	1.02
(CML494/CML550)DH136	7.51	71.73	0.60	259.96	134.38	0.51	1.02
(CML494/CML550)DH142	7.62	70.45	0.78	246.23	122.48	0.51	1.02
(CML494/CML550)DH144	7.46	70.89	0.65	252.06	127.14	0.51	1.02
(CML494/CML550)DH148	7.69	70.18	0.74	243.37	116.76	0.49	1.02
(CML494/CML550)DH149	8.11	70.77	0.67	251.95	123.40	0.50	1.02
(CML494/CML550)DH153	7.42	71.73	0.63	254.16	129.21	0.51	1.02
(CML494/CML550)DH154	7.24	70.86	0.79	248.58	123.26	0.51	1.02
(CML494/CML550)DH158	7.28	70.87	0.63	249.65	125.66	0.51	1.02
(CML494/CML550)DH163	7.31	70.90	0.96	251.12	126.88	0.51	1.02
(CML494/CML550)DH169	7.63	72.07	0.67	258.82	135.55	0.52	1.02
(CML494/CML550)DH170	7.58	70.91	0.63	256.53	130.96	0.51	1.02
(CML494/CML550)DH171	7.34	70.63	0.75	255.13	122.46	0.48	1.02
(CML494/CML550)DH174	7.32	70.80	0.70	249.05	119.02	0.49	1.02
(CML494/CML550)DH175	7.77	71.66	0.88	255.92	134.99	0.52	1.02
(CML494/CML550)DH179	7.61	71.34	0.70	255.31	128.14	0.50	1.02
(CML494/CML550)DH190	7.37	70.62	0.61	253.64	125.54	0.50	1.02
(CML494/CML550)DH192	7.93	70.01	0.62	251.20	127.83	0.51	1.02
(CML494/CML550)DH193	7.36	70.68	0.63	246.81	126.33	0.52	1.02
(CML494/CML550)DH194	7.30	70.92	0.60	248.89	123.74	0.51	1.02
(CML494/CML550)DH197	7.28	71.53	0.50	255.80	125.96	0.50	1.02
(CML494/CML550)DH198	7.33	71.21	0.76	252.62	127.53	0.51	1.02
(CML494/CML550)DH199	7.14	71.34	0.55	254.37	124.87	0.50	1.02
(CML494/CML550)DH200	7.91	71.34	0.53	254.91	134.47	0.52	1.02
(CML494/CML550)DH201	7.97	71.06	0.73	256.68	129.55	0.50	1.02
(CML494/CML550)DH206	7.44	70.77	0.54	250.33	125.12	0.50	1.02
(CML494/CML550)DH207	7.75	71.66	0.68	261.20	134.70	0.51	1.02
(CML494/CML550)DH208	7.83	71.30	0.70	253.17	129.21	0.51	1.02
(CML494/CML550)DH209	7.73	71.27	0.90	250.66	126.77	0.51	1.02
(CML494/CML550)DH211	7.74	71.41	0.77	252.78	127.34	0.51	1.02
(CML494/CML550)DH212	7.80	71.41	0.63	258.67	132.09	0.51	1.02
(CML494/CML550)DH215	7.76	71.68	0.46	253.37	131.32	0.52	1.02
(CML494/CML550)DH216	7.61	71.42	0.55	261.51	128.60	0.50	1.02
(CML494/CML550)DH219	7.90	71.42	0.61	254.60	131.02	0.51	1.02
(CML494/CML550)DH220	7.16	70.58	0.72	253.79	126.64	0.50	1.02
(CML494/CML550)DH221	7.74	70.11	0.63	251.75	125.26	0.50	1.02
(CML494/CML550)DH223	7.66	71.13	0.69	251.82	129.19	0.52	1.02
(CML494/CML550)DH224	7.75	71.20	0.61	253.09	129.85	0.51	1.02
(CML494/CML550)DH225	7.74	71.16	0.67	251.89	123.24	0.49	1.02
(CML494/CML550)DH226	7.06	71.47	0.72	250.60	127.22	0.51	1.02
(CML494/CML550)DH228	7.46	71.24	0.59	251.45	123.91	0.49	1.02
(CML494/CML550)DH232	7.98	70.78	0.53	251.26	121.11	0.49	1.02
(CML494/CML550)DH235	7.68	71.55	0.68	253.34	130.93	0.52	1.02
CML312/CML494	7.93	71.74	0.66	254.36	127.06	0.50	1.02
CML312/CML550	8.21	69.90	0.59	244.95	120.98	0.51	1.02
Locations	**3**	**3**	**3**	**3**	**3**	**3**	**3**
Replications	**2**	**2**	**2**	**2**	**2**	**2**	**2**
Error Variance	**1.60**	**1.20**	**0.99**	**95.59**	**75.73**	**0.00**	**0.02**
Genotypic Variance	**0.23**	**0.53**	**0.04**	**26.54**	**32.58**	**0.00**	**0.00**
GenxEnv Variance	**0.33**	**0.28**	**0.02**	**2.27**	**0.00**	**0.00**	**0.00**
Location Variance	**1.05**	**129.81**	**0.23**	**1271.99**	**134.14**	**0.00**	**0.00**
Heritability	**0.38**	**0.64**	**0.19**	**0.61**	**0.72**	**0.53**	**0.00**
Grand Mean	**7.58**	**70.95**	**0.66**	**252.04**	**126.67**	**0.51**	**1.02**
LSD	**2.48**	**2.15**	**1.95**	**19.16**	**17.06**	**0.06**	**0.30**
CV	**16.70**	**1.55**	**151.11**	**3.88**	**6.87**	**6.39**	**14.86**

GY, Grain yield; AD, Anthesis date; ASI, Anthesis silking interval; PH, Plant height; EH, ear height; EPO, ear position; EPP, ears per plant; LSD, least significant difference; CV, coefficient of variation; GenxEnv; genotype by environment interaction

Appendix Table 5. Mean GY, AD, ASI, PH, EH, EPO and SEN of 212 doubled haploid test cross progenies (CML504/CML550) and three commercial checks evaluated in three low N stress sites in Kenya (Alupe, Embu and Kakamega) during the main season of 2014.

Genotype	GYF	AD	ASI	PH	EH	EPO	SEN
(CLWN201/CML504)DH1	3.53	72.02	1.96	202.35	96.24	0.47	3.15
(CLWN201/CML504)DH2	3.25	72.38	2.09	208.82	96.38	0.46	3.16
(CLWN201/CML504)DH3	3.17	73.91	1.71	207.43	98.15	0.46	3.17

Genotype	GYF	AD	ASI	PH	EH	EPO	SEN
(CLWN201/CML504)DH4	3.33	72.37	1.92	206.02	100.69	0.48	3.26
(CLWN201/CML504)DH5	3.29	74.71	2.27	216.02	97.38	0.45	3.17
(CLWN201/CML504)DH6	3.53	72.20	1.93	200.83	91.17	0.45	3.15
(CLWN201/CML504)DH7	3.45	72.63	2.42	204.68	95.88	0.47	3.16
(CLWN201/CML504)DH8	3.24	74.01	2.39	225.63	106.36	0.47	3.20
(CLWN201/CML504)DH9	3.33	73.27	2.21	197.81	87.14	0.44	3.14
(CLWN201/CML504)DH10	3.37	73.05	1.46	201.64	91.37	0.45	3.14
(CLWN201/CML504)DH11	3.24	71.14	1.68	197.42	89.59	0.45	3.18
(CLWN201/CML504)DH12	3.32	72.72	2.10	213.26	101.45	0.47	3.17
(CLWN201/CML504)DH13	3.31	73.52	2.03	211.75	95.16	0.45	3.14
(CLWN201/CML504)DH14	3.14	74.36	1.90	206.62	94.04	0.45	3.21
(CLWN201/CML504)DH15	3.69	71.20	2.16	217.40	100.44	0.46	3.11
(CLWN201/CML504)DH16	3.54	71.80	1.27	203.19	97.98	0.47	3.12
(CLWN201/CML504)DH17	3.29	70.33	1.92	197.98	89.83	0.45	3.08
(CLWN201/CML504)DH18	3.52	73.20	1.52	219.41	99.53	0.46	3.20
(CLWN201/CML504)DH19	3.00	72.01	1.91	200.09	89.37	0.44	3.16
(CLWN201/CML504)DH20	3.42	72.23	1.69	218.96	102.14	0.46	3.17
(CLWN201/CML504)DH21	3.51	73.57	1.99	210.07	96.08	0.45	3.17
(CLWN201/CML504)DH22	3.16	72.40	2.35	185.93	87.60	0.46	3.13
(CLWN201/CML504)DH23	3.36	70.97	1.60	200.84	97.93	0.48	3.16
(CLWN201/CML504)DH24	3.22	72.47	2.16	199.50	92.27	0.46	3.15
(CLWN201/CML504)DH25	3.07	72.02	2.06	200.26	88.00	0.44	3.16
(CLWN201/CML504)DH26	2.94	72.32	1.79	201.51	94.82	0.46	3.12
(CLWN201/CML504)DH27	3.22	72.66	1.77	211.44	94.06	0.45	3.19
(CLWN201/CML504)DH28	3.38	72.12	1.83	210.70	98.26	0.46	3.18
(CLWN201/CML504)DH29	3.20	71.62	2.42	211.71	93.25	0.44	3.14
(CLWN201/CML504)DH30	3.30	72.95	1.96	215.91	98.18	0.45	3.23
(CLWN201/CML504)DH31	3.49	71.27	2.18	208.68	94.39	0.45	3.12
(CLWN201/CML504)DH32	3.38	71.71	1.89	201.91	96.31	0.47	3.18
(CLWN201/CML504)DH33	3.52	72.25	1.54	204.73	91.82	0.45	3.16
(CLWN201/CML504)DH34	3.57	71.38	2.06	209.07	95.00	0.45	3.18
(CLWN201/CML504)DH35	3.07	72.83	2.36	208.08	98.28	0.47	3.19
(CLWN201/CML504)DH37	3.48	71.73	1.41	208.86	93.63	0.45	3.13
(CLWN201/CML504)DH38	3.55	70.10	1.83	202.68	92.21	0.45	3.14
(CLWN201/CML504)DH39	3.40	73.22	1.87	207.23	95.03	0.45	3.15
(CLWN201/CML504)DH40	3.13	70.95	2.18	200.15	91.81	0.46	3.20
(CLWN201/CML504)DH41	3.43	70.94	1.97	204.96	91.65	0.45	3.22
(CLWN201/CML504)DH42	3.42	71.94	1.93	212.83	99.89	0.46	3.17
(CLWN201/CML504)DH43	3.36	72.27	1.73	211.66	103.84	0.48	3.12
(CLWN201/CML504)DH44	3.30	72.98	1.63	216.80	99.88	0.46	3.13
(CLWN201/CML504)DH45	3.35	72.46	2.12	213.12	98.53	0.46	3.21
(CLWN201/CML504)DH46	3.46	70.85	2.03	199.60	93.24	0.46	3.21
(CLWN201/CML504)DH47	3.36	72.41	1.54	209.66	98.24	0.47	3.19
(CLWN201/CML504)DH48	3.49	72.09	1.88	198.84	90.40	0.45	3.12
(CLWN201/CML504)DH49	3.23	73.36	1.81	216.30	108.22	0.48	3.18
(CLWN201/CML504)DH50	3.06	72.28	1.77	213.68	97.68	0.46	3.25
(CLWN201/CML504)DH51	3.38	73.02	2.15	212.33	98.12	0.46	3.23
(CLWN201/CML504)DH52	3.56	74.50	2.52	207.38	96.88	0.46	3.16
(CLWN201/CML504)DH53	3.33	74.22	2.22	209.98	96.24	0.46	3.12
(CLWN201/CML504)DH54	3.55	74.28	1.83	211.59	101.13	0.47	3.13
(CLWN201/CML504)DH55	3.36	72.32	2.49	200.93	90.54	0.45	3.08
(CLWN201/CML504)DH56	3.26	72.13	2.09	194.76	87.59	0.45	3.12
(CLWN201/CML504)DH57	3.31	71.72	1.68	193.43	87.18	0.45	3.15
(CLWN201/CML504)DH58	3.44	72.90	2.84	214.26	102.35	0.47	3.19
(CLWN201/CML504)DH59	3.36	73.67	1.50	213.22	97.59	0.46	3.12
(CLWN201/CML504)DH60	3.37	73.88	2.08	214.34	100.58	0.46	3.18
(CLWN201/CML504)DH61	3.52	72.28	1.50	205.04	93.27	0.45	3.15
(CLWN201/CML504)DH63	3.28	70.82	1.48	196.31	85.99	0.44	3.10
(CLWN201/CML504)DH64	3.41	73.05	1.65	212.67	103.81	0.48	3.18
(CLWN201/CML504)DH65	3.66	72.89	1.90	215.93	100.03	0.46	3.16
(CLWN201/CML504)DH66	3.39	73.61	1.83	213.10	96.01	0.45	3.16
(CLWN201/CML504)DH67	3.42	72.27	2.13	207.36	94.50	0.45	3.19
(CLWN201/CML504)DH68	3.32	73.25	1.77	212.36	95.67	0.45	3.11
(CLWN201/CML504)DH69	3.72	71.88	1.99	212.75	97.39	0.46	3.15
(CLWN201/CML504)DH70	3.34	73.54	1.86	203.60	93.47	0.46	3.17
(CLWN201/CML504)DH71	3.13	73.28	2.89	209.30	92.59	0.45	3.17
(CLWN201/CML504)DH72	3.28	73.35	2.10	211.08	98.37	0.46	3.15
(CLWN201/CML504)DH73	3.74	73.23	1.99	208.42	95.89	0.46	3.17

Genotype	GYF	AD	ASI	PH	EH	EPO	SEN
(CLWN201/CML504)DH74	3.06	73.76	2.25	204.07	93.60	0.46	3.20
(CLWN201/CML504)DH75	3.50	72.44	2.59	204.51	98.94	0.47	3.19
(CLWN201/CML504)DH76	3.13	72.76	2.51	189.27	84.29	0.45	3.19
(CLWN201/CML504)DH77	3.11	71.12	1.97	197.69	90.41	0.46	3.14
(CLWN201/CML504)DH78	3.54	73.75	2.23	220.84	99.78	0.45	3.21
(CLWN201/CML504)DH79	3.50	71.96	2.14	198.38	92.37	0.46	3.13
(CLWN201/CML504)DH80	3.31	71.66	1.85	200.48	92.92	0.46	3.15
(CLWN201/CML504)DH81	3.34	69.08	1.74	193.23	89.22	0.46	3.10
(CLWN201/CML504)DH82	3.29	72.13	2.19	217.18	100.41	0.46	3.16
(CLWN201/CML504)DH83	3.73	73.38	2.70	210.67	100.06	0.47	3.13
(CLWN201/CML504)DH84	3.57	71.80	2.36	222.58	106.87	0.48	3.18
(CLWN201/CML504)DH85	3.49	72.41	2.22	199.67	90.99	0.45	3.11
(CLWN201/CML504)DH86	3.68	73.63	1.81	218.62	103.01	0.47	3.14
(CLWN201/CML504)DH87	3.52	73.07	1.99	207.25	95.04	0.46	3.15
(CLWN201/CML504)DH88	2.96	72.95	2.60	201.25	90.30	0.44	3.20
(CLWN201/CML504)DH89	3.44	72.77	1.63	208.03	91.55	0.44	3.17
(CLWN201/CML504)DH90	3.55	72.33	2.24	208.13	96.39	0.46	3.18
(CLWN201/CML504)DH91	3.49	74.20	2.13	216.74	94.27	0.44	3.13
(CLWN201/CML504)DH92	3.45	71.79	2.42	203.69	91.23	0.45	3.20
(CLWN201/CML504)DH93	3.35	72.65	1.98	218.04	101.81	0.47	3.17
(CLWN201/CML504)DH94	3.31	72.89	1.99	201.26	88.67	0.44	3.14
(CLWN201/CML504)DH95	3.45	72.88	2.01	217.30	101.13	0.46	3.16
(CLWN201/CML504)DH96	3.45	74.08	1.92	210.13	102.14	0.48	3.12
(CLWN201/CML504)DH97	3.47	71.17	1.50	203.28	87.05	0.43	3.16
(CLWN201/CML504)DH98	3.38	72.77	2.10	209.66	91.92	0.44	3.15
(CLWN201/CML504)DH99	3.63	72.69	2.11	212.29	93.89	0.44	3.13
(CLWN201/CML504)DH100	3.54	73.44	1.45	208.55	92.62	0.45	3.17
(CLWN201/CML504)DH101	3.51	72.84	1.94	217.35	104.77	0.48	3.16
(CLWN201/CML504)DH102	3.34	71.85	2.00	208.33	102.62	0.48	3.18
(CLWN201/CML504)DH103	3.49	73.59	1.79	212.72	101.04	0.47	3.18
(CLWN201/CML504)DH104	3.31	70.07	2.04	201.93	99.86	0.48	3.21
(CLWN201/CML504)DH105	3.64	72.34	1.65	206.52	90.23	0.44	3.15
(CLWN201/CML504)DH106	3.60	73.31	2.25	216.90	100.52	0.46	3.22
(CLWN201/CML504)DH107	3.54	72.56	2.17	206.97	95.19	0.46	3.16
(CLWN201/CML504)DH108	3.55	73.30	1.86	227.45	107.70	0.47	3.09
(CLWN201/CML504)DH109	3.51	71.15	2.01	203.16	94.00	0.46	3.14
(CLWN201/CML504)DH110	3.40	73.00	1.74	213.30	98.58	0.46	3.19
(CLWN201/CML504)DH111	3.47	72.67	2.09	202.25	90.00	0.45	3.12
(CLWN201/CML504)DH112	3.81	72.54	1.47	208.21	97.77	0.47	3.12
(CLWN201/CML504)DH113	3.51	71.87	2.19	220.71	100.85	0.46	3.16
(CLWN201/CML504)DH114	3.22	71.40	1.80	221.59	106.20	0.48	3.19
(CLWN201/CML504)DH115	3.00	67.17	2.29	190.42	76.61	0.42	3.19
(CLWN201/CML504)DH116	3.30	72.97	2.02	202.63	89.36	0.44	3.17
(CLWN201/CML504)DH117	3.44	72.37	2.21	216.58	106.08	0.48	3.17
(CLWN201/CML504)DH118	3.42	73.12	1.58	205.91	89.06	0.44	3.17
(CLWN201/CML504)DH119	3.40	71.04	2.35	209.18	92.45	0.44	3.14
(CLWN201/CML504)DH120	3.46	71.76	1.52	209.63	100.95	0.47	3.14
(CLWN201/CML504)DH121	3.11	72.91	2.32	204.45	92.41	0.45	3.16
(CLWN201/CML504)DH122	3.24	71.30	2.27	209.35	93.70	0.45	3.19
(CLWN201/CML504)DH124	3.26	72.79	1.32	217.39	100.39	0.46	3.15
(CLWN201/CML504)DH125	3.70	72.35	1.58	213.48	98.27	0.46	3.16
(CLWN201/CML504)DH126	3.26	74.36	2.36	220.95	100.83	0.45	3.22
(CLWN201/CML504)DH127	3.28	72.29	1.81	188.24	87.14	0.46	3.11
(CLWN201/CML504)DH128	3.40	73.16	2.13	216.42	102.70	0.47	3.19
(CLWN201/CML504)DH129	3.28	70.96	1.91	205.50	91.46	0.45	3.17
(CLWN201/CML504)DH130	3.37	71.59	2.10	197.35	84.62	0.43	3.10
(CLWN201/CML504)DH131	3.31	72.17	2.50	194.49	86.61	0.44	3.14
(CLWN201/CML504)DH132	3.42	71.22	2.11	201.17	92.22	0.45	3.19
(CLWN201/CML504)DH133	3.54	73.18	2.44	212.21	99.86	0.47	3.13
(CLWN201/CML504)DH134	3.29	73.09	1.91	218.93	90.81	0.42	3.17
(CLWN201/CML504)DH135	3.32	72.22	1.83	207.60	97.28	0.46	3.14
(CLWN201/CML504)DH136	3.47	73.65	1.62	203.53	93.51	0.46	3.17
(CLWN201/CML504)DH137	3.39	72.55	2.37	204.64	93.32	0.45	3.13
(CLWN201/CML504)DH139	3.13	67.78	1.84	204.52	82.46	0.41	3.15
(CLWN201/CML504)DH141	3.35	73.16	1.62	205.96	96.87	0.46	3.17
(CLWN201/CML504)DH142	3.47	72.08	1.86	221.73	103.65	0.46	3.20
(CLWN201/CML504)DH143	3.39	71.71	1.50	208.34	101.31	0.48	3.09
(CLWN201/CML504)DH146	3.49	72.02	2.65	216.02	97.74	0.45	3.21

Genotype	GYF	AD	ASI	PH	EH	EPO	SEN
(CLWN201/CML504)DH147	3.63	73.08	1.87	223.85	105.79	0.47	3.17
(CLWN201/CML504)DH148	3.53	72.76	1.51	209.87	90.29	0.44	3.12
(CLWN201/CML504)DH149	3.75	74.04	1.68	209.02	98.71	0.47	3.10
(CLWN201/CML504)DH151	3.48	71.55	1.98	195.66	90.50	0.46	3.12
(CLWN201/CML504)DH152	3.14	67.87	1.91	199.70	90.83	0.46	3.23
(CLWN201/CML504)DH154	3.24	73.05	2.73	213.70	99.53	0.46	3.17
(CLWN201/CML504)DH156	3.59	72.34	2.38	218.38	100.08	0.46	3.20
(CLWN201/CML504)DH157	3.31	73.53	1.38	216.26	100.35	0.46	3.25
(CLWN201/CML504)DH158	3.38	72.97	2.10	206.35	98.00	0.47	3.16
(CLWN201/CML504)DH159	3.30	72.19	2.47	220.50	101.20	0.46	3.17
(CLWN201/CML504)DH160	3.43	72.45	1.80	220.36	103.73	0.47	3.17
(CLWN201/CML504)DH161	3.54	71.10	1.53	211.66	98.38	0.46	3.19
(CLWN201/CML504)DH162	3.43	72.81	1.39	210.78	93.78	0.44	3.17
(CLWN201/CML504)DH163	3.75	71.98	1.68	217.33	96.54	0.45	3.17
(CLWN201/CML504)DH164	3.39	71.44	2.27	214.79	96.50	0.45	3.15
(CLWN201/CML504)DH165	3.29	71.06	1.79	207.63	91.36	0.44	3.14
(CLWN201/CML504)DH166	3.73	72.62	1.90	217.51	99.22	0.45	3.16
(CLWN201/CML504)DH167	3.33	71.67	1.97	206.66	94.66	0.45	3.17
(CLWN201/CML504)DH168	3.34	72.93	2.20	211.75	98.95	0.47	3.13
(CLWN201/CML504)DH169	3.29	72.52	2.23	202.03	98.59	0.48	3.21
(CLWN201/CML504)DH170	3.69	72.62	2.62	209.74	92.08	0.44	3.15
(CLWN201/CML504)DH171	3.36	73.18	2.14	215.88	102.27	0.47	3.16
(CLWN201/CML504)DH172	3.37	71.38	1.56	203.75	98.70	0.48	3.22
(CLWN201/CML504)DH173	3.37	71.18	1.91	197.41	88.95	0.45	3.17
(CLWN201/CML504)DH174	3.17	73.64	2.59	207.26	88.51	0.43	3.19
(CLWN201/CML504)DH175	3.31	72.61	1.95	207.18	95.03	0.46	3.15
(CLWN201/CML504)DH176	3.62	73.03	1.94	214.73	105.17	0.48	3.20
(CLWN201/CML504)DH177	3.49	71.57	1.52	197.08	91.68	0.46	3.15
(CLWN201/CML504)DH178	3.35	71.51	2.00	204.85	93.74	0.46	3.14
(CLWN201/CML504)DH179	3.61	71.58	1.94	208.90	100.86	0.48	3.13
(CLWN201/CML504)DH180	3.35	71.35	1.90	202.95	93.48	0.46	3.15
(CLWN201/CML504)DH181	3.20	72.97	2.26	206.76	96.09	0.46	3.17
(CLWN201/CML504)DH182	3.37	71.42	1.55	205.98	85.31	0.42	3.15
(CLWN201/CML504)DH183	3.27	71.28	1.48	201.25	93.63	0.46	3.15
(CLWN201/CML504)DH184	3.24	71.88	1.75	192.74	84.34	0.44	3.13
(CLWN201/CML504)DH185	3.40	71.67	1.76	214.88	93.67	0.44	3.16
(CLWN201/CML504)DH186	3.40	74.37	2.48	224.94	106.59	0.47	3.19
(CLWN201/CML504)DH187	3.14	71.55	1.72	209.38	98.29	0.46	3.19
(CLWN201/CML504)DH188	3.57	73.41	2.40	228.85	108.19	0.47	3.16
(CLWN201/CML504)DH189	3.36	70.32	2.06	212.09	103.13	0.48	3.18
(CLWN201/CML504)DH194	3.22	71.02	1.73	210.01	95.51	0.45	3.14
(CLWN201/CML504)DH195	3.14	70.59	1.88	196.76	86.82	0.44	3.14
(CLWN201/CML504)DH196	3.67	71.31	2.01	214.48	92.28	0.44	3.19
(CLWN201/CML504)DH197	3.14	70.43	1.81	205.11	89.71	0.44	3.20
(CLWN201/CML504)DH198	3.41	72.15	1.86	217.15	98.35	0.45	3.14
(CLWN201/CML504)DH199	3.55	70.56	1.86	210.28	96.52	0.46	3.16
(CLWN201/CML504)DH200	3.58	73.18	2.55	204.38	89.21	0.44	3.14
(CLWN201/CML504)DH201	3.21	73.94	2.16	220.85	104.23	0.47	3.20
(CLWN201/CML504)DH202	3.19	70.63	2.29	209.61	97.94	0.46	3.19
(CLWN201/CML504)DH204	3.52	71.87	1.84	218.52	100.55	0.46	3.22
(CLWN201/CML504)DH205	3.40	72.24	1.86	204.95	94.36	0.46	3.12
(CLWN201/CML504)DH206	3.47	71.73	2.09	202.35	92.74	0.45	3.11
(CLWN201/CML504)DH207	3.25	71.51	1.62	210.39	92.28	0.44	3.14
(CLWN201/CML504)DH210	3.26	71.19	1.21	200.13	85.85	0.43	3.13
(CLWN201/CML504)DH211	3.08	71.71	1.20	191.56	85.68	0.44	3.11
(CLWN201/CML504)DH212	3.47	71.41	1.83	213.88	94.39	0.44	3.19
(CLWN201/CML504)DH213	3.21	72.17	1.81	221.48	104.58	0.47	3.18
(CLWN201/CML504)DH214	3.28	71.30	2.20	207.33	90.37	0.44	3.16
(CLWN201/CML504)DH215	3.61	73.05	1.74	210.60	95.34	0.45	3.16
(CLWN201/CML504)DH216	3.27	71.86	1.87	203.20	91.33	0.45	3.17
(CLWN201/CML504)DH217	3.10	73.52	1.91	203.32	93.01	0.46	3.15
(CLWN201/CML504)DH218	3.30	73.72	2.15	215.85	100.58	0.46	3.14
(CLWN201/CML504)DH220	3.16	73.21	1.83	198.67	88.84	0.45	3.15
(CLWN201/CML504)DH221	3.57	72.97	2.26	214.71	99.09	0.46	3.18
(CLWN201/CML504)DH224	3.49	72.30	2.42	224.99	109.92	0.48	3.16
(CLWN201/CML504)DH226	3.54	71.92	1.34	208.01	96.26	0.46	3.10
(CLWN201/CML504)DH227	3.49	72.78	1.72	215.89	94.09	0.44	3.25
(CLWN201/CML504)DH228	3.55	72.27	2.64	223.86	104.45	0.47	3.22

Genotype	GYF	AD	ASI	PH	EH	EPO	SEN
(CLWN201/CML504)DH229	3.38	72.10	2.27	209.24	98.54	0.46	3.17
(CLWN201/CML504)DH231	3.60	72.72	1.63	208.05	91.46	0.45	3.17
(CLWN201/CML504)DH233	3.43	75.70	2.85	226.32	105.25	0.47	3.15
(CLWN201/CML504)DH234	3.41	69.92	2.10	202.17	90.81	0.45	3.19
(CLWN201/CML504)DH235	3.57	71.97	2.42	208.91	101.91	0.48	3.17
CZH0616	3.36	71.89	1.86	202.96	94.45	0.46	3.13
PAN53	3.36	72.85	2.64	223.11	104.99	0.47	3.15
WH507	3.35	76.04	2.36	214.54	105.86	0.48	3.14
n Locs	**3**	**3**	**3**	**3**	**3**	**3**	**3**
n Reps	**2**	**2**	**2**	**2**	**2**	**2**	**2**
Error Var	**0.60**	**2.30**	**1.81**	**91.94**	**73.81**	**0.00**	**0.11**
Genotypic Var	**0.08**	**1.81**	**0.26**	**79.89**	**43.22**	**0.00**	**0.01**
GenxEnv Var	**0.11**	**0.27**	**0.07**	**0.00**	**0.00**	**0.00**	**0.02**
Location Var	**0.81**	**19.78**	**3.58**	**841.66**	**325.99**	**0.00**	**0.00**
Heritability	**0.38**	**0.79**	**0.45**	**0.84**	**0.78**	**0.62**	**0.23**
Grand Mean	**3.39**	**72.34**	**1.98**	**208.77**	**95.87**	**0.46**	**3.16**
LSD	**1.52**	**2.97**	**2.64**	**18.79**	**16.84**	**0.06**	**0.66**
CV	**22.87**	**2.10**	**67.87**	**4.59**	**8.96**	**7.11**	**10.64**

GY, Grain yield; AD, Anthesis date; ASI, Anthesis silking interval; PH, Plant height; EH, ear height; EPO, ear position; SEN, leaf scenescence; LSD, least significant difference; CV, coefficient of variation; GenxEnv; genotype by environment interaction

Appendix Table 6. Mean GY, AD, ASI, PH, EH, EPO and SEN of 207 doubled haploid test cross progenies (CML504/CML550) and four commercial checks evaluated in two low N stress sites in Kenya (Kiboko) during off season of 2014.

Genotype	GYF	AD	ASI	PH	EH	EPO	SEN
(CLWN201/CML504)DH1	2.06	66.75	2.07	142.45	53.80	0.38	5.20
(CLWN201/CML504)DH2	1.80	67.98	1.87	147.46	55.24	0.37	5.38
(CLWN201/CML504)DH3	2.73	66.72	2.31	141.79	54.00	0.38	5.54
(CLWN201/CML504)DH4	3.03	68.01	1.58	144.82	54.32	0.37	5.65
(CLWN201/CML504)DH5	2.79	67.43	2.28	146.71	53.66	0.36	5.32
(CLWN201/CML504)DH6	1.90	67.78	1.95	146.65	54.90	0.38	5.12
(CLWN201/CML504)DH7	2.10	68.18	1.99	142.41	52.92	0.37	5.45
(CLWN201/CML504)DH8	2.99	68.33	2.64	148.47	54.22	0.37	5.48
(CLWN201/CML504)DH9	2.48	66.82	2.36	134.89	49.16	0.36	5.50
(CLWN201/CML504)DH10	2.58	67.75	1.69	139.20	50.85	0.36	5.47
(CLWN201/CML504)DH11	2.18	67.65	1.84	142.65	52.33	0.36	5.64
(CLWN201/CML504)DH12	2.66	68.88	1.61	149.98	57.05	0.38	5.53
(CLWN201/CML504)DH13	1.68	66.75	1.82	148.11	56.57	0.38	5.65
(CLWN201/CML504)DH14	1.95	69.12	2.09	141.00	53.65	0.38	5.58
(CLWN201/CML504)DH15	2.81	65.23	3.07	148.66	54.02	0.36	5.49
(CLWN201/CML504)DH16	2.62	66.84	2.09	138.40	51.69	0.37	5.81
(CLWN201/CML504)DH17	2.76	66.18	2.15	142.84	52.10	0.36	5.28
(CLWN201/CML504)DH18	2.32	67.83	1.86	149.44	54.38	0.36	5.48
(CLWN201/CML504)DH19	2.06	66.29	1.76	137.04	53.57	0.39	5.84
(CLWN201/CML504)DH20	2.02	67.33	2.02	145.25	54.05	0.37	5.70
(CLWN201/CML504)DH21	1.69	66.86	1.61	139.34	50.90	0.36	5.62
(CLWN201/CML504)DH22	2.70	66.75	1.96	138.36	52.02	0.37	5.56
(CLWN201/CML504)DH23	2.57	68.15	1.76	143.57	53.88	0.38	5.63
(CLWN201/CML504)DH24	2.57	67.34	2.23	144.80	53.36	0.37	5.31
(CLWN201/CML504)DH25	2.56	66.68	2.03	138.12	51.23	0.37	5.52
(CLWN201/CML504)DH26	3.13	65.72	2.07	143.68	54.12	0.37	5.79
(CLWN201/CML504)DH27	2.42	66.74	1.52	148.18	54.03	0.37	5.78
(CLWN201/CML504)DH28	2.74	66.58	1.93	150.32	56.08	0.37	5.56
(CLWN201/CML504)DH29	2.29	67.38	2.58	148.45	56.63	0.38	5.51
(CLWN201/CML504)DH30	2.53	66.99	1.90	150.49	57.15	0.38	5.65
(CLWN201/CML504)DH31	2.22	67.45	1.57	140.13	50.91	0.36	5.22
(CLWN201/CML504)DH32	2.72	65.23	2.61	148.59	57.52	0.39	5.89
(CLWN201/CML504)DH33	2.43	67.11	1.81	143.27	54.65	0.38	5.72
(CLWN201/CML504)DH34	3.02	65.46	2.19	146.26	52.86	0.36	5.68
(CLWN201/CML504)DH35	2.78	67.23	2.44	148.47	57.49	0.39	5.55
(CLWN201/CML504)DH36	2.23	65.81	1.88	141.46	54.92	0.39	5.78
(CLWN201/CML504)DH37	1.98	65.93	1.47	145.45	52.82	0.37	5.56
(CLWN201/CML504)DH38	2.99	64.59	2.40	142.31	53.81	0.38	5.79
(CLWN201/CML504)DH39	2.76	66.72	2.41	146.65	56.43	0.38	5.58
(CLWN201/CML504)DH40	2.77	65.72	2.51	148.06	56.18	0.38	5.84
(CLWN201/CML504)DH41	2.24	66.25	2.24	140.70	52.70	0.37	5.67
(CLWN201/CML504)DH42	2.43	68.30	1.17	149.23	56.82	0.38	5.63
(CLWN201/CML504)DH43	2.78	66.47	1.99	146.36	54.63	0.37	5.59
(CLWN201/CML504)DH44	1.94	68.42	1.68	150.80	56.49	0.37	5.74
(CLWN201/CML504)DH45	2.45	66.06	1.73	149.42	58.18	0.39	5.98
(CLWN201/CML504)DH46	3.03	65.68	1.87	144.49	55.25	0.38	5.55
(CLWN201/CML504)DH47	2.74	66.45	2.32	145.17	53.72	0.37	5.56
(CLWN201/CML504)DH48	3.22	66.13	1.81	146.45	53.78	0.37	5.65
(CLWN201/CML504)DH49	1.96	68.55	2.40	150.47	59.45	0.39	5.66
(CLWN201/CML504)DH50	2.01	66.55	1.98	144.15	53.99	0.37	5.79
(CLWN201/CML504)DH51	2.11	67.83	2.07	153.32	58.07	0.38	5.43
(CLWN201/CML504)DH52	2.89	68.33	2.32	149.75	55.88	0.37	5.36
(CLWN201/CML504)DH53	2.45	68.13	1.92	149.50	58.30	0.39	5.35
(CLWN201/CML504)DH54	2.60	68.85	2.02	150.03	57.18	0.38	5.34
(CLWN201/CML504)DH55	2.49	66.99	1.94	141.46	54.42	0.38	5.29
(CLWN201/CML504)DH56	1.98	67.47	2.11	145.02	51.78	0.36	5.38
(CLWN201/CML504)DH57	2.19	67.30	1.37	142.17	54.68	0.38	5.50
(CLWN201/CML504)DH58	2.77	67.12	2.42	149.67	57.72	0.38	5.11
(CLWN201/CML504)DH59	2.05	66.98	2.61	142.47	54.01	0.38	5.40
(CLWN201/CML504)DH60	2.68	68.68	2.12	149.28	54.78	0.37	5.39
(CLWN201/CML504)DH61	2.74	67.76	2.21	141.56	53.25	0.37	5.54
(CLWN201/CML504)DH62	2.48	67.36	2.37	146.08	54.69	0.37	5.55
(CLWN201/CML504)DH63	3.03	66.44	2.17	144.98	53.76	0.37	5.46
(CLWN201/CML504)DH64	2.18	69.13	2.20	150.87	57.78	0.38	5.29
(CLWN201/CML504)DH65	2.22	67.21	1.87	150.27	56.47	0.38	5.53

Genotype	GYF	AD	ASI	PH	EH	EPO	SEN
(CLWN201/CML504)DH66	2.26	69.02	1.82	143.83	54.55	0.38	5.54
(CLWN201/CML504)DH67	2.49	68.62	2.00	145.67	54.90	0.38	5.51
(CLWN201/CML504)DH68	2.93	66.83	2.21	142.54	49.92	0.36	5.66
(CLWN201/CML504)DH69	2.13	66.79	1.87	146.23	52.20	0.36	5.48
(CLWN201/CML504)DH70	2.57	66.97	2.08	146.82	55.15	0.37	5.50
(CLWN201/CML504)DH71	2.75	68.24	2.43	147.82	55.89	0.38	5.44
(CLWN201/CML504)DH72	2.95	66.94	2.21	149.09	55.47	0.37	5.54
(CLWN201/CML504)DH73	3.06	67.35	2.12	144.28	54.72	0.38	5.41
(CLWN201/CML504)DH74	2.43	67.75	1.92	141.77	53.38	0.37	5.63
(CLWN201/CML504)DH75	2.83	67.60	2.19	142.11	54.62	0.38	5.43
(CLWN201/CML504)DH76	2.87	66.15	2.57	140.08	53.42	0.38	5.59
(CLWN201/CML504)DH77	2.60	66.98	2.15	142.90	54.13	0.38	5.54
(CLWN201/CML504)DH78	2.24	68.41	2.47	151.38	58.27	0.38	5.57
(CLWN201/CML504)DH79	1.44	68.19	1.65	142.45	54.13	0.38	5.51
(CLWN201/CML504)DH80	2.37	66.53	2.64	144.91	56.07	0.38	5.58
(CLWN201/CML504)DH81	2.67	64.05	1.76	139.55	52.78	0.38	5.84
(CLWN201/CML504)DH82	2.56	67.17	2.52	146.22	54.88	0.37	5.69
(CLWN201/CML504)DH83	2.55	68.35	2.51	147.91	57.36	0.39	5.22
(CLWN201/CML504)DH84	2.14	67.89	2.40	147.67	56.03	0.38	5.53
(CLWN201/CML504)DH85	3.05	67.42	2.00	144.59	54.73	0.38	5.27
(CLWN201/CML504)DH86	2.71	68.45	1.88	148.72	58.26	0.39	5.43
(CLWN201/CML504)DH87	2.70	68.41	1.64	148.39	57.61	0.39	5.39
(CLWN201/CML504)DH88	2.78	67.23	2.41	146.85	54.47	0.37	5.58
(CLWN201/CML504)DH89	2.46	67.26	2.26	147.24	51.75	0.35	5.16
(CLWN201/CML504)DH90	2.93	66.60	1.94	143.03	56.71	0.39	5.70
(CLWN201/CML504)DH91	2.87	68.23	2.52	148.06	51.87	0.35	5.31
(CLWN201/CML504)DH92	2.99	65.19	2.39	140.24	52.87	0.37	5.42
(CLWN201/CML504)DH93	2.45	67.04	2.02	148.37	54.79	0.37	5.63
(CLWN201/CML504)DH94	2.93	67.30	2.52	146.75	55.29	0.37	5.50
(CLWN201/CML504)DH95	3.21	66.10	2.55	152.63	57.31	0.38	5.49
(CLWN201/CML504)DH96	2.71	68.28	1.75	146.24	56.29	0.38	5.19
(CLWN201/CML504)DH97	2.65	66.49	2.21	144.37	54.93	0.38	5.73
(CLWN201/CML504)DH98	2.31	66.17	2.71	143.58	51.58	0.36	5.62
(CLWN201/CML504)DH99	2.89	68.10	2.15	147.44	53.29	0.36	5.50
(CLWN201/CML504)DH100	2.77	67.61	2.27	145.68	53.32	0.37	5.36
(CLWN201/CML504)DH101	2.87	66.35	2.54	148.26	56.44	0.38	5.75
(CLWN201/CML504)DH102	2.72	66.13	2.46	142.10	53.63	0.38	5.64
(CLWN201/CML504)DH103	2.93	67.14	2.16	149.32	57.98	0.39	5.51
(CLWN201/CML504)DH104	2.76	64.71	2.69	143.17	53.57	0.37	5.98
(CLWN201/CML504)DH105	3.07	66.28	2.54	146.24	56.66	0.39	5.72
(CLWN201/CML504)DH106	3.00	67.00	2.57	153.83	55.91	0.36	5.64
(CLWN201/CML504)DH107	3.25	67.57	2.22	141.19	52.50	0.37	5.47
(CLWN201/CML504)DH108	3.21	67.50	2.22	153.73	60.20	0.39	5.34
(CLWN201/CML504)DH109	2.82	66.10	2.32	143.72	50.71	0.35	5.35
(CLWN201/CML504)DH110	2.80	66.62	1.91	147.01	55.39	0.38	5.84
(CLWN201/CML504)DH111	2.34	66.77	2.27	141.09	53.44	0.38	5.76
(CLWN201/CML504)DH112	2.99	66.97	2.26	144.64	55.33	0.38	5.54
(CLWN201/CML504)DH113	2.43	67.15	2.35	147.87	53.17	0.36	5.48
(CLWN201/CML504)DH114	2.53	64.95	3.16	149.75	53.21	0.36	5.71
(CLWN201/CML504)DH116	2.74	67.06	2.11	143.59	51.27	0.35	5.51
(CLWN201/CML504)DH117	2.62	66.85	2.18	144.60	55.90	0.39	5.63
(CLWN201/CML504)DH118	2.22	67.74	1.62	143.47	51.54	0.36	5.85
(CLWN201/CML504)DH120	2.34	65.82	2.75	147.46	51.88	0.36	5.71
(CLWN201/CML504)DH122	2.65	66.53	2.19	142.59	53.58	0.37	5.37
(CLWN201/CML504)DH123	3.28	65.69	2.45	142.31	52.15	0.37	5.48
(CLWN201/CML504)DH124	2.54	67.64	2.07	147.65	54.44	0.37	5.56
(CLWN201/CML504)DH125	3.01	65.90	2.52	145.72	53.56	0.37	5.65
(CLWN201/CML504)DH126	2.93	67.10	2.33	149.00	55.25	0.37	5.71
(CLWN201/CML504)DH127	2.56	66.37	1.92	138.73	54.10	0.39	5.50
(CLWN201/CML504)DH128	2.49	67.92	1.86	146.00	55.52	0.38	5.50
(CLWN201/CML504)DH130	3.08	64.42	2.26	142.49	52.65	0.37	5.81
(CLWN201/CML504)DH131	2.60	66.93	2.35	139.86	51.46	0.36	5.47
(CLWN201/CML504)DH132	3.16	66.36	2.08	145.55	52.84	0.36	5.78
(CLWN201/CML504)DH133	2.64	67.50	2.19	143.83	54.81	0.38	5.67
(CLWN201/CML504)DH134	2.86	65.61	2.23	154.73	53.90	0.35	5.81
(CLWN201/CML504)DH135	2.37	66.66	2.15	143.79	51.61	0.36	5.40
(CLWN201/CML504)DH136	2.88	67.05	2.46	143.55	52.92	0.37	5.71
(CLWN201/CML504)DH137	2.91	66.75	2.66	149.32	54.90	0.37	5.64
(CLWN201/CML504)DH139	2.37	61.14	3.02	136.79	47.59	0.35	6.03
(CLWN201/CML504)DH141	2.20	65.87	2.55	147.19	57.12	0.39	5.74

Genotype	GYF	AD	ASI	PH	EH	EPO	SEN
(CLWN201/CML504)DH142	2.55	67.56	2.37	150.45	58.02	0.39	5.73
(CLWN201/CML504)DH143	2.50	66.91	2.37	144.36	55.02	0.38	5.42
(CLWN201/CML504)DH146	2.82	66.59	2.50	154.02	57.79	0.38	5.57
(CLWN201/CML504)DH147	3.32	67.08	2.46	150.72	54.53	0.36	5.64
(CLWN201/CML504)DH148	3.23	67.36	2.37	140.56	51.22	0.36	5.57
(CLWN201/CML504)DH150	3.11	67.60	2.10	153.19	58.82	0.38	5.58
(CLWN201/CML504)DH151	2.90	66.60	2.49	144.97	54.68	0.38	5.33
(CLWN201/CML504)DH152	2.15	63.81	2.53	141.20	52.15	0.37	6.33
(CLWN201/CML504)DH154	2.61	67.56	2.38	147.80	53.33	0.36	5.51
(CLWN201/CML504)DH156	2.96	67.25	2.90	149.44	56.77	0.38	5.43
(CLWN201/CML504)DH157	2.85	66.71	1.93	153.51	58.39	0.38	5.80
(CLWN201/CML504)DH158	2.86	65.81	2.75	147.53	52.94	0.36	5.74
(CLWN201/CML504)DH159	2.63	66.37	2.97	152.58	55.66	0.37	5.76
(CLWN201/CML504)DH160	2.64	66.53	2.51	149.00	55.93	0.38	5.70
(CLWN201/CML504)DH161	3.17	65.55	2.23	146.91	55.88	0.38	5.55
(CLWN201/CML504)DH162	2.96	66.01	2.13	143.97	53.54	0.37	5.84
(CLWN201/CML504)DH163	2.66	66.41	2.27	147.19	54.13	0.37	5.84
(CLWN201/CML504)DH164	2.80	65.13	2.66	145.00	54.39	0.38	5.77
(CLWN201/CML504)DH165	3.25	64.45	1.92	145.34	51.59	0.36	5.63
(CLWN201/CML504)DH166	2.79	66.16	2.12	149.26	57.49	0.38	5.76
(CLWN201/CML504)DH168	2.97	68.19	2.07	153.69	57.73	0.38	5.50
(CLWN201/CML504)DH169	2.52	67.65	1.84	143.88	57.88	0.40	5.66
(CLWN201/CML504)DH170	3.14	67.05	2.92	147.12	51.92	0.35	5.32
(CLWN201/CML504)DH171	2.83	66.10	2.72	147.43	55.38	0.38	5.62
(CLWN201/CML504)DH172	2.57	66.61	2.36	141.61	53.60	0.38	5.40
(CLWN201/CML504)DH173	2.56	66.60	2.54	142.43	51.74	0.36	5.77
(CLWN201/CML504)DH174	2.25	66.92	1.60	148.02	54.21	0.37	5.60
(CLWN201/CML504)DH175	2.99	67.00	2.44	147.10	54.53	0.37	5.35
(CLWN201/CML504)DH176	3.42	65.98	2.69	149.88	58.06	0.39	5.63
(CLWN201/CML504)DH177	2.53	66.24	2.16	146.41	54.22	0.37	5.67
(CLWN201/CML504)DH178	2.71	66.11	2.36	148.37	54.40	0.37	5.62
(CLWN201/CML504)DH179	3.12	65.86	2.36	146.61	56.80	0.38	5.55
(CLWN201/CML504)DH180	2.67	66.08	1.90	143.98	52.96	0.37	5.58
(CLWN201/CML504)DH182	3.09	65.85	2.69	147.15	53.41	0.37	5.61
(CLWN201/CML504)DH183	2.62	65.43	1.78	141.90	54.56	0.38	5.60
(CLWN201/CML504)DH184	2.72	65.73	2.52	138.51	50.10	0.36	5.57
(CLWN201/CML504)DH185	2.70	65.88	2.17	149.28	53.60	0.36	5.67
(CLWN201/CML504)DH186	2.97	67.27	2.49	147.86	54.96	0.37	5.60
(CLWN201/CML504)DH187	2.98	65.24	2.66	143.90	51.89	0.36	5.54
(CLWN201/CML504)DH188	2.79	68.78	2.30	155.79	56.83	0.37	5.39
(CLWN201/CML504)DH189	2.47	66.04	2.17	144.31	55.62	0.38	5.72
(CLWN201/CML504)DH191	2.89	65.49	2.84	145.80	51.92	0.36	5.60
(CLWN201/CML504)DH195	3.02	65.32	2.51	140.16	54.28	0.39	5.70
(CLWN201/CML504)DH197	2.60	65.47	2.16	147.37	53.76	0.36	5.88
(CLWN201/CML504)DH198	2.59	67.43	2.20	148.73	53.59	0.36	5.43
(CLWN201/CML504)DH199	2.68	65.34	1.98	150.01	54.70	0.36	5.77
(CLWN201/CML504)DH200	3.44	66.84	2.51	148.26	55.03	0.37	5.29
(CLWN201/CML504)DH201	2.21	67.11	2.19	153.91	58.03	0.38	5.55
(CLWN201/CML504)DH202	2.38	64.99	2.12	140.70	52.29	0.37	5.95
(CLWN201/CML504)DH204	2.96	65.93	2.55	150.35	56.16	0.38	5.73
(CLWN201/CML504)DH205	2.69	67.09	2.31	147.74	53.84	0.36	5.57
(CLWN201/CML504)DH206	2.45	65.35	2.07	139.56	52.29	0.37	5.65
(CLWN201/CML504)DH207	2.34	65.82	2.15	146.07	52.48	0.36	5.76
(CLWN201/CML504)DH208	2.88	64.86	2.47	146.48	54.31	0.37	5.36
(CLWN201/CML504)DH211	2.47	64.83	1.86	137.49	48.05	0.35	5.79
(CLWN201/CML504)DH212	2.36	66.34	2.34	150.18	54.63	0.36	5.78
(CLWN201/CML504)DH213	2.50	66.67	2.08	147.04	54.64	0.37	5.91
(CLWN201/CML504)DH215	2.89	66.89	2.17	147.29	55.47	0.38	5.54
(CLWN201/CML504)DH216	2.65	65.99	1.83	146.04	53.67	0.37	5.66
(CLWN201/CML504)DH217	3.27	67.67	2.12	147.92	56.10	0.38	5.09
(CLWN201/CML504)DH218	2.55	68.73	2.03	150.33	56.84	0.38	5.39
(CLWN201/CML504)DH220	2.97	66.22	2.67	143.44	54.07	0.37	5.55
(CLWN201/CML504)DH221	3.18	66.74	2.78	152.19	56.87	0.38	5.63
(CLWN201/CML504)DH225	2.67	66.83	2.74	147.56	54.74	0.37	5.53
(CLWN201/CML504)DH226	2.55	66.62	2.34	140.35	50.56	0.36	5.68
(CLWN201/CML504)DH227	2.91	67.06	2.78	149.67	53.14	0.36	5.50
(CLWN201/CML504)DH228	2.70	65.78	2.39	152.41	58.91	0.39	5.85
(CLWN201/CML504)DH229	2.30	66.86	2.18	147.96	56.13	0.38	5.47
(CLWN201/CML504)DH231	3.00	66.03	1.97	145.50	52.81	0.36	5.67
(CLWN201/CML504)DH234	2.28	65.23	2.66	140.27	52.14	0.37	5.79

Genotype	GYF	AD	ASI	PH	EH	EPO	SEN
(CLWN201/CML504)DH235	3.14	67.17	2.36	149.60	57.75	0.38	5.66
DK8031	2.26	66.39	2.46	150.17	58.10	0.39	6.03
PAN53	3.42	68.31	2.23	156.45	61.15	0.39	5.44
WH403	2.93	68.84	2.03	146.59	56.41	0.38	5.52
WH507	3.34	68.27	1.77	153.48	59.79	0.39	5.47
Locations	**2**	**2**	**2**	**2**	**2**	**2**	**2**
Replications	**3**	**3**	**3**	**3**	**3**	**3**	**3**
Error Variance	**0.45**	**1.99**	**1.07**	**68.56**	**33.51**	**0.00**	**0.34**
Genotypic Variance	**0.20**	**1.55**	**0.22**	**25.72**	**8.98**	**0.00**	**0.07**
GenxEnv Variance	**0.01**	**0.00**	**0.00**	**1.71**	**0.00**	**0.00**	**0.00**
Location Variance	**0.13**	**0.44**	**0.00**	**29.02**	**1.12**	**0.00**	**0.08**
Heritability	**0.71**	**0.82**	**0.55**	**0.68**	**0.62**	**0.58**	**0.55**
Grand Mean	**2.65**	**66.80**	**2.22**	**146.05**	**54.56**	**0.37**	**5.58**
LSD	**1.32**	**2.77**	**2.02**	**16.23**	**11.35**	**0.06**	**1.14**
CV	**25.30**	**2.11**	**46.60**	**5.67**	**10.61**	**7.60**	**10.44**

GY, Grain yield; AD, Anthesis date; ASI, Anthesis silking interval; PH, Plant height; EH, ear height; EPO, ear position; SEN, leaf scenescence; LSD, least significant difference; CV, coefficient of variation; GenxEnv; genotype by environment interaction

Appendix Table 7. Mean GY, AD, ASI, PH, EH, EPO and SEN of 213 doubled haploid test cross progenies (CML504/CML550) and three commercial checks evaluated in five optimum sites in Kenya (Kiboko, Kitale and Kakamega) during the main season of 2014.

Genotype	GYF	AD	ASI	PH	EH	EPO	SEN
(CLWN201/CML504)DH1	6.92	71.56	1.55	232.67	124.40	0.53	2.92
(CLWN201/CML504)DH2	6.68	73.66	1.38	228.04	121.52	0.53	3.02
(CLWN201/CML504)DH3	7.08	73.17	1.54	232.35	126.00	0.53	2.92
(CLWN201/CML504)DH4	6.95	73.45	1.13	238.91	129.89	0.54	3.09
(CLWN201/CML504)DH5	7.15	73.47	1.55	239.33	124.42	0.52	2.91
(CLWN201/CML504)DH6	7.09	73.29	1.19	240.31	122.90	0.51	2.99
(CLWN201/CML504)DH7	6.39	74.06	1.44	232.61	120.68	0.52	3.13
(CLWN201/CML504)DH8	7.25	73.47	2.08	245.85	130.28	0.53	3.04
(CLWN201/CML504)DH9	6.54	73.10	1.38	225.88	113.40	0.51	2.95
(CLWN201/CML504)DH10	6.46	73.46	1.41	237.56	123.11	0.52	2.97
(CLWN201/CML504)DH11	5.94	72.19	1.32	227.89	115.84	0.51	3.00
(CLWN201/CML504)DH12	7.07	73.10	1.38	236.90	124.10	0.52	2.90
(CLWN201/CML504)DH13	5.16	74.43	1.34	238.28	126.80	0.53	2.87
(CLWN201/CML504)DH14	5.99	75.01	1.66	230.30	119.30	0.52	2.97
(CLWN201/CML504)DH15	6.51	71.54	1.79	241.13	125.14	0.52	2.81
(CLWN201/CML504)DH16	6.26	71.14	1.51	229.52	122.76	0.53	2.95
(CLWN201/CML504)DH17	6.72	71.07	1.37	229.91	117.94	0.51	2.79
(CLWN201/CML504)DH18	6.96	72.36	1.45	245.27	125.57	0.52	2.96
(CLWN201/CML504)DH19	6.07	73.17	1.27	228.33	118.91	0.52	3.10
(CLWN201/CML504)DH20	7.19	72.35	1.47	237.79	121.67	0.52	3.06
(CLWN201/CML504)DH21	6.68	71.89	1.45	238.29	129.37	0.54	2.99
(CLWN201/CML504)DH22	6.26	72.16	1.44	227.22	120.50	0.53	2.97
(CLWN201/CML504)DH23	6.23	72.79	1.36	229.49	119.40	0.52	3.12
(CLWN201/CML504)DH24	6.30	73.57	1.69	227.96	116.77	0.52	3.01
(CLWN201/CML504)DH25	6.30	72.02	1.35	228.55	118.20	0.52	2.94
(CLWN201/CML504)DH26	6.25	71.75	1.36	234.22	122.22	0.52	3.09
(CLWN201/CML504)DH27	5.93	72.53	1.47	239.09	123.36	0.52	2.90
(CLWN201/CML504)DH28	6.71	72.16	1.46	238.68	128.06	0.53	2.94
(CLWN201/CML504)DH29	6.19	72.39	1.95	237.86	123.53	0.52	3.00
(CLWN201/CML504)DH30	7.24	73.11	1.46	247.41	127.43	0.52	3.13
(CLWN201/CML504)DH31	5.82	71.03	1.67	230.92	114.30	0.50	3.01
(CLWN201/CML504)DH32	6.56	72.14	1.49	236.05	124.81	0.53	2.95
(CLWN201/CML504)DH33	7.02	72.38	1.33	238.84	127.10	0.53	2.94
(CLWN201/CML504)DH34	6.56	71.96	1.51	229.17	121.07	0.53	2.93
(CLWN201/CML504)DH35	6.88	73.18	1.60	240.78	129.34	0.54	2.83
(CLWN201/CML504)DH37	6.50	74.06	1.73	238.86	122.00	0.51	2.98
(CLWN201/CML504)DH38	5.57	70.68	1.60	228.10	119.09	0.52	3.12
(CLWN201/CML504)DH39	7.14	72.67	1.39	239.38	126.70	0.53	2.99
(CLWN201/CML504)DH40	6.23	72.11	1.32	235.36	123.17	0.53	3.00
(CLWN201/CML504)DH41	6.82	71.96	1.39	232.58	123.81	0.53	3.00
(CLWN201/CML504)DH42	6.66	75.41	1.47	237.49	127.01	0.53	2.99
(CLWN201/CML504)DH43	6.53	72.10	1.34	238.93	125.55	0.52	2.89
(CLWN201/CML504)DH44	6.55	73.87	1.12	237.72	125.55	0.52	3.12
(CLWN201/CML504)DH45	6.67	71.27	1.40	238.68	124.12	0.52	2.95
(CLWN201/CML504)DH46	7.12	71.13	1.13	230.60	119.71	0.52	2.92
(CLWN201/CML504)DH47	6.23	73.13	1.44	239.90	122.58	0.51	2.89
(CLWN201/CML504)DH48	6.79	71.57	1.48	227.38	120.74	0.53	2.97
(CLWN201/CML504)DH49	6.26	72.92	1.67	232.22	127.68	0.54	2.99
(CLWN201/CML504)DH50	6.24	73.59	1.25	232.72	123.13	0.53	3.06
(CLWN201/CML504)DH51	7.46	72.55	1.61	242.27	130.14	0.53	2.98
(CLWN201/CML504)DH52	6.93	72.58	1.73	239.72	123.19	0.51	2.92
(CLWN201/CML504)DH53	6.60	74.68	1.38	238.00	128.17	0.54	2.92
(CLWN201/CML504)DH54	6.61	75.23	1.37	241.57	130.15	0.53	3.09
(CLWN201/CML504)DH55	5.84	74.17	1.53	224.57	116.66	0.52	2.91
(CLWN201/CML504)DH56	5.86	75.27	1.61	228.75	116.77	0.51	3.03
(CLWN201/CML504)DH57	6.74	72.52	1.25	232.42	121.24	0.52	2.95
(CLWN201/CML504)DH58	6.40	73.57	1.57	236.61	124.28	0.52	3.02
(CLWN201/CML504)DH59	6.01	73.25	1.45	233.20	119.18	0.51	2.97
(CLWN201/CML504)DH60	6.86	73.36	1.42	236.75	120.49	0.51	3.02
(CLWN201/CML504)DH61	6.03	72.07	1.39	234.56	125.48	0.53	3.15
(CLWN201/CML504)DH63	6.15	71.90	1.40	225.01	113.57	0.51	2.93
(CLWN201/CML504)DH64	7.43	71.70	1.26	240.01	126.52	0.53	3.01

Genotype	GYF	AD	ASI	PH	EH	EPO	SEN
(CLWN201/CML504)DH65	6.61	72.63	1.46	239.38	125.44	0.52	2.68
(CLWN201/CML504)DH66	6.57	72.88	1.52	234.72	122.21	0.52	3.04
(CLWN201/CML504)DH67	6.41	73.65	1.34	229.13	121.87	0.53	2.93
(CLWN201/CML504)DH68	6.23	72.17	1.46	232.48	119.22	0.51	3.07
(CLWN201/CML504)DH69	6.50	73.47	1.50	242.26	127.30	0.53	3.01
(CLWN201/CML504)DH70	7.07	72.51	2.01	236.90	126.24	0.53	3.08
(CLWN201/CML504)DH71	7.59	73.91	1.85	246.73	129.17	0.52	3.03
(CLWN201/CML504)DH72	6.92	73.52	1.48	228.27	123.48	0.54	2.87
(CLWN201/CML504)DH73	6.90	74.23	1.04	232.54	123.16	0.53	2.82
(CLWN201/CML504)DH74	6.46	72.41	1.86	236.77	123.44	0.52	2.95
(CLWN201/CML504)DH75	7.04	71.90	1.86	239.37	128.93	0.54	3.13
(CLWN201/CML504)DH76	6.31	72.07	1.66	226.01	118.02	0.52	2.95
(CLWN201/CML504)DH77	6.37	71.78	1.32	227.66	120.91	0.53	2.95
(CLWN201/CML504)DH78	6.16	74.70	1.51	243.29	128.81	0.53	3.26
(CLWN201/CML504)DH79	6.12	71.80	1.95	226.79	119.13	0.53	2.84
(CLWN201/CML504)DH80	6.52	71.64	1.50	234.43	121.78	0.52	3.01
(CLWN201/CML504)DH81	6.40	69.79	1.43	222.11	112.01	0.51	2.91
(CLWN201/CML504)DH82	7.55	73.59	1.75	245.88	133.70	0.54	3.04
(CLWN201/CML504)DH83	7.11	74.82	1.72	242.86	130.96	0.54	2.99
(CLWN201/CML504)DH84	6.49	71.23	1.87	247.25	132.24	0.53	3.03
(CLWN201/CML504)DH85	7.04	72.63	1.59	232.63	119.87	0.52	2.97
(CLWN201/CML504)DH86	6.47	75.06	1.70	245.19	129.39	0.53	3.16
(CLWN201/CML504)DH87	6.58	73.55	2.05	234.50	122.65	0.52	2.94
(CLWN201/CML504)DH88	6.58	73.06	1.67	234.68	120.21	0.51	3.00
(CLWN201/CML504)DH89	6.27	72.37	1.66	234.76	117.06	0.50	2.87
(CLWN201/CML504)DH90	6.47	73.88	1.33	228.49	123.93	0.54	2.93
(CLWN201/CML504)DH91	6.97	73.56	1.74	243.74	124.13	0.51	3.10
(CLWN201/CML504)DH92	7.03	73.01	1.87	237.42	122.48	0.52	3.00
(CLWN201/CML504)DH93	5.98	72.90	1.72	231.64	122.47	0.53	3.06
(CLWN201/CML504)DH94	6.50	71.59	1.47	231.06	123.58	0.53	2.78
(CLWN201/CML504)DH95	7.21	72.67	1.34	240.73	124.17	0.51	2.97
(CLWN201/CML504)DH96	6.19	73.26	1.38	236.02	125.62	0.53	3.09
(CLWN201/CML504)DH97	6.99	70.74	1.32	238.64	121.32	0.51	2.94
(CLWN201/CML504)DH98	6.35	73.13	1.72	232.99	116.77	0.51	3.12
(CLWN201/CML504)DH99	6.87	73.48	1.31	239.62	124.62	0.52	2.90
(CLWN201/CML504)DH100	6.77	72.72	1.52	233.91	121.55	0.52	2.95
(CLWN201/CML504)DH101	7.25	72.50	1.19	249.05	130.50	0.52	3.03
(CLWN201/CML504)DH102	6.62	71.96	1.39	226.82	123.15	0.54	2.81
(CLWN201/CML504)DH103	6.92	74.41	1.55	239.19	127.71	0.53	2.98
(CLWN201/CML504)DH104	6.86	71.08	1.60	232.60	123.13	0.53	2.80
(CLWN201/CML504)DH105	6.49	72.90	1.60	232.74	123.13	0.53	2.99
(CLWN201/CML504)DH106	7.24	72.86	2.30	245.27	128.62	0.52	3.03
(CLWN201/CML504)DH107	6.64	73.71	1.31	232.90	120.59	0.52	3.05
(CLWN201/CML504)DH108	7.36	75.33	1.17	236.39	124.35	0.53	2.93
(CLWN201/CML504)DH109	6.40	72.78	1.66	234.00	120.84	0.52	2.86
(CLWN201/CML504)DH110	7.15	71.94	1.57	237.09	123.07	0.52	2.99
(CLWN201/CML504)DH111	6.75	72.53	1.53	231.94	120.56	0.52	2.85
(CLWN201/CML504)DH112	6.57	72.47	1.25	230.08	124.10	0.55	2.94
(CLWN201/CML504)DH113	6.63	72.96	1.71	237.97	120.88	0.51	3.05
(CLWN201/CML504)DH114	6.18	73.25	1.20	247.33	129.01	0.52	3.03
(CLWN201/CML504)DH115	5.65	69.77	1.24	226.05	109.46	0.49	3.05
(CLWN201/CML504)DH116	6.20	73.32	1.60	230.52	120.51	0.52	2.88
(CLWN201/CML504)DH117	6.30	73.87	1.47	240.50	134.00	0.55	3.06
(CLWN201/CML504)DH118	6.50	73.07	1.20	237.90	124.84	0.52	3.00
(CLWN201/CML504)DH119	6.81	72.08	1.29	241.16	123.24	0.51	2.83
(CLWN201/CML504)DH120	6.25	71.16	1.17	236.51	119.64	0.51	3.00
(CLWN201/CML504)DH121	6.27	72.44	1.42	228.86	120.30	0.52	2.88
(CLWN201/CML504)DH122	6.20	72.11	1.61	232.82	119.78	0.51	2.88
(CLWN201/CML504)DH124	6.91	72.16	1.48	237.19	122.63	0.52	2.93
(CLWN201/CML504)DH125	6.58	72.33	1.41	229.25	122.65	0.53	2.91
(CLWN201/CML504)DH126	6.53	73.85	1.39	237.32	124.24	0.52	3.03
(CLWN201/CML504)DH127	6.19	71.79	1.02	221.98	115.50	0.52	2.78
(CLWN201/CML504)DH128	6.50	73.46	1.26	239.48	123.30	0.52	3.02
(CLWN201/CML504)DH129	6.38	72.45	1.39	240.01	125.75	0.52	3.12
(CLWN201/CML504)DH130	6.57	71.39	1.31	232.09	117.86	0.51	2.95
(CLWN201/CML504)DH131	6.06	72.94	1.60	223.33	114.09	0.51	2.97

Genotype	GYF	AD	ASI	PH	EH	EPO	SEN
(CLWN201/CML504)DH132	6.92	73.03	1.58	231.68	120.51	0.52	3.13
(CLWN201/CML504)DH133	7.20	73.21	1.29	237.76	127.79	0.54	2.98
(CLWN201/CML504)DH134	7.03	73.65	1.89	248.86	123.22	0.50	3.03
(CLWN201/CML504)DH135	6.71	72.02	1.27	234.20	121.95	0.52	2.97
(CLWN201/CML504)DH136	7.06	73.82	1.48	231.97	120.06	0.52	2.88
(CLWN201/CML504)DH137	6.69	72.85	1.65	235.26	124.21	0.53	2.89
(CLWN201/CML504)DH139	5.57	68.05	1.32	222.80	111.18	0.50	2.90
(CLWN201/CML504)DH141	6.21	71.54	1.66	237.33	125.83	0.53	3.00
(CLWN201/CML504)DH142	8.05	72.43	1.45	245.23	130.39	0.53	2.86
(CLWN201/CML504)DH143	6.51	72.83	1.27	232.37	127.05	0.54	3.00
(CLWN201/CML504)DH146	6.70	73.41	1.54	244.34	126.60	0.52	3.22
(CLWN201/CML504)DH147	6.93	73.43	2.06	241.33	121.11	0.51	3.10
(CLWN201/CML504)DH148	6.97	72.01	1.17	234.66	124.02	0.53	2.99
(CLWN201/CML504)DH149	7.15	74.63	1.59	241.08	127.46	0.53	3.01
(CLWN201/CML504)DH151	6.69	71.35	1.92	228.90	119.93	0.53	3.03
(CLWN201/CML504)DH152	5.41	68.47	1.42	220.95	111.81	0.51	3.21
(CLWN201/CML504)DH154	6.52	74.15	1.82	239.67	125.50	0.52	3.05
(CLWN201/CML504)DH156	6.87	72.61	1.65	241.85	125.82	0.52	2.99
(CLWN201/CML504)DH157	6.86	73.52	1.02	243.10	131.62	0.54	3.13
(CLWN201/CML504)DH158	7.19	72.31	1.72	239.61	126.24	0.53	2.90
(CLWN201/CML504)DH159	7.31	72.75	1.81	244.35	125.69	0.52	2.99
(CLWN201/CML504)DH160	6.76	72.52	1.45	237.98	125.35	0.53	3.20
(CLWN201/CML504)DH161	6.68	72.30	1.25	235.13	123.30	0.52	2.98
(CLWN201/CML504)DH162	6.66	72.41	1.53	236.65	122.83	0.52	2.98
(CLWN201/CML504)DH163	7.68	72.65	1.59	239.18	124.78	0.52	3.02
(CLWN201/CML504)DH164	6.68	72.12	1.81	241.58	125.76	0.52	3.01
(CLWN201/CML504)DH165	6.52	70.82	1.14	231.64	117.19	0.51	2.83
(CLWN201/CML504)DH166	7.43	72.45	1.30	240.72	125.35	0.52	2.85
(CLWN201/CML504)DH167	6.81	72.44	1.29	236.98	126.24	0.53	3.12
(CLWN201/CML504)DH168	6.53	73.43	1.42	234.08	123.26	0.53	2.85
(CLWN201/CML504)DH169	7.01	72.96	1.60	236.38	128.56	0.54	3.04
(CLWN201/CML504)DH170	6.20	74.61	1.59	240.85	122.01	0.51	3.11
(CLWN201/CML504)DH171	6.85	72.95	1.72	227.93	120.59	0.53	3.05
(CLWN201/CML504)DH172	6.48	71.97	1.19	230.00	124.58	0.54	2.89
(CLWN201/CML504)DH173	6.71	73.15	1.78	230.87	118.99	0.51	2.91
(CLWN201/CML504)DH174	6.04	73.84	1.47	242.45	122.26	0.51	3.13
(CLWN201/CML504)DH175	6.59	72.82	1.79	229.34	119.54	0.52	2.94
(CLWN201/CML504)DH176	7.29	74.08	1.30	238.95	129.24	0.53	2.93
(CLWN201/CML504)DH177	5.79	72.66	1.30	220.67	113.62	0.51	3.09
(CLWN201/CML504)DH178	6.24	72.50	1.40	229.73	118.36	0.52	2.89
(CLWN201/CML504)DH179	6.33	72.32	1.32	234.51	122.24	0.52	2.85
(CLWN201/CML504)DH180	5.73	71.29	1.56	227.54	118.83	0.52	2.91
(CLWN201/CML504)DH181	6.83	73.00	1.92	240.10	128.88	0.53	3.00
(CLWN201/CML504)DH182	6.69	71.30	1.28	235.80	119.76	0.51	2.99
(CLWN201/CML504)DH183	5.89	71.28	1.55	229.25	118.53	0.52	3.03
(CLWN201/CML504)DH184	6.30	72.05	1.32	227.80	116.75	0.51	2.95
(CLWN201/CML504)DH185	6.52	70.36	1.64	238.61	124.08	0.52	2.99
(CLWN201/CML504)DH186	7.34	73.78	1.86	243.07	129.32	0.53	2.92
(CLWN201/CML504)DH187	6.79	71.79	1.60	247.13	128.30	0.52	3.12
(CLWN201/CML504)DH188	6.82	74.01	2.16	236.34	120.99	0.51	3.09
(CLWN201/CML504)DH189	6.58	71.23	1.58	234.76	126.09	0.53	2.88
(CLWN201/CML504)DH194	6.11	72.90	1.18	240.10	125.62	0.52	3.10
(CLWN201/CML504)DH195	6.39	70.86	1.27	226.70	116.95	0.52	2.86
(CLWN201/CML504)DH196	6.60	74.13	1.49	236.57	117.93	0.50	3.07
(CLWN201/CML504)DH197	6.13	71.48	1.26	240.69	126.69	0.53	3.08
(CLWN201/CML504)DH198	6.72	72.02	1.67	237.98	122.32	0.52	2.89
(CLWN201/CML504)DH199	6.66	71.40	1.20	241.61	124.66	0.52	2.92
(CLWN201/CML504)DH200	7.05	72.99	2.00	229.90	118.51	0.52	2.84
(CLWN201/CML504)DH201	6.79	73.26	1.54	247.79	129.85	0.52	3.03
(CLWN201/CML504)DH202	6.63	70.70	1.39	236.04	126.58	0.53	3.12
(CLWN201/CML504)DH204	6.72	73.15	1.32	247.24	125.98	0.51	3.07
(CLWN201/CML504)DH205	6.74	71.89	1.49	239.38	123.29	0.52	3.00
(CLWN201/CML504)DH206	5.65	72.73	1.41	222.66	113.45	0.51	2.96
(CLWN201/CML504)DH207	6.03	71.32	1.32	234.45	119.10	0.51	3.00
(CLWN201/CML504)DH210	5.05	73.55	1.06	222.11	108.45	0.50	2.95
(CLWN201/CML504)DH211	6.09	70.57	1.48	228.07	115.00	0.51	2.82

Genotype	GYF	AD	ASI	PH	EH	EPO	SEN
(CLWN201/CML504)DH212	6.62	72.75	1.66	238.32	121.01	0.51	2.96
(CLWN201/CML504)DH213	6.98	72.58	1.24	238.44	128.97	0.53	3.06
(CLWN201/CML504)DH214	6.11	71.50	1.43	237.72	122.40	0.52	2.89
(CLWN201/CML504)DH215	6.59	73.85	1.57	231.53	121.15	0.52	2.91
(CLWN201/CML504)DH216	6.60	70.58	1.01	239.25	124.80	0.52	3.09
(CLWN201/CML504)DH217	6.05	74.05	1.44	231.21	120.39	0.52	2.86
(CLWN201/CML504)DH218	7.04	73.58	1.53	244.35	132.93	0.54	3.01
(CLWN201/CML504)DH220	6.35	73.82	1.70	231.22	121.45	0.52	2.86
(CLWN201/CML504)DH221	6.72	73.70	1.59	238.90	123.90	0.52	3.27
(CLWN201/CML504)DH224	7.38	72.22	1.54	240.90	128.14	0.53	2.92
(CLWN201/CML504)DH226	5.93	72.02	1.48	229.98	118.51	0.52	2.88
(CLWN201/CML504)DH227	6.83	73.20	1.19	248.20	129.38	0.52	3.01
(CLWN201/CML504)DH228	6.70	72.58	1.76	240.52	128.49	0.53	3.09
(CLWN201/CML504)DH229	6.73	73.51	1.21	235.70	123.12	0.52	3.00
(CLWN201/CML504)DH231	7.11	72.09	1.69	233.53	122.89	0.53	2.81
(CLWN201/CML504)DH233	6.59	75.81	1.36	241.29	123.73	0.52	2.94
(CLWN201/CML504)DH234	5.98	70.50	1.45	233.21	120.45	0.51	2.99
(CLWN201/CML504)DH235	7.34	71.37	1.88	235.99	127.41	0.53	2.97
CZH0616	7.22	70.72	1.41	231.46	123.90	0.53	2.65
PAN53	6.74	74.09	1.48	243.05	125.73	0.52	2.91
WH507	6.42	76.17	1.21	236.88	128.85	0.54	3.01
Locations	**5**	**5**	**5**	**5**	**5**	**5**	**5**
Replications	**2**	**2**	**2**	**2**	**2**	**2**	**2**
Error Variance	**1.33**	**4.95**	**1.89**	**148.55**	**90.46**	**0.00**	**0.19**
Genotypic Variance	**0.34**	**1.94**	**0.13**	**50.57**	**28.55**	**0.00**	**0.03**
GenxEnv Variance	**0.24**	**0.46**	**0.00**	**0.00**	**0.00**	**0.00**	**0.03**
Location Variance	**5.32**	**111.57**	**0.56**	**384.13**	**172.29**	**0.00**	**0.51**
Heritability	**0.65**	**0.77**	**0.41**	**0.77**	**0.76**	**0.59**	**0.51**
Grand Mean	**6.60**	**72.68**	**1.49**	**235.59**	**123.10**	**0.52**	**2.98**
LSD	**2.26**	**4.36**	**2.70**	**23.89**	**18.64**	**0.06**	**0.86**
CV	**17.50**	**3.06**	**92.18**	**5.17**	**7.73**	**5.92**	**14.78**

GY, Grain yield; AD, Anthesis date; ASI, Anthesis silking interval; PH, Plant height; EH, ear height; EPO, ear position; SEN, leaf scenescence; LSD, least significant difference; CV, coefficient of variation; GenxEnv; genotype by environment interaction

Appendix Table 8. Mean GY, AD, ASI, PH, EH, EPO and SEN of 107 doubled haploid test cross progenies (CML504/CML550) and three commercial checks evaluated in three low N sites in Kenya (Alupe, Kakamega and Mtwapa) during the main season of 2014.

Genotype	GYF	AD	ASI	PH	EH	EPO	SEN
(CLWN201/CML511)DH1	2.73	67.79	2.27	168.52	72.51	0.41	3.07
(CLWN201/CML511)DH2	3.06	66.91	1.49	175.85	75.24	0.41	3.07
(CLWN201/CML511)DH3	2.62	68.58	1.81	166.26	66.07	0.38	2.98
(CLWN201/CML511)DH4	2.61	66.69	1.86	167.63	66.13	0.39	3.08
(CLWN201/CML511)DH5	2.50	67.50	1.89	173.06	71.22	0.40	2.96
(CLWN201/CML511)DH6	2.60	67.35	1.41	173.21	69.85	0.39	3.11
(CLWN201/CML511)DH7	2.54	67.84	1.98	170.15	68.95	0.39	3.10
(CLWN201/CML511)DH8	2.71	66.73	1.98	173.75	74.50	0.41	3.06
(CLWN201/CML511)DH9	3.01	67.83	2.00	177.65	77.19	0.42	3.11
(CLWN201/CML511)DH10	2.67	65.80	1.85	169.33	69.68	0.40	3.03
(CLWN201/CML511)DH11	2.74	68.09	1.96	172.00	70.11	0.40	3.03
(CLWN201/CML511)DH12	2.48	67.73	2.31	170.46	70.27	0.40	3.14
(CLWN201/CML511)DH13	2.92	67.61	1.90	169.38	72.44	0.42	3.01
(CLWN201/CML511)DH14	3.10	67.36	2.53	173.19	72.20	0.40	3.13
(CLWN201/CML511)DH15	2.34	67.73	2.10	164.84	68.35	0.40	3.11
(CLWN201/CML511)DH16	2.76	66.11	2.27	172.94	73.68	0.41	3.10
(CLWN201/CML511)DH17	2.96	65.36	1.98	169.37	76.92	0.43	3.10
(CLWN201/CML511)DH18	2.59	67.42	2.16	166.38	67.36	0.39	3.05
(CLWN201/CML511)DH19	2.94	66.77	1.66	167.88	65.03	0.38	3.01
(CLWN201/CML511)DH20	2.37	63.27	1.39	152.96	56.40	0.36	3.12
(CLWN201/CML511)DH21	2.68	67.40	1.54	176.66	74.19	0.41	3.10
(CLWN201/CML511)DH23	2.71	66.58	2.20	166.96	64.54	0.38	3.06
(CLWN201/CML511)DH24	2.72	68.43	1.79	174.34	71.22	0.39	3.09
(CLWN201/CML511)DH25	2.57	67.72	1.54	164.62	68.39	0.40	3.03
(CLWN201/CML511)DH26	2.69	67.93	1.60	165.95	68.13	0.39	3.12
(CLWN201/CML511)DH27	2.72	68.10	1.78	169.96	71.36	0.41	3.11
(CLWN201/CML511)DH28	2.53	66.10	2.10	173.50	70.27	0.39	3.09
(CLWN201/CML511)DH29	2.28	68.86	1.76	171.32	69.50	0.39	3.00
(CLWN201/CML511)DH30	2.42	66.57	1.83	167.49	67.69	0.39	3.07
(CLWN201/CML511)DH31	2.65	66.52	2.09	177.37	75.97	0.41	3.08
(CLWN201/CML511)DH32	2.74	67.59	1.90	167.90	70.54	0.41	3.03
(CLWN201/CML511)DH33	3.00	66.77	2.15	173.64	73.59	0.41	2.98
(CLWN201/CML511)DH34	2.79	66.50	1.43	164.71	65.68	0.39	3.03
(CLWN201/CML511)DH35	2.42	67.80	1.82	164.09	66.94	0.40	3.08
(CLWN201/CML511)DH36	2.63	67.46	1.87	173.26	71.76	0.40	3.06
(CLWN201/CML511)DH37	2.54	68.65	2.09	170.73	72.57	0.40	3.03
(CLWN201/CML511)DH38	2.88	67.47	2.13	171.55	70.76	0.40	3.02
(CLWN201/CML511)DH39	2.46	66.99	2.47	170.71	72.30	0.40	3.13
(CLWN201/CML511)DH40	2.62	69.78	1.65	171.78	72.94	0.41	3.02
(CLWN201/CML511)DH42	2.51	68.58	1.38	172.19	75.60	0.42	3.12
(CLWN201/CML511)DH43	2.92	66.81	1.49	173.43	70.23	0.40	3.10
(CLWN201/CML511)DH44	2.73	66.16	1.78	165.59	69.53	0.41	3.13
(CLWN201/CML511)DH45	3.01	67.23	1.68	175.00	73.91	0.41	3.12
(CLWN201/CML511)DH46	2.79	66.35	2.21	170.15	69.84	0.40	3.07
(CLWN201/CML511)DH47	2.72	67.85	1.83	173.95	72.93	0.41	3.01
(CLWN201/CML511)DH48	2.01	70.23	2.27	167.70	68.82	0.39	3.01
(CLWN201/CML511)DH49	2.44	66.60	2.01	163.72	65.38	0.39	3.08
(CLWN201/CML511)DH51	2.34	65.52	2.46	168.17	69.55	0.40	3.11
(CLWN201/CML511)DH52	2.69	69.19	2.26	169.71	70.81	0.41	3.14
(CLWN201/CML511)DH53	2.60	69.74	1.85	178.30	77.06	0.40	3.09
(CLWN201/CML511)DH54	2.29	68.71	1.83	171.19	72.27	0.41	3.13
(CLWN201/CML511)DH55	2.43	67.57	2.24	166.72	71.62	0.41	3.04
(CLWN201/CML511)DH56	2.28	68.33	2.16	171.74	70.64	0.40	3.11
(CLWN201/CML511)DH57	2.43	68.53	1.96	170.20	70.01	0.39	3.07
(CLWN201/CML511)DH58	2.67	68.09	1.37	169.80	75.87	0.42	3.06
(CLWN201/CML511)DH59	2.32	70.36	1.92	166.14	71.96	0.41	2.90
(CLWN201/CML511)DH60	2.71	68.30	1.84	170.89	70.34	0.40	3.05
(CLWN201/CML511)DH61	2.33	66.01	1.54	171.52	70.90	0.40	3.10
(CLWN201/CML511)DH62	2.84	66.79	2.42	167.70	67.24	0.39	3.03
(CLWN201/CML511)DH63	2.67	67.70	1.99	162.58	66.12	0.39	3.05
(CLWN201/CML511)DH64	2.59	68.06	1.96	171.84	69.88	0.40	3.08

Genotype	GYF	AD	ASI	PH	EH	EPO	SEN
(CLWN201/CML511)DH65	2.63	68.11	1.51	172.24	72.42	0.40	3.03
(CLWN201/CML511)DH66	2.74	67.47	2.62	171.02	69.29	0.39	3.10
(CLWN201/CML511)DH67	2.74	67.43	2.24	166.96	69.46	0.40	3.11
(CLWN201/CML511)DH69	2.52	66.75	2.01	171.07	65.08	0.38	3.03
(CLWN201/CML511)DH70	2.91	67.74	1.86	174.63	75.79	0.41	3.05
(CLWN201/CML511)DH71	2.84	68.17	1.56	174.49	76.31	0.42	3.01
(CLWN201/CML511)DH72	2.83	67.90	2.61	171.86	69.43	0.39	3.11
(CLWN201/CML511)DH73	2.55	67.40	2.10	170.26	70.44	0.40	3.06
(CLWN201/CML511)DH74	2.78	69.01	1.55	173.71	69.75	0.39	2.96
(CLWN201/CML511)DH75	2.50	66.12	2.29	166.98	66.52	0.39	3.08
(CLWN201/CML511)DH76	2.50	68.78	2.14	168.79	70.62	0.41	3.01
(CLWN201/CML511)DH77	2.59	68.59	2.74	178.92	77.57	0.41	3.12
(CLWN201/CML511)DH78	2.92	66.32	2.60	180.27	73.83	0.40	3.10
(CLWN201/CML511)DH79	2.71	67.52	2.01	164.71	63.01	0.38	3.09
(CLWN201/CML511)DH80	2.62	67.54	1.89	169.82	70.30	0.40	3.09
(CLWN201/CML511)DH81	2.11	70.60	1.91	166.50	65.11	0.38	3.05
(CLWN201/CML511)DH82	2.72	66.59	1.51	173.17	70.46	0.40	3.14
(CLWN201/CML511)DH83	2.78	69.21	1.35	168.55	70.41	0.40	2.99
(CLWN201/CML511)DH84	2.55	66.32	2.14	168.91	65.84	0.38	3.07
(CLWN201/CML511)DH85	2.58	65.85	1.52	167.61	71.68	0.41	3.15
(CLWN201/CML511)DH86	2.34	66.50	2.02	166.10	64.30	0.38	3.12
(CLWN201/CML511)DH88	2.97	66.51	1.78	167.21	67.04	0.39	3.12
(CLWN201/CML511)DH89	2.40	68.87	1.76	166.50	67.02	0.39	3.08
(CLWN201/CML511)DH90	2.81	66.95	2.16	177.35	75.58	0.41	3.13
(CLWN201/CML511)DH91	2.47	68.36	1.51	176.66	73.99	0.41	3.06
(CLWN201/CML511)DH92	2.43	69.27	1.68	173.88	78.28	0.42	3.06
(CLWN201/CML511)DH93	2.52	67.96	2.07	167.51	66.32	0.39	3.04
(CLWN201/CML511)DH94	2.60	67.13	1.63	171.04	72.16	0.41	3.01
(CLWN201/CML511)DH95	2.50	68.31	1.69	172.95	67.63	0.38	3.04
(CLWN201/CML511)DH96	2.41	69.85	1.32	170.79	70.14	0.39	3.00
(CLWN201/CML511)DH98	2.78	67.51	2.52	174.40	71.01	0.40	3.01
(CLWN201/CML511)DH101	2.32	67.55	1.68	165.78	73.07	0.42	3.05
(CLWN201/CML511)DH102	2.57	68.19	2.21	169.68	70.93	0.40	3.01
(CLWN201/CML511)DH103	2.80	67.93	2.16	168.31	70.44	0.40	2.90
(CLWN201/CML511)DH104	2.49	67.82	2.19	165.86	64.67	0.38	3.10
(CLWN201/CML511)DH105	2.35	70.82	2.36	164.08	67.75	0.40	3.06
(CLWN201/CML511)DH106	2.38	67.76	2.27	172.50	68.21	0.38	2.98
(CLWN201/CML511)DH107	2.52	68.96	1.44	169.70	69.27	0.39	2.92
(CLWN201/CML511)DH108	2.50	67.53	1.73	170.71	68.92	0.38	3.03
(CLWN201/CML511)DH110	2.36	70.03	2.68	169.55	70.12	0.40	3.10
(CLWN201/CML511)DH111	2.93	68.12	2.59	177.31	76.44	0.42	3.01
(CLWN201/CML511)DH112	2.92	66.92	2.10	170.79	67.63	0.38	3.12
(CLWN201/CML511)DH113	2.77	67.77	2.09	169.13	65.08	0.37	3.02
(CLWN201/CML511)DH118	2.78	67.56	1.78	175.96	75.98	0.41	3.04
(CLWN201/CML511)DH119	2.26	69.32	2.25	164.01	66.54	0.39	3.03
(CLWN201/CML511)DH120	2.24	68.83	1.89	166.70	67.88	0.39	3.04
CZH0616	2.42	68.35	2.25	167.37	70.04	0.40	3.01
PAN53	2.55	69.61	2.09	178.13	75.02	0.41	3.07
WH507	2.45	71.07	2.18	171.87	75.62	0.42	3.04
n Locs	**3**	**3**	**3**	**3**	**3**	**3**	**3**
n Reps	**2**	**2**	**2**	**2**	**2**	**2**	**2**
Error Var	**0.50**	**2.97**	**2.57**	**113.48**	**80.44**	**0.00**	**0.16**
Genotypic Var	**0.11**	**2.12**	**0.30**	**32.15**	**25.33**	**0.00**	**0.01**
GenxEnv Var	**0.16**	**0.59**	**0.16**	**14.19**	**18.45**	**0.00**	**0.02**
Location Var	**0.00**	**135.30**	**0.87**	**2351.52**	**884.13**	**0.01**	**0.05**
Heritability	**0.45**	**0.75**	**0.38**	**0.58**	**0.56**	**0.43**	**0.29**
Grand Mean	**2.61**	**67.74**	**1.96**	**170.29**	**70.40**	**0.40**	**3.06**
LSD	**1.39**	**3.38**	**3.14**	**20.88**	**17.58**	**0.10**	**0.79**
CV	**27.06**	**2.54**	**82.02**	**6.26**	**12.74**	**12.53**	**13.16**

GY, Grain yield; AD, Anthesis date; ASI, Anthesis silking interval; PH, Plant height; EH, ear height; EPO, ear position; SEN, leaf scenescence; LSD, least significant difference; CV, coefficient of variation; GenxEnv; genotype by environment interaction

Appendix Table 9 Mean GY, AD, ASI, PH, EH, EPO and SEN of 105 doubled haploid test cross progenies (CML504/CML550) and five commercial checks evaluated in two low N sites in Kenya (Kiboko) during the off season of 2014.

Genotype	GYF	AD	ASI	PH	EH	EPO	SEN
(CLWN201/CML511)DH1	2.36	66.81	1.89	132.45	51.30	0.38	5.68
(CLWN201/CML511)DH2	2.47	67.12	1.38	139.43	53.44	0.38	5.68
(CLWN201/CML511)DH3	2.74	66.98	1.95	133.40	50.60	0.38	5.68
(CLWN201/CML511)DH4	2.04	66.41	1.63	130.64	49.70	0.38	5.68
(CLWN201/CML511)DH5	2.73	67.14	1.85	133.73	51.59	0.38	5.68
(CLWN201/CML511)DH6	2.35	67.78	2.02	138.76	53.79	0.39	5.68
(CLWN201/CML511)DH7	2.28	68.57	1.55	134.22	52.99	0.39	5.68
(CLWN201/CML511)DH8	2.52	67.36	1.92	135.08	49.74	0.37	5.68
(CLWN201/CML511)DH9	2.92	68.08	1.55	138.04	57.14	0.41	5.68
(CLWN201/CML511)DH10	2.77	66.14	2.02	137.97	52.45	0.38	5.68
(CLWN201/CML511)DH11	2.46	68.69	1.67	138.42	54.29	0.39	5.68
(CLWN201/CML511)DH12	2.49	67.70	2.32	142.86	52.94	0.37	5.68
(CLWN201/CML511)DH13	2.45	66.26	1.80	139.30	52.87	0.38	5.68
(CLWN201/CML511)DH14	2.50	67.72	1.86	139.31	53.48	0.38	5.68
(CLWN201/CML511)DH15	2.15	66.71	1.76	127.81	48.37	0.38	5.68
(CLWN201/CML511)DH18	2.69	67.41	1.84	131.91	50.45	0.38	5.68
(CLWN201/CML511)DH19	2.91	66.84	1.91	138.68	53.95	0.39	5.68
(CLWN201/CML511)DH20	2.16	61.01	2.50	118.64	43.96	0.37	5.68
(CLWN201/CML511)DH21	2.42	66.45	2.17	139.33	50.54	0.37	5.68
(CLWN201/CML511)DH22	2.39	67.24	1.59	136.83	51.59	0.38	5.68
(CLWN201/CML511)DH23	3.00	65.63	1.57	138.15	53.08	0.38	5.68
(CLWN201/CML511)DH24	2.51	68.14	2.13	142.93	56.14	0.39	5.68
(CLWN201/CML511)DH25	2.64	66.03	1.42	133.28	52.70	0.39	5.68
(CLWN201/CML511)DH26	2.38	66.32	2.12	131.51	50.39	0.38	5.68
(CLWN201/CML511)DH27	2.45	68.47	1.92	141.00	55.90	0.39	5.68
(CLWN201/CML511)DH28	2.61	66.38	2.04	140.18	53.60	0.38	5.68
(CLWN201/CML511)DH29	2.62	67.33	1.69	143.01	53.68	0.37	5.68
(CLWN201/CML511)DH30	2.04	67.46	1.97	131.35	49.96	0.38	5.68
(CLWN201/CML511)DH31	2.22	68.27	2.09	135.16	53.82	0.39	5.68
(CLWN201/CML511)DH32	2.57	67.17	1.89	135.45	51.71	0.38	5.68
(CLWN201/CML511)DH33	3.03	67.87	2.17	149.29	60.50	0.40	5.68
(CLWN201/CML511)DH34	2.48	68.53	1.59	134.29	52.28	0.39	5.68
(CLWN201/CML511)DH35	2.40	66.41	1.68	137.14	53.30	0.38	5.68
(CLWN201/CML511)DH36	2.94	67.14	2.06	141.01	54.02	0.38	5.68
(CLWN201/CML511)DH37	2.69	67.98	1.74	142.87	54.24	0.38	5.68
(CLWN201/CML511)DH38	2.64	66.59	2.16	138.00	54.01	0.39	5.68
(CLWN201/CML511)DH39	2.16	67.04	1.92	136.65	51.63	0.38	5.68
(CLWN201/CML511)DH40	2.32	68.69	1.45	134.89	53.96	0.40	5.68
(CLWN201/CML511)DH41	2.18	67.32	1.95	135.24	48.20	0.36	5.68
(CLWN201/CML511)DH42	2.85	67.82	1.81	138.05	54.85	0.39	5.68
(CLWN201/CML511)DH43	2.97	66.64	1.88	141.88	54.89	0.38	5.68
(CLWN201/CML511)DH44	2.25	66.59	2.02	136.20	49.23	0.36	5.68
(CLWN201/CML511)DH45	2.35	67.37	1.89	139.70	53.67	0.38	5.68
(CLWN201/CML511)DH46	2.61	66.55	1.95	134.87	49.05	0.37	5.68
(CLWN201/CML511)DH47	2.63	66.47	2.02	144.95	54.48	0.37	5.68
(CLWN201/CML511)DH48	1.78	68.43	1.58	136.46	53.66	0.39	5.68
(CLWN201/CML511)DH49	1.98	66.49	1.75	130.67	48.06	0.37	5.68
(CLWN201/CML511)DH50	2.14	68.03	2.11	133.58	49.39	0.37	5.68
(CLWN201/CML511)DH51	2.43	66.57	2.30	133.22	49.84	0.38	5.68
(CLWN201/CML511)DH52	2.74	67.88	1.93	142.18	56.63	0.40	5.68
(CLWN201/CML511)DH53	2.53	67.77	1.72	138.92	55.95	0.40	5.68
(CLWN201/CML511)DH54	2.22	67.44	2.15	134.00	52.33	0.39	5.68
(CLWN201/CML511)DH55	2.43	67.15	2.17	126.60	49.26	0.39	5.68
(CLWN201/CML511)DH56	2.00	66.75	1.94	129.64	49.02	0.37	5.68
(CLWN201/CML511)DH58	2.44	69.25	1.49	135.50	52.42	0.39	5.68
(CLWN201/CML511)DH59	2.53	69.39	1.76	137.31	51.55	0.38	5.68
(CLWN201/CML511)DH60	2.70	67.28	1.58	133.22	51.30	0.39	5.68
(CLWN201/CML511)DH61	2.54	66.12	1.91	133.95	50.94	0.38	5.68
(CLWN201/CML511)DH62	2.59	66.53	2.09	137.22	52.95	0.39	5.68
(CLWN201/CML511)DH63	2.61	67.50	1.80	132.50	51.32	0.39	5.68
(CLWN201/CML511)DH64	2.08	66.75	1.70	132.03	49.24	0.37	5.68
(CLWN201/CML511)DH65	2.70	67.76	1.89	140.35	53.49	0.38	5.68
(CLWN201/CML511)DH66	2.58	66.88	2.17	135.94	50.97	0.38	5.68

Genotype	GYF	AD	ASI	PH	EH	EPO	SEN
(CLWN201/CML511)DH67	2.53	67.74	1.84	133.55	53.07	0.39	5.68
(CLWN201/CML511)DH69	2.35	66.81	1.94	136.99	49.39	0.37	5.68
(CLWN201/CML511)DH70	2.15	67.89	2.21	139.97	55.79	0.39	5.68
(CLWN201/CML511)DH71	2.24	68.09	1.77	137.66	52.53	0.38	5.68
(CLWN201/CML511)DH72	2.20	68.90	1.90	130.31	48.06	0.37	5.68
(CLWN201/CML511)DH73	2.56	66.85	2.14	140.08	54.08	0.39	5.68
(CLWN201/CML511)DH74	2.29	68.68	1.84	140.14	52.78	0.38	5.68
(CLWN201/CML511)DH75	2.45	66.59	2.44	135.16	48.85	0.36	5.68
(CLWN201/CML511)DH76	2.10	68.90	1.87	132.90	50.62	0.38	5.68
(CLWN201/CML511)DH77	2.52	67.73	1.93	144.62	57.45	0.39	5.68
(CLWN201/CML511)DH78	2.57	66.29	2.68	141.70	50.48	0.36	5.68
(CLWN201/CML511)DH79	2.57	67.02	1.68	135.01	49.31	0.37	5.68
(CLWN201/CML511)DH80	2.58	67.21	2.05	141.79	56.19	0.39	5.68
(CLWN201/CML511)DH81	2.62	69.15	1.78	140.24	52.92	0.38	5.68
(CLWN201/CML511)DH82	2.36	67.35	2.07	135.17	50.93	0.38	5.68
(CLWN201/CML511)DH83	2.16	67.23	1.95	133.85	53.13	0.39	5.68
(CLWN201/CML511)DH84	2.46	65.87	1.90	132.25	48.13	0.37	5.68
(CLWN201/CML511)DH85	2.80	65.24	1.85	134.50	52.84	0.39	5.68
(CLWN201/CML511)DH86	2.49	65.85	1.77	136.04	49.26	0.37	5.68
(CLWN201/CML511)DH88	2.82	66.53	2.45	139.97	52.21	0.38	5.68
(CLWN201/CML511)DH89	2.40	66.67	1.73	136.15	52.53	0.39	5.68
(CLWN201/CML511)DH90	2.66	68.48	2.10	142.27	54.87	0.39	5.68
(CLWN201/CML511)DH91	2.74	66.21	2.03	139.05	53.32	0.38	5.68
(CLWN201/CML511)DH92	2.15	68.55	1.63	139.36	53.86	0.38	5.68
(CLWN201/CML511)DH93	2.44	66.64	1.79	132.14	48.67	0.37	5.68
(CLWN201/CML511)DH95	2.90	66.93	1.73	143.69	56.61	0.39	5.68
(CLWN201/CML511)DH96	2.46	67.99	1.70	138.31	52.17	0.38	5.68
(CLWN201/CML511)DH98	2.55	67.86	2.29	138.25	51.01	0.37	5.68
(CLWN201/CML511)DH101	2.44	66.35	2.02	137.87	53.73	0.39	5.68
(CLWN201/CML511)DH102	2.63	66.80	1.98	141.07	54.55	0.39	5.68
(CLWN201/CML511)DH103	2.61	67.38	2.19	139.72	54.29	0.39	5.68
(CLWN201/CML511)DH104	2.65	66.42	1.76	135.34	51.53	0.38	5.68
(CLWN201/CML511)DH105	2.39	70.05	1.77	134.21	54.24	0.40	5.68
(CLWN201/CML511)DH106	2.24	67.32	1.87	134.04	49.47	0.37	5.68
(CLWN201/CML511)DH108	2.55	66.27	2.16	135.67	48.50	0.36	5.68
(CLWN201/CML511)DH110	2.00	68.05	2.23	131.51	51.63	0.39	5.68
(CLWN201/CML511)DH111	2.49	69.36	2.05	140.74	54.22	0.38	5.68
(CLWN201/CML511)DH112	2.67	66.49	2.30	138.06	50.73	0.37	5.68
(CLWN201/CML511)DH113	2.46	67.04	1.93	139.43	51.35	0.37	5.68
(CLWN201/CML511)DH118	2.46	67.45	1.63	135.84	52.96	0.39	5.68
(CLWN201/CML511)DH119	2.28	67.48	2.03	129.08	48.64	0.38	5.68
(CLWN201/CML511)DH120	2.10	67.92	2.09	135.74	54.20	0.40	5.68
DK8031	1.91	65.81	2.58	133.81	51.92	0.38	5.68
PAN53	2.98	68.63	2.33	146.65	59.44	0.40	5.68
PHB3253	1.94	68.33	2.03	135.71	56.60	0.41	5.68
WH403	2.63	69.22	1.85	148.85	69.44	0.45	5.68
WH507	2.82	69.34	1.72	145.65	60.77	0.41	5.68
Locations	**2**	**2**	**2**	**2**	**2**	**2**	**2**
Replications	**3**	**3**	**3**	**3**	**3**	**3**	**3**
Error Variance	**0.38**	**1.60**	**0.82**	**83.07**	**33.56**	**0.00**	**0.11**
Genotypic Variance	**0.11**	**1.54**	**0.13**	**33.99**	**15.88**	**0.00**	**0.00**
GenxEnv Variance	**0.01**	**0.00**	**0.00**	**5.68**	**4.01**	**0.00**	**0.02**
Location Variance	**0.21**	**1.08**	**0.41**	**0.00**	**0.00**	**0.00**	**0.00**
Heritability	**0.63**	**0.85**	**0.49**	**0.67**	**0.68**	**0.60**	**0.00**
Grand Mean	**2.47**	**67.30**	**1.93**	**136.88**	**52.56**	**0.38**	**5.68**
LSD	**1.21**	**2.48**	**1.77**	**17.86**	**11.35**	**0.06**	**0.66**
CV	**24.91**	**1.88**	**46.89**	**6.66**	**11.02**	**7.86**	**5.93**

GY, Grain yield; AD, Anthesis date; ASI, Anthesis silking interval; PH, Plant height; EH, ear height; EPO, ear position; SEN, leaf scenescence; LSD, least significant difference; CV, coefficient of variation; GenxEnv; genotype by environment interaction

Appendix Table 10. Mean GY, AD, ASI, PH, EH, EPO and SEN of 107 doubled haploid test cross progenies (CML504/CML550) and three commercial checks evaluated in five optimum sites in Kenya (Kiboko, Kitale and Kakamega) during the main season of 2014.

Genotype	GYF	AD	ASI	PH	EH	EPO	SEN
(CLWN201/CML511)DH1	6.69	72.97	1.78	226.52	109.28	0.49	2.69
(CLWN201/CML511)DH2	6.28	73.49	0.84	231.12	111.59	0.48	2.69
(CLWN201/CML511)DH3	6.12	73.21	1.52	205.41	95.35	0.47	2.69
(CLWN201/CML511)DH4	6.16	72.11	1.26	218.39	105.82	0.48	2.65
(CLWN201/CML511)DH5	6.45	72.08	0.73	232.36	113.31	0.49	2.66
(CLWN201/CML511)DH6	6.37	73.48	0.64	229.58	114.19	0.50	2.73
(CLWN201/CML511)DH7	5.96	73.99	1.00	232.79	110.04	0.47	2.71
(CLWN201/CML511)DH8	6.07	73.34	1.13	233.62	110.73	0.48	2.80
(CLWN201/CML511)DH9	6.96	74.09	0.71	231.66	116.39	0.50	2.66
(CLWN201/CML511)DH10	6.54	71.66	1.02	227.12	108.04	0.48	2.75
(CLWN201/CML511)DH11	6.81	72.88	0.80	231.13	110.70	0.48	2.67
(CLWN201/CML511)DH12	6.58	73.74	0.88	234.39	112.11	0.48	2.72
(CLWN201/CML511)DH13	6.70	72.70	1.13	233.24	111.61	0.48	2.64
(CLWN201/CML511)DH14	6.75	73.83	0.47	226.82	113.31	0.50	2.61
(CLWN201/CML511)DH15	6.12	73.47	0.52	214.84	104.39	0.49	2.71
(CLWN201/CML511)DH16	6.62	72.64	2.28	221.00	107.29	0.48	2.69
(CLWN201/CML511)DH17	6.25	72.12	1.03	226.55	113.52	0.50	2.70
(CLWN201/CML511)DH18	6.39	73.44	1.23	222.17	105.28	0.48	2.67
(CLWN201/CML511)DH19	6.51	71.88	0.73	221.76	103.99	0.47	2.60
(CLWN201/CML511)DH20	5.54	67.04	0.94	201.18	87.34	0.44	2.68
(CLWN201/CML511)DH21	6.26	72.58	0.58	237.75	113.88	0.48	2.74
(CLWN201/CML511)DH23	6.32	73.08	0.91	226.68	109.93	0.48	2.63
(CLWN201/CML511)DH24	6.99	73.26	1.20	252.19	120.14	0.48	2.68
(CLWN201/CML511)DH25	6.53	72.35	0.76	222.12	106.73	0.49	2.67
(CLWN201/CML511)DH26	6.97	72.05	1.09	225.29	109.29	0.49	2.65
(CLWN201/CML511)DH27	6.71	73.15	0.92	244.26	120.45	0.49	2.63
(CLWN201/CML511)DH28	6.54	71.66	1.22	228.51	105.08	0.46	2.76
(CLWN201/CML511)DH29	6.12	74.15	1.01	239.98	112.37	0.47	2.68
(CLWN201/CML511)DH30	5.53	72.99	1.37	217.66	102.89	0.47	2.77
(CLWN201/CML511)DH31	6.48	73.85	1.10	229.78	114.16	0.50	2.67
(CLWN201/CML511)DH32	7.07	72.24	0.57	227.41	112.26	0.50	2.65
(CLWN201/CML511)DH33	6.51	73.99	0.62	241.15	118.45	0.49	2.60
(CLWN201/CML511)DH34	6.53	73.38	0.14	220.44	115.40	0.52	2.64
(CLWN201/CML511)DH35	6.01	72.92	0.69	229.76	106.04	0.46	2.67
(CLWN201/CML511)DH36	6.83	72.81	2.08	227.49	110.10	0.48	2.64
(CLWN201/CML511)DH37	6.45	72.94	1.02	232.35	110.50	0.48	2.70
(CLWN201/CML511)DH38	7.13	72.54	1.57	233.82	115.38	0.49	2.65
(CLWN201/CML511)DH39	6.03	71.83	0.92	220.89	105.66	0.48	2.72
(CLWN201/CML511)DH40	6.40	74.98	0.60	229.82	115.95	0.50	2.70
(CLWN201/CML511)DH42	6.48	72.49	0.94	229.75	113.60	0.49	2.64
(CLWN201/CML511)DH43	6.52	73.57	0.34	237.48	113.36	0.48	2.70
(CLWN201/CML511)DH44	6.46	72.16	1.19	221.44	102.68	0.46	2.69
(CLWN201/CML511)DH45	6.50	72.15	1.19	233.82	113.94	0.49	2.75
(CLWN201/CML511)DH46	6.13	72.80	1.16	222.90	102.92	0.46	2.68
(CLWN201/CML511)DH47	6.37	72.25	0.78	233.16	106.40	0.46	2.65
(CLWN201/CML511)DH48	5.40	76.00	1.46	224.45	111.22	0.49	2.67
(CLWN201/CML511)DH49	5.90	72.67	1.14	218.69	100.13	0.46	2.64
(CLWN201/CML511)DH51	6.47	71.59	1.00	227.29	109.69	0.49	2.75
(CLWN201/CML511)DH52	6.73	74.31	0.89	234.03	116.72	0.50	2.72
(CLWN201/CML511)DH53	6.18	73.60	0.76	236.91	118.68	0.50	2.72
(CLWN201/CML511)DH54	6.42	73.11	1.48	227.26	113.50	0.50	2.72
(CLWN201/CML511)DH55	6.13	72.98	1.75	222.47	109.48	0.49	2.66
(CLWN201/CML511)DH56	6.00	72.47	1.22	226.48	107.55	0.48	2.66
(CLWN201/CML511)DH57	6.18	73.45	1.03	223.63	109.59	0.49	2.70
(CLWN201/CML511)DH58	6.63	73.40	0.92	225.26	111.42	0.49	2.69
(CLWN201/CML511)DH59	5.72	75.35	0.64	220.84	111.69	0.51	2.68
(CLWN201/CML511)DH60	6.67	73.31	0.54	216.97	107.63	0.49	2.66
(CLWN201/CML511)DH61	6.19	71.50	1.11	224.64	110.73	0.49	2.66
(CLWN201/CML511)DH62	6.37	72.99	0.89	226.76	106.74	0.47	2.68
(CLWN201/CML511)DH63	6.36	72.45	0.98	218.06	105.50	0.49	2.64
(CLWN201/CML511)DH64	5.96	73.96	1.01	229.60	111.81	0.49	2.70
(CLWN201/CML511)DH65	6.94	72.56	0.70	247.25	124.48	0.50	2.68
(CLWN201/CML511)DH66	6.47	72.01	1.44	225.19	107.55	0.48	2.68
(CLWN201/CML511)DH67	6.63	74.01	1.28	226.41	110.19	0.49	2.67

Genotype	GYF	AD	ASI	PH	EH	EPO	SEN
(CLWN201/CML511)DH69	6.78	71.81	1.23	219.28	99.10	0.46	2.65
(CLWN201/CML511)DH70	7.10	73.71	0.72	244.06	123.88	0.51	2.66
(CLWN201/CML511)DH71	6.75	72.90	0.22	231.59	113.76	0.49	2.65
(CLWN201/CML511)DH72	6.83	73.22	0.86	236.13	115.06	0.49	2.66
(CLWN201/CML511)DH73	6.56	73.10	1.21	234.35	112.98	0.48	2.68
(CLWN201/CML511)DH74	6.26	73.69	0.57	231.95	109.15	0.47	2.63
(CLWN201/CML511)DH75	6.52	71.11	1.93	220.70	102.81	0.47	2.70
(CLWN201/CML511)DH76	6.01	73.26	0.76	223.41	109.37	0.49	2.67
(CLWN201/CML511)DH77	6.41	73.59	1.28	231.64	114.44	0.49	2.68
(CLWN201/CML511)DH78	6.56	72.67	1.64	236.93	107.12	0.46	2.69
(CLWN201/CML511)DH79	6.43	72.88	0.64	228.50	101.77	0.45	2.71
(CLWN201/CML511)DH80	6.25	73.20	0.73	238.41	116.06	0.49	2.71
(CLWN201/CML511)DH81	5.91	73.78	1.20	222.22	106.30	0.48	2.73
(CLWN201/CML511)DH82	6.19	73.15	1.10	230.56	111.99	0.49	2.70
(CLWN201/CML511)DH83	6.59	73.69	1.06	232.18	115.64	0.50	2.70
(CLWN201/CML511)DH84	6.27	71.54	1.43	222.73	100.02	0.45	2.63
(CLWN201/CML511)DH85	6.25	70.90	0.76	219.60	106.07	0.48	2.70
(CLWN201/CML511)DH86	6.12	71.57	0.82	222.65	101.97	0.46	2.61
(CLWN201/CML511)DH88	6.36	72.57	1.38	223.23	106.95	0.48	2.66
(CLWN201/CML511)DH89	6.20	72.94	1.21	222.94	107.31	0.48	2.67
(CLWN201/CML511)DH90	6.70	72.91	1.18	243.87	116.46	0.48	2.72
(CLWN201/CML511)DH91	6.37	72.77	0.76	227.38	112.18	0.49	2.61
(CLWN201/CML511)DH92	6.33	73.29	1.01	242.16	120.11	0.50	2.78
(CLWN201/CML511)DH93	6.07	72.33	1.58	223.20	106.67	0.48	2.71
(CLWN201/CML511)DH94	6.59	72.38	1.12	234.52	115.43	0.49	2.69
(CLWN201/CML511)DH95	6.66	73.54	0.58	238.93	118.26	0.49	2.66
(CLWN201/CML511)DH96	6.32	74.79	0.62	232.30	114.97	0.49	2.64
(CLWN201/CML511)DH98	6.91	73.79	1.41	235.46	110.99	0.47	2.69
(CLWN201/CML511)DH101	6.29	72.48	1.17	227.39	114.59	0.50	2.67
(CLWN201/CML511)DH102	6.26	72.23	1.00	226.38	104.11	0.46	2.68
(CLWN201/CML511)DH103	6.05	72.95	1.76	228.68	106.39	0.47	2.70
(CLWN201/CML511)DH104	6.10	72.94	1.77	219.70	100.62	0.46	2.68
(CLWN201/CML511)DH105	6.68	74.59	0.83	228.88	116.06	0.51	2.68
(CLWN201/CML511)DH106	6.23	73.55	0.97	235.83	114.90	0.49	2.73
(CLWN201/CML511)DH107	6.29	73.11	0.88	222.70	103.38	0.47	2.62
(CLWN201/CML511)DH108	6.07	72.44	0.72	219.08	100.65	0.46	2.68
(CLWN201/CML511)DH110	6.19	74.90	1.07	230.87	113.44	0.49	2.72
(CLWN201/CML511)DH111	7.06	73.77	1.29	241.90	118.68	0.49	2.69
(CLWN201/CML511)DH112	6.44	71.87	1.56	227.51	106.41	0.47	2.68
(CLWN201/CML511)DH113	6.02	73.40	0.97	231.18	105.52	0.46	2.68
(CLWN201/CML511)DH118	6.54	72.32	0.87	230.25	119.64	0.52	2.66
(CLWN201/CML511)DH119	6.52	72.63	0.72	223.54	111.32	0.50	2.68
(CLWN201/CML511)DH120	6.21	74.12	0.96	230.77	119.77	0.52	2.71
CZH0616	6.64	72.23	0.63	220.88	112.09	0.50	2.59
PAN53	6.98	74.79	1.11	233.30	113.43	0.49	2.64
WH507	6.25	74.91	0.53	233.25	123.28	0.53	2.63
Locations	**5**	**5**	**5**	**5**	**5**	**5**	**5**
Replications	**2**	**2**	**2**	**2**	**2**	**2**	**2**
Error Variance	**1.28**	**4.03**	**1.66**	**130.11**	**54.12**	**0.00**	**0.18**
Genotypic Variance	**0.22**	**1.59**	**0.26**	**75.04**	**43.57**	**0.00**	**0.01**
GenxEnv Variance	**0.45**	**0.63**	**0.16**	**0.00**	**5.05**	**0.00**	**0.09**
Location Variance	**5.15**	**137.85**	**0.45**	**724.79**	**197.31**	**0.00**	**0.11**
Heritability	**0.51**	**0.75**	**0.57**	**0.85**	**0.87**	**0.81**	**0.23**
Grand Mean	**6.40**	**72.98**	**1.02**	**228.48**	**110.43**	**0.48**	**2.68**
LSD	**2.22**	**3.94**	**2.52**	**22.36**	**14.42**	**0.05**	**0.84**
CV	**17.68**	**2.75**	**125.80**	**4.99**	**6.66**	**5.29**	**15.98**

GY, Grain yield; AD, Anthesis date; ASI, Anthesis silking interval; PH, Plant height; EH, ear height; EPO, ear position; SEN, leaf scenescence; LSD, least significant difference; CV, coefficient of variation; GenxEnv; genotype by environment interaction

Appendix Table 11. Mean GY, AD, ASI, PH, EH, EPO and EPP of 167 doubled haploid test cross progenies (CML50/LPS) and seven commercial checks evaluated at one low N stress sites in Kenya (Kiboko) during the main season of 2014.

Genotype	GYF	AD	ASI	PH	EH	EPO	EPP
CKDHL140302	4.41	68.49	2.23	180.98	98.14	0.54	0.83
CKDHL140303	4.29	68.57	2.27	175.14	99.27	0.58	0.84
CKDHL140304	4.38	68.18	2.36	177.88	98.52	0.56	0.85
CKDHL140305	4.44	68.79	2.47	180.75	101.71	0.57	0.85
CKDHL140307	4.13	66.71	2.57	177.46	98.30	0.55	0.82
CKDHL140308	4.65	68.61	2.71	178.54	97.73	0.54	0.82
CKDHL140310	4.18	69.25	2.44	177.16	96.82	0.54	0.84
CKDHL140313	4.28	68.46	2.20	185.66	100.70	0.54	0.85
CKDHL140314	4.31	70.57	1.98	177.90	98.17	0.56	0.84
CKDHL140315	4.48	69.73	2.56	168.80	98.28	0.61	0.84
CKDHL140316	4.44	68.35	2.31	180.59	100.14	0.56	0.83
CKDHL140317	4.48	68.65	2.01	177.37	99.69	0.57	0.85
CKDHL140318	4.16	70.28	2.41	171.27	92.71	0.52	0.84
CKDHL140319	4.42	68.63	2.48	181.67	99.23	0.54	0.84
CKDHL140320	4.28	68.78	2.13	182.74	104.86	0.59	0.85
CKDHL140321	4.13	69.18	2.61	177.68	96.74	0.54	0.85
CKDHL140324	4.30	68.84	2.37	179.03	97.81	0.54	0.85
CKDHL140325	4.34	68.04	2.28	175.25	95.88	0.54	0.85
CKDHL140327	3.95	68.60	2.37	178.43	99.09	0.55	0.88
CKDHL140328	4.33	68.38	2.08	182.89	101.17	0.56	0.85
CKDHL140332	4.35	70.32	2.44	177.86	98.49	0.55	0.84
CKDHL140335	4.28	68.24	2.26	180.05	98.89	0.55	0.84
CKDHL140336	4.24	69.76	2.34	181.77	102.74	0.58	0.86
CKDHL140342	4.35	69.00	2.06	184.18	103.08	0.57	0.84
CKDHL140343	4.61	67.89	2.38	182.09	99.29	0.54	0.82
CKDHL140345	4.31	68.84	2.23	178.19	101.54	0.58	0.82
CKDHL140346	4.34	68.77	2.28	179.17	98.31	0.55	0.83
CKDHL140350	4.25	68.76	2.59	177.94	99.49	0.56	0.85
CKDHL140352	4.42	68.69	2.21	177.49	99.33	0.56	0.86
CKDHL140355	4.33	69.55	2.58	176.56	94.67	0.52	0.83
CKDHL140357	4.21	67.74	2.22	183.09	101.96	0.56	0.83
CKDHL140359	4.03	69.31	2.23	181.40	100.20	0.56	0.84
CKDHL140360	4.30	69.23	2.59	181.70	101.37	0.56	0.84
CKDHL140363	4.18	67.60	2.54	179.04	100.26	0.56	0.85
CKDHL140364	4.30	67.26	2.47	180.99	99.33	0.55	0.88
CKDHL140367	4.25	68.18	2.25	179.54	99.40	0.56	0.85
CKDHL140369	4.37	67.68	2.11	183.83	98.64	0.52	0.82
CKDHL140370	4.42	69.09	2.00	181.21	99.36	0.55	0.86
CKDHL140373	4.14	69.33	2.00	175.59	96.93	0.55	0.84
CKDHL140375	4.59	68.36	2.15	176.33	98.46	0.56	0.85
CKDHL140376	4.04	69.79	1.72	177.78	99.59	0.57	0.90
CKDHL140377	4.54	68.55	2.22	179.70	98.91	0.55	0.84
CKDHL140378	3.84	68.72	2.23	176.44	96.35	0.53	0.87
CKDHL140379	4.02	68.35	2.55	176.29	95.34	0.53	0.80
CKDHL140380	4.09	67.30	2.35	177.66	96.82	0.54	0.82
CKDHL140381	4.57	67.91	2.35	182.16	101.06	0.56	0.83
CKDHL140383	4.40	68.53	2.26	183.33	101.24	0.55	0.82
CKDHL140385	4.21	69.63	2.04	177.72	97.08	0.54	0.93
CKDHL140387	4.25	69.00	2.26	175.22	96.96	0.55	0.84
CKDHL140389	4.69	66.41	2.66	179.61	99.04	0.55	0.85
CKDHL140394	4.30	68.20	2.78	183.60	100.01	0.54	0.88
CKDHL140396	4.73	67.56	2.39	180.75	98.51	0.54	0.86
CKDHL140400	4.79	68.96	2.05	184.74	102.61	0.56	0.83
CKDHL140403	4.23	69.20	2.47	176.76	97.98	0.55	0.84
CKDHL141822	4.11	67.03	2.49	177.56	96.61	0.54	0.82
CKDHL140405	4.47	69.79	2.42	177.26	96.60	0.54	0.84
CKDHL141823	4.30	69.92	2.15	181.05	100.98	0.56	0.84
CKDHL140409	4.47	68.46	2.49	176.64	98.99	0.56	0.84
CKDHL140410	4.85	67.63	2.60	183.24	100.37	0.54	0.85
CKDHL140412	4.13	70.49	2.10	185.12	101.65	0.55	0.84
CKDHL140413	4.37	68.25	2.11	180.89	101.66	0.57	0.85
CKDHL140415	4.52	68.45	2.28	179.74	99.74	0.56	0.86
CKDHL140416	4.24	66.24	2.24	179.15	97.63	0.53	0.81
CKDHL140417	4.41	68.27	2.55	180.03	100.92	0.57	0.82
CKDHL140422	4.17	68.45	2.24	184.22	100.47	0.54	0.83
CKDHL140423	4.20	67.42	2.72	175.42	95.26	0.53	0.84
CKDHL140425	4.37	68.63	2.25	185.13	102.66	0.56	0.84

Genotype	GYF	AD	ASI	PH	EH	EPO	EPP
CKDHL140427	4.84	68.22	2.18	180.12	99.81	0.56	0.84
CKDHL140431	4.30	69.16	2.69	181.50	99.45	0.55	0.84
CKDHL140433	4.17	69.73	2.67	179.57	100.62	0.57	0.82
CKDHL140435	4.51	69.22	2.10	178.42	97.32	0.54	0.83
CKDHL140438	4.46	68.63	2.46	188.73	102.76	0.54	0.84
CKDHL140442	4.32	67.43	2.75	182.79	100.27	0.55	0.85
CKDHL140443	4.65	69.88	2.03	175.27	100.25	0.58	0.86
CKDHL140444	3.99	68.58	2.15	181.70	100.88	0.56	0.86
CKDHL141825	4.34	67.69	2.26	181.75	101.12	0.56	0.84
CKDHL140450	4.02	68.55	2.00	178.67	99.31	0.56	0.88
CKDHL140451	4.54	69.16	2.05	177.26	101.02	0.58	0.85
CKDHL140452	4.21	68.83	2.10	182.93	101.05	0.55	0.85
CKDHL140453	4.25	68.28	2.15	181.41	98.94	0.54	0.84
CKDHL140457	4.40	69.47	2.03	184.08	103.08	0.56	0.85
CKDHL140458	4.19	69.86	2.06	181.45	101.66	0.57	0.85
CKDHL140459	4.05	68.27	2.35	175.52	95.52	0.53	0.85
CKDHL140460	4.35	69.30	2.37	181.99	101.66	0.56	0.83
CKDHL140461	4.61	68.10	2.35	176.93	97.32	0.54	0.83
CKDHL140462	4.73	69.05	2.35	181.34	101.08	0.56	0.82
CKDHL140463	4.49	68.81	2.10	179.67	99.05	0.55	0.86
CKDHL140464	4.35	69.96	2.21	187.65	103.67	0.56	0.82
CKDHL140465	4.24	68.55	2.63	179.70	98.25	0.54	0.84
CKDHL140469	4.32	69.75	2.24	185.15	103.62	0.57	0.83
CKDHL140470	4.62	68.96	2.17	178.31	98.51	0.56	0.81
CKDHL141830	4.00	68.86	2.82	174.61	98.44	0.57	0.86
CKDHL141831	4.30	68.69	2.30	180.99	98.81	0.54	0.83
CKDHL141832	4.10	68.60	2.73	183.45	100.23	0.55	0.85
CKDHL141833	4.22	68.94	2.23	181.42	100.66	0.56	0.83
CKDHL141834	4.08	68.84	2.11	184.71	105.60	0.59	0.83
CKDHL141835	4.22	66.97	2.34	178.89	96.64	0.53	0.85
CKDHL141838	4.30	68.47	2.25	183.61	101.66	0.56	0.82
CKDHL141844	4.08	68.46	2.63	173.28	97.49	0.57	0.83
CKDHL141845	4.29	67.15	2.69	176.40	99.01	0.57	0.83
CKDHL141846	4.18	67.30	2.49	181.32	97.94	0.54	0.83
CKDHL141847	4.43	69.46	2.46	177.28	96.53	0.54	0.82
CKDHL141848	4.72	68.99	2.01	178.10	96.72	0.54	0.85
CKDHL141849	4.26	69.41	2.21	179.24	99.60	0.56	0.85
CKDHL141850	4.37	67.87	2.46	181.71	100.70	0.56	0.83
CKDHL141851	4.10	67.45	2.59	174.73	94.79	0.53	0.83
CKDHL141855	4.05	68.59	2.44	182.16	98.17	0.53	0.84
CKDHL140472	4.26	68.90	2.07	175.19	99.47	0.58	0.83
CKDHL141859	4.27	68.92	2.36	179.31	98.98	0.55	0.81
CKDHL141860	3.88	70.39	2.22	179.25	100.71	0.57	0.94
CKDHL141861	4.57	68.66	2.12	190.09	104.16	0.55	0.82
CKDHL141868	4.42	68.60	2.15	183.46	104.02	0.58	0.84
CKDHL141871	4.06	68.44	2.15	180.44	97.82	0.54	0.84
CKDHL141872	4.06	68.79	2.48	180.99	100.98	0.56	0.86
CKDHL141873	4.25	68.84	2.41	183.89	102.56	0.57	0.84
CKDHL141874	4.34	68.34	2.10	177.35	97.31	0.55	0.85
CKDHL141875	4.16	68.60	2.05	184.03	103.64	0.57	0.86
CKDHL141878	4.08	69.81	2.14	179.45	98.94	0.55	0.88
CKDHL141883	4.32	68.89	2.59	179.89	99.97	0.56	0.85
CKDHL141889	4.21	68.11	2.28	182.85	100.44	0.55	0.83
CKDHL141895	4.11	68.93	2.24	185.35	99.85	0.53	0.84
CKDHL141901	4.39	68.87	2.35	176.45	99.89	0.57	0.82
CKDHL141905	4.24	68.57	2.12	181.08	99.16	0.54	0.85
CKDHL141907	4.26	68.83	2.34	176.85	96.19	0.53	0.83
CKDHL141908	4.10	68.52	2.28	175.88	98.73	0.57	0.83
CKDHL141912	4.34	68.10	2.13	178.57	98.73	0.55	0.85
CKDHL141913	4.49	67.58	2.38	179.45	97.09	0.53	0.83
CKDHL141914	4.11	69.78	1.78	175.97	99.26	0.57	0.84
CKDHL141916	4.34	69.39	2.34	179.77	99.59	0.55	0.85
CKDHL141918	3.86	70.73	2.47	177.31	98.01	0.55	0.84
CKDHL141919	4.28	68.95	2.14	183.16	99.54	0.54	0.84
CKDHL141920	4.09	69.59	2.19	173.14	95.24	0.54	0.83
CKDHL141922	4.19	68.90	2.60	174.85	96.37	0.54	0.84
CKDHL141923	4.34	67.75	2.37	180.75	98.34	0.54	0.84
CKDHL141924	4.22	69.22	2.21	182.14	102.16	0.56	0.83
CKDHL141925	4.36	69.93	2.13	178.10	100.16	0.57	0.86
CKDHL141929	4.20	69.53	2.38	182.08	100.92	0.56	0.82

Genotype	GYF	AD	ASI	PH	EH	EPO	EPP
CKDHL141931	4.24	69.09	2.14	181.74	102.76	0.57	0.86
CKDHL141935	4.34	67.75	2.38	173.48	98.59	0.58	0.86
CKDHL141936	4.43	69.56	2.13	174.56	96.87	0.55	0.88
CKDHL141939	4.18	68.56	2.13	174.59	97.74	0.56	0.84
CKDHL141940	4.22	68.57	2.14	183.90	104.07	0.58	0.84
CKDHL141941	4.63	69.58	2.03	178.40	97.98	0.54	0.83
CKDHL141946	4.48	68.88	2.58	187.36	102.16	0.55	0.83
CKDHL141947	4.19	69.21	2.26	177.41	99.36	0.57	0.88
CKDHL141951	4.61	67.20	2.11	182.16	100.94	0.56	0.84
CKDHL141952	4.19	68.85	2.12	179.12	100.24	0.57	0.85
CKDHL141953	4.21	68.27	2.37	178.09	96.67	0.54	0.86
CKDHL141954	4.21	67.17	2.61	177.59	96.85	0.54	0.84
CKDHL141955	4.37	68.57	2.11	178.52	99.67	0.56	0.85
CKDHL141956	4.79	68.45	2.28	179.65	100.24	0.56	0.84
CKDHL141958	4.27	69.05	2.31	179.11	97.29	0.54	0.84
CKDHL141959	4.31	66.86	2.26	177.83	96.08	0.53	0.84
CKDHL141968	4.24	69.25	2.00	184.97	101.33	0.55	0.83
CKDHL141969	4.54	68.72	2.22	181.03	102.69	0.58	0.83
CKDHL141973	4.17	68.58	2.09	175.92	96.53	0.54	0.85
CKDHL141974	4.17	68.42	1.94	175.46	99.14	0.57	0.84
CKDHL141987	4.47	67.02	2.32	180.10	100.08	0.56	0.86
CKDHL141991	4.29	68.03	2.38	180.43	96.37	0.52	0.83
CKDHL141998	4.96	67.25	2.36	182.21	99.45	0.54	0.84
CKDHL142005	4.00	68.95	2.41	181.58	102.20	0.57	0.83
CKDHL142007	4.40	68.30	2.27	177.61	94.61	0.52	0.86
CKDHL142008	4.28	69.05	2.21	181.00	100.86	0.56	0.84
CKDHL142012	4.08	68.65	2.57	180.71	99.69	0.55	0.89
CKDHL142014	3.99	69.75	2.15	182.08	97.18	0.53	0.87
CKDHL142017	4.38	69.08	2.11	180.12	99.04	0.55	0.85
CKDHL142020	4.43	68.07	2.30	177.33	97.00	0.54	0.85
(CML395/CML444)//LPS	4.55	70.62	1.78	185.22	101.03	0.54	0.87
(CML395/CML444)//CML505	4.57	66.12	2.95	183.26	96.98	0.52	0.83
WE1101	4.03	68.76	2.36	182.12	99.01	0.54	0.93
H517	4.14	71.04	2.82	193.07	106.26	0.55	0.84
DK8031	4.13	67.46	2.66	185.63	100.58	0.54	0.85
Duma 43	4.14	66.07	3.03	185.61	96.42	0.50	0.86
WH505	4.14	69.96	2.15	188.27	101.94	0.54	0.85
Location	**1**	**1**	**1**	**1**	**1**	**1**	**1**
Replication	**2**	**2**	**2**	**2**	**2**	**2**	**2**
Error Variance	**1.03**	**2.18**	**1.42**	**96.13**	**51.15**	**0.00**	**0.03**
Genotypic Variance	**0.17**	**1.51**	**0.22**	**34.45**	**15.75**	**0.00**	**0.00**
Heritability	**0.25**	**0.58**	**0.24**	**0.42**	**0.38**	**0.64**	**0.15**
Grand Mean	**4.29**	**68.66**	**2.31**	**179.97**	**99.38**	**0.55**	**0.84**
LSD	**1.99**	**2.89**	**2.33**	**19.22**	**14.02**	**0.04**	**0.33**
CV	**23.65**	**2.15**	**51.60**	**5.45**	**7.20**	**4.01**	**20.18**

GY, Grain yield; AD, Anthesis date; ASI, Anthesis silking interval; PH, Plant height; EH, ear height; EPO, ear position; EPP, ears per plant; LSD, least significant difference; CV, coefficient of variation; GenxEnv; genotype by environment interaction; LPS, La Posta Seq C7-F64-2-6-2-2-B-B-B-B-B

Appendix Table 12. Mean GY, AD, ASI, PH, EH, EPO and EPP of 167 doubled haploid test cross progenies (CML50/LPS) and seven commercial checks evaluated at one low N stress sites in Kenya (Kiboko) during off season of 2014.

Genotype	GYF	AD	ASI	PH	EH	EPO	EPP
CKDHL140302	0.84	62.72	5.37	125.04	61.40	0.49	0.50
CKDHL140303	1.16	60.08	5.61	126.00	64.18	0.51	0.54
CKDHL140304	0.99	63.14	5.31	118.56	60.37	0.51	0.49
CKDHL140305	1.12	62.82	5.10	129.48	66.28	0.52	0.50
CKDHL140307	0.98	61.19	4.83	119.19	60.00	0.50	0.50
CKDHL140308	1.01	61.54	5.33	127.73	64.98	0.51	0.52
CKDHL140310	1.11	63.55	5.24	120.83	60.69	0.49	0.53
CKDHL140313	1.00	62.00	5.66	124.17	60.61	0.49	0.42
CKDHL140314	1.26	63.26	5.06	130.62	63.91	0.49	0.52
CKDHL140315	1.00	63.78	5.49	127.29	64.92	0.51	0.54
CKDHL140316	1.08	63.88	5.29	115.93	60.45	0.52	0.42
CKDHL140317	1.11	62.48	4.53	124.12	61.58	0.49	0.55
CKDHL140318	1.29	62.77	4.77	120.73	62.36	0.52	0.53
CKDHL140319	0.95	62.79	5.12	127.74	65.27	0.52	0.51
CKDHL140320	0.94	60.73	4.12	121.12	61.63	0.50	0.50
CKDHL140321	1.50	60.95	5.19	127.19	62.22	0.49	0.52
CKDHL140324	1.12	61.87	5.70	123.08	62.93	0.51	0.50
CKDHL140325	1.09	61.21	5.45	122.02	60.38	0.49	0.50
CKDHL140327	1.12	61.97	4.84	126.63	61.74	0.48	0.47
CKDHL140328	0.96	62.52	5.60	125.03	63.20	0.50	0.48
CKDHL140332	1.13	62.36	5.41	127.23	64.39	0.51	0.52
CKDHL140335	1.15	62.35	5.00	129.03	64.99	0.51	0.52
CKDHL140336	1.11	62.26	5.19	128.15	65.06	0.51	0.52
CKDHL140342	1.01	62.83	5.81	124.15	63.31	0.51	0.54
CKDHL140343	0.84	62.71	5.37	122.09	59.79	0.48	0.43
CKDHL140345	1.33	62.26	4.99	125.79	63.77	0.51	0.51
CKDHL140346	1.08	62.73	5.48	131.73	65.49	0.50	0.51
CKDHL140350	0.98	61.92	5.91	125.29	63.34	0.51	0.46
CKDHL140352	1.44	61.81	4.53	124.85	63.84	0.51	0.54
CKDHL140355	0.93	62.90	5.25	116.08	58.25	0.49	0.54
CKDHL140357	1.08	62.56	4.89	119.25	60.35	0.50	0.54
CKDHL140359	0.97	63.65	5.68	122.75	61.09	0.50	0.52
CKDHL140360	1.03	63.17	5.59	118.15	59.05	0.49	0.47
CKDHL140363	1.00	60.35	6.06	121.20	63.29	0.53	0.47
CKDHL140364	1.22	60.50	5.18	126.83	62.70	0.49	0.51
CKDHL140367	1.36	61.28	5.25	123.18	62.21	0.51	0.58
CKDHL140369	1.24	62.30	5.60	133.53	64.29	0.48	0.47
CKDHL140370	0.98	61.70	4.80	130.54	62.89	0.49	0.49
CKDHL140373	0.95	64.20	5.25	114.11	60.36	0.53	0.46
CKDHL140375	1.27	61.79	4.39	123.89	63.45	0.51	0.52
CKDHL140376	1.08	62.86	5.04	125.32	63.97	0.51	0.53
CKDHL140377	1.10	63.08	4.95	126.05	60.68	0.48	0.54
CKDHL140378	0.88	61.01	5.49	121.61	60.20	0.48	0.49
CKDHL140379	1.07	60.36	5.11	124.75	61.67	0.49	0.53
CKDHL140380	0.86	61.07	4.75	122.37	60.30	0.49	0.54
CKDHL140381	1.23	61.48	5.21	123.23	62.04	0.50	0.54
CKDHL140383	1.26	62.16	4.41	130.74	67.82	0.53	0.54
CKDHL140385	1.18	63.57	4.82	124.57	62.36	0.50	0.50
CKDHL140387	1.26	61.90	4.99	123.15	64.06	0.52	0.54
CKDHL140389	0.97	61.36	5.71	126.12	62.45	0.49	0.46
CKDHL140394	1.42	62.55	4.67	135.20	66.13	0.50	0.55
CKDHL140396	1.07	62.54	5.23	126.13	62.17	0.49	0.47
CKDHL140400	1.16	63.18	5.34	124.00	64.11	0.52	0.54
CKDHL140403	1.09	62.48	5.39	121.57	62.88	0.52	0.55
CKDHL141822	0.79	63.13	5.10	110.74	57.00	0.49	0.46
CKDHL140405	1.09	62.57	4.96	122.59	61.77	0.50	0.55
CKDHL141823	1.24	64.50	5.52	123.54	62.43	0.51	0.53
CKDHL140409	1.20	62.04	4.73	122.94	62.65	0.51	0.52
CKDHL140410	0.86	64.17	5.37	122.85	60.16	0.48	0.51
CKDHL140412	1.08	63.98	5.35	124.80	62.61	0.50	0.54
CKDHL140413	1.12	63.13	4.75	132.33	66.70	0.51	0.53

Genotype	GYF	AD	ASI	PH	EH	EPO	EPP
CKDHL140415	1.22	62.64	4.99	132.42	66.47	0.51	0.48
CKDHL140416	1.17	62.67	4.97	114.29	58.76	0.50	0.51
CKDHL140417	0.98	62.44	5.66	123.77	62.14	0.50	0.47
CKDHL140422	0.96	61.57	5.62	123.70	62.15	0.50	0.47
CKDHL140423	1.18	60.31	5.37	127.37	64.68	0.52	0.51
CKDHL140425	1.17	61.88	5.11	134.95	64.84	0.49	0.54
CKDHL140427	1.01	62.62	5.03	119.59	61.75	0.51	0.49
CKDHL140431	1.12	63.53	5.35	128.95	62.89	0.49	0.50
CKDHL140433	0.88	63.36	5.36	114.12	58.53	0.50	0.45
CKDHL140435	0.99	63.99	4.82	128.18	61.48	0.48	0.49
CKDHL140438	1.21	62.81	5.07	124.98	61.72	0.49	0.51
CKDHL140442	0.98	62.62	5.59	121.86	62.51	0.51	0.52
CKDHL140443	1.28	63.05	4.92	129.04	63.34	0.49	0.51
CKDHL140444	1.42	62.33	4.65	127.34	64.73	0.51	0.51
CKDHL141825	1.25	60.79	5.60	119.66	61.18	0.51	0.51
CKDHL140450	1.25	62.61	4.34	134.38	67.87	0.51	0.52
CKDHL140451	1.21	62.25	5.02	123.62	63.35	0.51	0.55
CKDHL140452	1.21	61.90	5.16	125.56	63.53	0.50	0.51
CKDHL140453	1.20	61.05	5.50	129.43	65.78	0.52	0.55
CKDHL140457	1.27	61.99	5.13	128.83	65.27	0.51	0.48
CKDHL140458	1.20	62.83	4.88	133.38	67.64	0.52	0.53
CKDHL140459	1.16	62.17	5.51	124.09	62.04	0.50	0.52
CKDHL140460	1.29	63.52	4.58	124.33	66.04	0.54	0.50
CKDHL140461	1.07	63.13	5.21	127.17	62.44	0.49	0.51
CKDHL140462	0.77	64.03	5.93	117.04	59.62	0.50	0.45
CKDHL140463	1.05	62.76	4.78	121.63	61.96	0.51	0.46
CKDHL140464	1.11	63.90	5.09	125.05	63.98	0.51	0.51
CKDHL140465	1.31	62.09	5.38	127.31	62.97	0.49	0.50
CKDHL140469	1.02	63.39	5.47	127.17	65.10	0.52	0.45
CKDHL140470	1.07	62.14	5.33	128.25	64.67	0.51	0.51
CKDHL141830	0.97	61.10	5.36	121.04	62.00	0.51	0.48
CKDHL141831	0.94	62.24	5.08	124.75	62.88	0.51	0.48
CKDHL141832	0.89	63.19	4.93	126.60	63.67	0.51	0.47
CKDHL141833	1.00	63.90	5.66	125.91	64.59	0.52	0.46
CKDHL141834	1.25	60.68	5.14	130.33	65.99	0.52	0.56
CKDHL141835	1.05	60.95	5.39	126.93	63.11	0.50	0.52
CKDHL141838	0.96	62.16	5.53	123.94	62.76	0.51	0.45
CKDHL141844	0.86	61.05	5.61	126.19	63.20	0.50	0.45
CKDHL141845	1.22	62.41	4.98	116.75	63.39	0.55	0.49
CKDHL141846	NA	63.94	5.37	106.50	55.62	0.49	0.38
CKDHL141847	0.89	63.73	5.76	115.01	60.06	0.51	0.44
CKDHL141848	1.04	61.03	5.57	120.82	58.21	0.47	0.47
CKDHL141849	1.04	61.76	5.32	123.84	63.07	0.51	0.47
CKDHL141850	1.22	62.03	4.87	129.16	64.04	0.50	0.51
CKDHL141851	1.41	60.48	5.35	118.22	58.26	0.48	0.57
CKDHL141855	1.10	61.79	5.25	130.08	63.41	0.49	0.50
CKDHL140472	0.95	63.52	5.38	119.44	62.87	0.53	0.50
CKDHL141859	0.93	64.32	5.01	119.92	60.67	0.50	0.47
CKDHL141860	1.22	62.56	4.88	133.81	63.60	0.48	0.54
CKDHL141861	1.08	64.02	4.76	125.69	62.00	0.49	0.52
CKDHL141868	1.10	63.75	4.97	122.85	63.10	0.52	0.49
CKDHL141871	1.10	62.13	4.87	122.75	60.48	0.49	0.50
CKDHL141872	1.11	62.38	5.40	124.24	64.36	0.52	0.52
CKDHL141873	1.39	63.43	4.74	132.45	66.66	0.51	0.53
CKDHL141874	1.13	62.67	5.00	121.56	60.87	0.50	0.52
CKDHL141875	0.94	63.04	5.48	126.09	62.34	0.49	0.44
CKDHL141878	1.36	62.02	4.57	135.43	67.35	0.51	0.50
CKDHL141883	1.07	61.89	5.35	120.62	63.95	0.54	0.52
CKDHL141889	1.47	61.72	4.77	129.97	64.85	0.50	0.53
CKDHL141895	1.17	62.22	5.48	128.51	64.28	0.50	0.53
CKDHL141901	1.10	62.16	4.87	125.15	61.57	0.49	0.49
CKDHL141905	1.40	61.35	4.99	131.77	65.56	0.50	0.51
CKDHL141907	1.40	60.87	4.83	122.94	63.00	0.52	0.55

Genotype	GYF	AD	ASI	PH	EH	EPO	EPP
CKDHL141908	0.98	61.78	5.84	118.40	61.81	0.52	0.53
CKDHL141912	1.13	61.56	5.28	123.23	62.93	0.51	0.50
CKDHL141913	1.07	62.12	5.37	124.01	62.28	0.50	0.47
CKDHL141914	1.23	63.89	4.68	120.14	63.29	0.53	0.51
CKDHL141916	1.38	63.78	4.95	129.53	67.18	0.53	0.50
CKDHL141918	1.12	62.14	5.33	125.61	63.79	0.51	0.53
CKDHL141919	1.17	62.16	5.22	127.70	62.24	0.49	0.50
CKDHL141920	0.81	61.73	5.83	120.24	61.74	0.51	0.51
CKDHL141922	1.00	62.91	4.94	119.72	60.52	0.50	0.52
CKDHL141923	0.75	61.77	5.83	113.93	60.56	0.54	0.42
CKDHL141924	0.84	61.28	5.71	122.47	63.91	0.53	0.47
CKDHL141925	1.33	64.10	5.11	120.96	64.13	0.53	0.49
CKDHL141929	1.11	63.46	5.34	115.87	58.29	0.49	0.49
CKDHL141931	1.18	61.94	5.15	130.97	65.52	0.51	0.51
CKDHL141935	1.43	60.63	4.53	129.47	64.56	0.50	0.52
CKDHL141936	1.05	62.18	5.22	117.08	60.33	0.51	0.51
CKDHL141939	1.23	62.87	4.80	117.47	62.24	0.53	0.51
CKDHL141940	1.39	62.07	5.01	126.60	64.07	0.51	0.52
CKDHL141941	1.16	61.56	5.07	129.10	63.54	0.50	0.54
CKDHL141946	1.13	63.52	4.60	127.23	63.96	0.50	0.54
CKDHL141947	1.21	63.39	5.26	129.88	65.32	0.51	0.52
CKDHL141951	1.13	61.70	5.05	127.93	62.41	0.49	0.52
CKDHL141952	1.05	62.15	5.17	123.18	62.97	0.51	0.55
CKDHL141953	0.99	62.26	4.89	121.14	62.24	0.51	0.45
CKDHL141954	1.26	61.92	4.74	132.19	64.11	0.49	0.49
CKDHL141955	1.18	63.00	5.30	126.19	63.94	0.51	0.54
CKDHL141956	1.36	60.64	4.83	130.99	66.57	0.52	0.52
CKDHL141958	0.78	63.22	4.71	107.58	56.75	0.51	0.44
CKDHL141959	1.18	62.11	5.13	126.73	63.28	0.50	0.49
CKDHL141968	1.12	61.39	5.47	125.21	63.60	0.51	0.45
CKDHL141969	0.91	63.14	5.37	115.27	59.20	0.50	0.47
CKDHL141973	1.04	60.81	4.97	128.55	64.56	0.51	0.52
CKDHL141974	1.31	61.48	5.43	123.33	64.37	0.53	0.52
CKDHL141987	1.20	62.44	4.96	120.95	62.67	0.52	0.50
CKDHL141991	1.10	62.39	4.81	121.81	61.02	0.50	0.50
CKDHL141998	0.96	62.31	5.33	117.98	57.56	0.47	0.46
CKDHL142005	0.88	64.11	5.04	120.03	60.92	0.50	0.48
CKDHL142007	1.29	62.28	4.85	126.22	64.35	0.52	0.57
CKDHL142008	1.27	61.45	5.12	115.74	59.50	0.51	0.56
CKDHL142012	0.88	63.37	4.84	114.61	59.89	0.51	0.49
CKDHL142014	0.99	64.27	5.44	127.73	63.69	0.50	0.49
CKDHL142017	1.19	63.73	5.49	121.35	61.04	0.49	0.47
CKDHL142020	1.32	62.60	4.65	124.09	61.91	0.49	0.56
(CML395/CML444)//LPS	1.19	63.89	4.72	119.23	60.94	0.51	0.55
(CML395/CML444)//CML505	0.97	60.90	5.01	131.38	63.63	0.49	0.50
WE1101	0.93	64.70	4.77	119.66	61.42	0.51	0.57
H517	1.15	66.34	4.98	123.18	62.83	0.51	0.58
DK8031	0.86	60.61	5.59	126.75	63.56	0.51	0.45
Duma 43	0.98	60.29	5.65	131.98	62.18	0.47	0.50
WH505	1.05	64.92	6.30	132.88	65.38	0.50	0.50
Location	**1**	**1**	**1**	**1**	**1**	**1**	**1**
Replication	**2**	**2**	**2**	**2**	**2**	**2**	**2**
Error Variance	**0.10**	**2.77**	**3.79**	**110.99**	**40.82**	**0.00**	**0.01**
Genotypic Variance	**0.05**	**2.10**	**0.60**	**58.71**	**13.74**	**0.00**	**0.00**
Heritability	**0.51**	**0.60**	**0.24**	**0.51**	**0.40**	**0.52**	**0.35**
Grand Mean	**1.11**	**62.43**	**5.22**	**124.34**	**62.73**	**0.50**	**0.50**
LSD	**0.63**	**3.26**	**3.82**	**20.65**	**12.52**	**0.05**	**0.22**
CV	**29.02**	**2.67**	**37.27**	**8.47**	**10.19**	**5.48**	**22.08**

GY, Grain yield; AD, Anthesis date; ASI, Anthesis silking interval; PH, Plant height; EH, ear height; EPO, ear position; EPP, ears per plant; LSD, least significant difference; CV, coefficient of variation; GenxEnv; genotype by environment interaction; LPS, La Posta Seq C7-F64-2-6-2-2-B-B-B-B-B

Appendix Table 13. Mean GY, AD, ASI, PH, EH, EPO and EPP of 167 doubled haploid test cross progenies (CML50/LPS) and seven commercial checks evaluated at three low N stress sites in Kenya (Kakamega and Kiboko) during the main season of 2014.

Genotype	GYF	AD	ASI	PH	EH	EPO	EPP
CKDHL140302	6.16	66.87	1.01	209.19	106.79	0.51	0.95
CKDHL140303	6.16	66.62	0.59	206.20	109.66	0.52	0.95
CKDHL140304	6.16	67.05	0.39	210.56	111.60	0.52	0.95
CKDHL140305	6.16	67.29	1.06	209.83	110.22	0.52	0.95
CKDHL140307	6.16	65.30	1.25	205.14	107.10	0.52	0.95
CKDHL140308	6.16	66.24	1.02	207.89	110.72	0.53	0.95
CKDHL140310	6.16	68.25	0.83	206.50	108.75	0.52	0.95
CKDHL140313	6.16	66.55	1.59	209.16	109.60	0.52	0.95
CKDHL140314	6.16	68.19	1.06	203.97	107.94	0.52	0.95
CKDHL140315	6.16	68.77	0.68	204.01	110.76	0.53	0.95
CKDHL140316	6.16	67.27	1.11	212.22	110.11	0.51	0.95
CKDHL140317	6.16	67.03	0.00	208.20	108.58	0.52	0.95
CKDHL140318	6.16	66.65	1.56	203.59	103.82	0.50	0.95
CKDHL140319	6.16	67.56	0.80	208.29	111.39	0.53	0.95
CKDHL140320	6.16	67.37	0.26	212.61	113.21	0.52	0.95
CKDHL140321	6.16	67.07	1.43	205.63	105.27	0.50	0.95
CKDHL140324	6.16	67.63	1.50	209.28	111.55	0.53	0.95
CKDHL140325	6.16	67.34	1.00	204.71	106.30	0.51	0.95
CKDHL140327	6.16	67.33	0.69	205.15	109.53	0.52	0.95
CKDHL140328	6.16	66.77	0.59	213.59	112.48	0.52	0.95
CKDHL140332	6.16	68.05	0.90	207.58	107.46	0.51	0.95
CKDHL140335	6.16	67.58	0.69	209.89	112.72	0.53	0.95
CKDHL140336	6.16	67.87	1.30	207.95	111.89	0.53	0.95
CKDHL140342	6.16	67.48	0.66	209.24	109.18	0.52	0.95
CKDHL140343	6.16	67.29	0.91	215.10	110.09	0.51	0.95
CKDHL140345	6.16	67.06	0.60	206.64	110.63	0.53	0.95
CKDHL140346	6.16	68.38	0.46	211.44	112.99	0.53	0.95
CKDHL140350	6.16	67.56	1.31	209.47	112.25	0.53	0.95
CKDHL140352	6.16	66.84	-0.39	206.07	110.73	0.53	0.95
CKDHL140355	6.16	68.33	1.12	209.76	110.44	0.52	0.95
CKDHL140357	6.16	66.62	1.36	204.50	109.24	0.52	0.95
CKDHL140359	6.16	67.26	0.57	213.08	111.10	0.52	0.95
CKDHL140360	6.16	67.69	1.10	204.15	104.39	0.50	0.95
CKDHL140363	6.16	66.52	1.13	203.56	109.92	0.53	0.95
CKDHL140364	6.16	66.32	1.65	211.39	110.99	0.52	0.95
CKDHL140367	6.16	67.03	0.36	204.47	107.81	0.52	0.95
CKDHL140369	6.16	66.79	0.28	216.36	112.50	0.52	0.95
CKDHL140370	6.16	66.01	0.35	209.99	108.16	0.51	0.95
CKDHL140373	6.16	68.04	0.27	205.70	106.70	0.51	0.95
CKDHL140375	6.16	65.98	0.05	201.54	108.89	0.53	0.95
CKDHL140376	6.16	67.36	0.41	206.57	109.60	0.52	0.95
CKDHL140377	6.16	65.92	0.97	209.65	109.34	0.52	0.95
CKDHL140378	6.16	65.86	0.88	204.33	105.48	0.51	0.95
CKDHL140379	6.16	65.15	1.33	209.50	111.57	0.52	0.95
CKDHL140380	6.16	66.94	0.96	205.30	108.29	0.52	0.95
CKDHL140381	6.16	66.64	0.32	205.90	107.78	0.52	0.95
CKDHL140383	6.16	67.07	0.64	213.78	111.73	0.52	0.95
CKDHL140385	6.16	68.47	1.11	205.09	109.54	0.53	0.95
CKDHL140387	6.16	68.21	0.50	200.44	106.26	0.52	0.95
CKDHL140389	6.16	65.15	1.69	204.92	106.79	0.51	0.95
CKDHL140394	6.16	67.29	0.90	213.18	112.20	0.52	0.95
CKDHL140396	6.16	68.06	0.10	206.98	109.57	0.52	0.95
CKDHL140400	6.16	66.89	-0.06	214.14	112.85	0.52	0.95
CKDHL140403	6.16	67.50	1.01	204.57	107.37	0.52	0.95
CKDHL141822	6.16	65.99	-0.12	207.01	107.16	0.51	0.95
CKDHL140405	6.16	67.94	0.44	203.94	107.25	0.52	0.95
CKDHL141823	6.16	68.21	0.81	209.62	113.37	0.53	0.95
CKDHL140409	6.16	66.59	0.65	200.57	105.04	0.51	0.95
CKDHL140410	6.16	66.41	0.13	211.83	111.24	0.52	0.95
CKDHL140412	6.16	68.57	0.34	219.27	116.00	0.53	0.95
CKDHL140413	6.16	66.79	1.01	207.66	112.12	0.53	0.95
CKDHL140415	6.16	67.27	0.62	213.59	110.86	0.52	0.95
CKDHL140416	6.16	66.22	0.79	193.59	101.89	0.51	0.95
CKDHL140417	6.16	68.08	1.09	212.20	113.33	0.53	0.95
CKDHL140422	6.16	67.52	-0.03	207.75	107.43	0.51	0.95
CKDHL140423	6.16	65.66	0.89	210.97	109.57	0.52	0.95
CKDHL140425	6.16	67.13	0.66	210.83	114.87	0.54	0.95
CKDHL140427	6.16	67.27	1.23	203.21	110.43	0.53	0.95

Genotype	GYF	AD	ASI	PH	EH	EPO	EPP
CKDHL140431	6.16	68.33	0.70	212.08	111.34	0.52	0.95
CKDHL140433	6.16	66.99	1.24	208.17	112.00	0.53	0.95
CKDHL140435	6.16	68.08	-0.04	208.40	109.87	0.52	0.95
CKDHL140438	6.16	68.25	0.56	215.35	114.56	0.53	0.95
CKDHL140442	6.16	66.95	0.36	213.78	112.97	0.52	0.95
CKDHL140443	6.16	68.58	0.19	205.12	110.86	0.53	0.95
CKDHL140444	6.16	66.57	0.03	204.39	108.34	0.52	0.95
CKDHL141825	6.16	66.12	1.55	201.73	107.78	0.52	0.95
CKDHL140450	6.16	67.30	-0.36	213.71	112.97	0.52	0.95
CKDHL140451	6.16	67.37	0.27	201.14	109.17	0.53	0.95
CKDHL140452	6.16	67.02	0.41	212.53	113.89	0.53	0.95
CKDHL140453	6.16	65.54	0.39	207.59	109.21	0.52	0.95
CKDHL140457	6.16	67.31	0.59	214.13	114.56	0.53	0.95
CKDHL140458	6.16	67.82	0.85	203.69	107.90	0.52	0.95
CKDHL140459	6.16	66.48	1.40	206.47	107.84	0.52	0.95
CKDHL140460	6.16	68.14	0.38	211.07	113.73	0.53	0.95
CKDHL140461	6.16	67.36	0.00	204.37	108.49	0.52	0.95
CKDHL140462	6.16	67.10	0.98	212.03	111.01	0.52	0.95
CKDHL140463	6.16	68.12	-0.19	210.49	110.65	0.52	0.95
CKDHL140464	6.16	68.71	1.72	210.72	112.04	0.52	0.95
CKDHL140465	6.16	67.65	0.28	207.13	109.96	0.52	0.95
CKDHL140469	6.16	67.08	0.52	211.75	113.27	0.53	0.95
CKDHL140470	6.16	66.76	0.37	206.06	109.58	0.52	0.95
CKDHL141830	6.16	66.83	1.02	205.86	110.21	0.53	0.95
CKDHL141831	6.16	67.98	0.60	205.18	110.28	0.53	0.95
CKDHL141832	6.16	67.81	1.56	209.97	108.14	0.51	0.95
CKDHL141833	6.16	67.77	1.74	214.60	115.24	0.53	0.95
CKDHL141834	6.16	67.49	0.50	210.55	117.46	0.55	0.95
CKDHL141835	6.16	65.91	0.72	211.24	109.55	0.51	0.95
CKDHL141838	6.16	67.10	0.93	207.86	109.93	0.52	0.95
CKDHL141844	6.16	66.15	1.96	210.31	111.99	0.53	0.95
CKDHL141845	6.16	67.24	0.25	201.81	112.42	0.54	0.95
CKDHL141846	6.16	66.65	0.74	208.46	105.26	0.50	0.95
CKDHL141847	6.16	67.67	0.86	213.47	109.83	0.51	0.95
CKDHL141848	6.16	66.68	0.58	202.91	104.64	0.51	0.95
CKDHL141849	6.16	67.73	1.31	199.73	106.90	0.53	0.95
CKDHL141850	6.16	67.06	1.18	206.15	109.02	0.52	0.95
CKDHL141851	6.16	66.52	0.07	198.79	106.69	0.53	0.95
CKDHL141855	6.16	66.85	0.57	214.85	113.95	0.52	0.95
CKDHL140472	6.16	66.35	0.35	202.08	106.68	0.52	0.95
CKDHL141859	6.16	67.27	0.41	205.36	105.70	0.51	0.95
CKDHL141860	6.16	69.27	0.57	213.20	114.96	0.53	0.95
CKDHL141861	6.16	66.47	0.81	216.66	115.34	0.53	0.95
CKDHL141868	6.16	68.36	0.34	214.72	114.13	0.53	0.95
CKDHL141871	6.16	66.88	1.12	214.50	113.02	0.52	0.95
CKDHL141872	6.16	67.27	0.88	211.06	114.35	0.53	0.95
CKDHL141873	6.16	66.72	0.14	213.63	112.87	0.52	0.95
CKDHL141874	6.16	68.15	1.10	200.94	106.42	0.52	0.95
CKDHL141875	6.16	68.24	0.80	214.84	116.01	0.53	0.95
CKDHL141878	6.16	66.76	0.08	208.15	110.44	0.52	0.95
CKDHL141883	6.16	67.04	0.86	212.26	113.89	0.53	0.95
CKDHL141889	6.16	66.27	1.07	206.91	108.55	0.52	0.95
CKDHL141895	6.16	66.94	0.19	208.29	110.92	0.53	0.95
CKDHL141901	6.16	67.49	0.22	208.28	113.26	0.53	0.95
CKDHL141905	6.16	68.00	0.56	209.35	110.22	0.52	0.95
CKDHL141907	6.16	66.98	0.34	206.82	107.72	0.51	0.95
CKDHL141908	6.16	67.10	1.00	199.47	107.92	0.53	0.95
CKDHL141912	6.16	67.33	0.94	205.13	107.08	0.52	0.95
CKDHL141913	6.16	66.46	1.09	212.16	110.63	0.52	0.95
CKDHL141914	6.16	67.72	0.30	204.15	106.92	0.52	0.95
CKDHL141916	6.16	67.54	-0.05	211.76	113.26	0.53	0.95
CKDHL141918	6.16	67.28	0.59	200.80	104.86	0.51	0.95
CKDHL141919	6.16	67.68	0.82	210.21	111.61	0.53	0.95
CKDHL141920	6.16	66.14	0.00	200.01	104.94	0.51	0.95
CKDHL141922	6.16	67.69	0.67	206.11	107.36	0.51	0.95
CKDHL141923	6.16	66.63	0.15	207.81	108.58	0.52	0.95
CKDHL141924	6.16	66.66	0.78	207.53	110.34	0.52	0.95
CKDHL141925	6.16	69.21	0.69	207.00	109.49	0.52	0.95
CKDHL141929	6.16	68.17	0.97	207.58	110.69	0.53	0.95
CKDHL141931	6.16	67.04	0.73	212.69	112.30	0.52	0.95

Genotype	GYF	AD	ASI	PH	EH	EPO	EPP
CKDHL141935	6.16	67.05	0.35	200.58	108.93	0.53	0.95
CKDHL141936	6.16	67.09	0.40	205.10	110.05	0.53	0.95
CKDHL141939	6.16	67.04	1.24	207.52	109.91	0.52	0.95
CKDHL141940	6.16	66.80	0.14	210.47	112.97	0.53	0.95
CKDHL141941	6.16	65.87	0.04	207.77	108.90	0.52	0.95
CKDHL141946	6.16	69.43	1.18	216.89	115.16	0.53	0.95
CKDHL141947	6.16	67.42	0.89	209.50	111.00	0.52	0.95
CKDHL141951	6.16	67.29	0.39	204.40	109.14	0.52	0.95
CKDHL141952	6.16	67.77	0.57	209.15	109.17	0.52	0.95
CKDHL141953	6.16	65.87	0.93	198.59	104.22	0.52	0.95
CKDHL141954	6.16	66.04	1.01	208.97	109.99	0.52	0.95
CKDHL141955	6.16	66.84	0.01	205.94	111.07	0.53	0.95
CKDHL141956	6.16	66.62	0.81	209.55	108.48	0.51	0.95
CKDHL141958	6.16	67.24	0.81	203.34	105.38	0.51	0.95
CKDHL141959	6.16	66.35	0.19	207.24	106.77	0.51	0.95
CKDHL141968	6.16	68.00	0.03	207.96	110.98	0.53	0.95
CKDHL141969	6.16	67.32	0.50	205.78	109.62	0.53	0.95
CKDHL141973	6.16	66.94	0.36	206.92	106.80	0.51	0.95
CKDHL141974	6.16	66.61	0.15	204.03	109.08	0.53	0.95
CKDHL141987	6.16	65.40	0.61	209.16	111.03	0.52	0.95
CKDHL141991	6.16	67.15	1.40	215.90	114.40	0.53	0.95
CKDHL141998	6.16	66.12	0.97	209.06	111.10	0.52	0.95
CKDHL142005	6.16	66.86	0.09	208.06	111.62	0.53	0.95
CKDHL142007	6.16	67.11	0.70	211.42	106.98	0.50	0.95
CKDHL142008	6.16	67.09	0.42	207.65	108.72	0.52	0.95
CKDHL142012	6.16	68.20	0.91	209.91	110.71	0.52	0.95
CKDHL142014	6.16	68.75	1.58	215.12	111.98	0.52	0.95
CKDHL142017	6.16	67.05	0.38	211.11	109.64	0.51	0.95
CKDHL142020	6.16	67.47	0.13	205.58	107.37	0.51	0.95
(CML395/CML444)//LPS	6.16	68.55	0.08	210.82	111.80	0.52	0.95
(CML395/CML444)//CML505	6.16	63.29	1.56	218.87	111.30	0.51	0.95
WE1101	6.16	68.05	1.02	207.19	106.70	0.51	0.95
H517	6.16	69.42	1.97	223.32	121.13	0.54	0.95
DK8031	6.16	65.35	2.91	217.97	110.73	0.51	0.95
Duma 43	6.16	62.60	2.95	217.75	103.65	0.48	0.95
WH505	6.16	69.19	1.08	215.18	113.18	0.52	0.95
Location	**3**	**3**	**3**	**3**	**3**	**3**	**3**
Replication	**2**	**2**	**2**	**2**	**2**	**2**	**2**
Error Variance	**1.21**	**1.88**	**1.08**	**71.32**	**36.59**	**0.00**	**0.02**
Genotypic Variance	**0.00**	**1.23**	**0.45**	**34.65**	**17.39**	**0.00**	**0.00**
GenxEnv Variance	**0.27**	**0.13**	**0.19**	**2.07**	**13.21**	**0.00**	**0.00**
Location Variance	**1.52**	**59.44**	**3.44**	**833.20**	**1336.38**	**0.01**	**0.01**
Heritability	**0.00**	**0.77**	**0.65**	**0.73**	**0.62**	**0.58**	**0.00**
Grand Mean	**6.16**	**67.16**	**0.73**	**208.43**	**110.03**	**0.52**	**0.94**
LSD	**2.15**	**2.68**	**2.04**	**16.55**	**11.86**	**0.04**	**0.25**
CV	**17.83**	**2.04**	**143.10**	**4.05**	**5.50**	**4.08**	**13.66**

GY, Grain yield; AD, Anthesis date; ASI, Anthesis silking interval; PH, Plant height; EH, ear height; EPO, ear position; EPP, ears per plant; LSD, least significant difference; CV, coefficient of variation; GenxEnv; genotype by environment interaction; LPS, La Posta Seq C7-F64-2-6-2-2-B-B-B-B-B

Appendix Table 14. Mean GY, AD, ASI, PH, EH, EPO and EPP of 120 doubled haploid test cross progenies (CML50/LPS) and ten commercial checks evaluated at one low N stress site in Kenya (Kiboko) during the main season of 2014.

Genotype	GYF	AD	ASI	PH	EH	EPP	SEN
CKDHL140864	3.90	73.03	2.17	185.54	96.35	0.83	5.15
CKDHL140865	4.83	72.96	1.77	195.13	103.12	0.83	5.15
CKDHL140866	3.99	73.79	1.53	190.79	98.68	0.83	5.15
CKDHL140868	4.81	72.62	1.51	203.21	108.02	0.83	5.15
CKDHL140870	4.43	72.83	1.53	197.38	104.22	0.83	5.15
CKDHL140873	4.23	72.02	1.89	188.92	97.35	0.83	5.15
CKDHL140875	4.65	73.00	1.66	196.40	99.52	0.83	5.15
CKDHL140876	4.11	72.50	1.62	196.02	101.10	0.83	5.15
CKDHL140877	4.44	73.55	1.68	203.26	105.25	0.83	5.15
CKDHL140879	4.06	74.55	1.49	197.28	102.40	0.83	5.15
CKDHL140880	4.97	71.64	1.46	196.16	104.74	0.83	5.15
CKDHL140881	4.76	72.69	1.22	203.30	105.49	0.83	5.15
CKDHL140884	4.66	71.38	1.57	191.32	101.97	0.83	5.15
CKDHL140888	4.59	73.27	1.49	197.03	101.99	0.83	5.15
CKDHL140892	4.30	74.27	1.48	196.51	103.60	0.83	5.15
CKDHL140893	3.96	72.99	1.84	198.15	103.39	0.83	5.15
CKDHL140895	4.31	72.34	1.80	198.26	103.98	0.83	5.15
CKDHL140896	3.81	73.75	1.63	191.31	99.44	0.83	5.15
CKDHL140897	4.52	72.35	1.64	196.19	105.54	0.83	5.15
CKDHL140898	5.32	71.99	1.67	196.35	107.48	0.83	5.15
CKDHL140899	4.67	72.19	2.03	196.14	100.97	0.83	5.15
CKDHL140900	4.78	72.86	1.66	196.08	100.07	0.83	5.15
CKDHL140901	4.15	72.23	1.83	202.86	103.76	0.83	5.15
CKDHL140902	4.34	72.04	1.44	189.54	96.57	0.83	5.15
CKDHL140903	4.18	72.78	1.96	201.71	104.68	0.83	5.15
CKDHL140904	4.43	72.98	1.65	200.18	106.39	0.83	5.15
CKDHL140908	4.53	73.09	1.70	194.01	100.82	0.83	5.15
CKDHL140910	4.27	72.59	1.70	186.79	96.42	0.83	5.15
CKDHL140911	4.29	72.28	2.04	203.44	107.87	0.83	5.15
CKDHL140912	3.63	73.95	1.67	195.08	98.32	0.83	5.15
CKDHL140913	4.73	72.68	1.53	202.29	105.56	0.83	5.15
CKDHL140914	4.03	72.41	1.51	198.74	102.10	0.83	5.15
CKDHL140915	3.80	73.03	2.00	194.48	102.38	0.83	5.15
CKDHL140919	4.48	73.61	1.69	193.99	98.63	0.83	5.15
CKDHL140920	5.13	73.26	1.76	194.45	100.07	0.83	5.15
CKDHL140925	3.99	72.42	1.49	192.99	100.04	0.83	5.15
CKDHL140928	4.37	72.93	1.18	192.59	103.30	0.83	5.15
CKDHL140929	4.74	72.08	1.58	197.72	105.03	0.83	5.15
CKDHL140932	3.68	73.16	1.96	191.11	98.94	0.83	5.15
CKDHL140933	4.18	73.89	1.63	193.64	102.57	0.83	5.15
CKDHL142733	3.56	72.25	1.89	190.63	97.84	0.83	5.15
CKDHL140935	4.69	70.71	1.94	198.72	107.64	0.83	5.15
CKDHL140936	4.12	72.76	1.58	202.04	100.65	0.83	5.15
CKDHL140938	3.87	74.19	0.01	190.81	96.53	0.83	5.15
CKDHL140940	4.08	73.98	1.63	199.96	106.43	0.83	5.15
CKDHL140941	4.38	72.84	1.13	206.20	106.34	0.83	5.15
CKDHL140944	4.06	72.55	1.31	202.48	107.71	0.83	5.15
CKDHL140945	4.05	74.31	1.96	197.74	106.57	0.83	5.15
CKDHL140951	4.25	73.04	1.81	201.87	100.83	0.83	5.15
CKDHL140953	3.91	73.78	1.81	198.15	102.93	0.83	5.15
CKDHL140955	4.53	72.13	1.71	192.18	97.58	0.83	5.15
CKDHL140957	4.69	72.26	1.78	200.98	104.98	0.83	5.15
CKDHL140958	4.69	71.77	2.06	197.58	104.87	0.83	5.15
CKDHL140959	4.39	72.92	1.49	197.33	102.56	0.83	5.15
CKDHL140960	4.64	72.27	1.50	197.04	100.33	0.83	5.15
Pioneer 30G19	3.79	70.51	2.95	190.23	93.70	0.83	5.15
WH505	3.99	74.19	1.45	198.55	103.18	0.83	5.15
CKDHL140966	3.74	73.68	1.79	189.31	99.94	0.83	5.15
CKDHL140967	4.73	72.62	1.66	196.70	99.07	0.83	5.15
CKDHL142735	4.62	72.51	1.84	187.05	95.13	0.83	5.15
CKDHL140970	4.41	73.11	1.62	206.70	111.40	0.83	5.15
CKDHL140974	4.09	73.40	1.03	191.46	97.82	0.83	5.15
CKDHL140976	4.44	73.79	1.67	201.41	101.76	0.83	5.15
CKDHL140979	4.24	73.92	1.85	199.98	105.99	0.83	5.15
CKDHL140981	4.49	72.46	1.52	200.45	104.42	0.83	5.15
CKDHL140982	4.23	71.39	1.92	193.33	102.65	0.83	5.15
CKDHL140983	3.81	72.51	2.16	188.28	93.89	0.83	5.15
CKDHL140984	4.57	72.45	1.46	198.17	102.36	0.83	5.15
CKDHL140985	4.67	71.19	2.08	194.79	103.38	0.83	5.15
CKDHL140988	3.49	72.35	1.86	195.10	98.98	0.83	5.15
CKDHL140991	4.44	72.65	1.85	191.86	100.14	0.83	5.15
CKDHL140992	4.07	74.11	1.51	199.29	103.65	0.83	5.15

Genotype	GYF	AD	ASI	PH	EH	EPP	SEN
CKDHL140994	4.59	73.23	1.80	198.73	104.70	0.83	5.15
CKDHL140995	4.55	73.28	1.80	202.18	106.32	0.83	5.15
CKDHL142737	4.74	72.17	1.41	208.02	112.46	0.83	5.15
Pioneer 2859	3.69	71.30	2.17	186.81	89.44	0.83	5.15
CKDHL142743	4.30	71.21	1.59	197.98	103.36	0.83	5.15
CKDHL142745	4.26	72.33	1.62	193.29	102.34	0.83	5.15
CKDHL142746	4.62	73.51	1.52	199.33	106.85	0.83	5.15
CKDHL142750	3.90	72.78	2.51	195.24	102.80	0.83	5.15
CKDHL142754	4.30	72.06	1.65	202.45	107.97	0.83	5.15
CKDHL142755	4.25	72.85	1.62	196.15	104.44	0.83	5.15
CKDHL142757	4.55	74.45	1.59	191.28	95.16	0.83	5.15
CKDHL142758	4.70	71.59	1.86	188.25	96.24	0.83	5.15
CKDHL142759	4.38	72.97	1.14	194.21	105.01	0.83	5.15
CKDHL142762	4.38	72.42	2.15	206.08	109.72	0.83	5.15
CKDHL142764	5.11	72.75	1.51	202.46	108.10	0.83	5.15
CKDHL142767	3.77	73.74	2.11	193.50	102.51	0.83	5.15
CKDHL142772	4.34	72.42	1.52	195.98	102.22	0.83	5.15
CKDHL142773	4.14	72.02	1.98	194.22	104.87	0.83	5.15
CKDHL142774	4.16	73.41	1.52	204.51	110.90	0.83	5.15
CKDHL142777	4.32	72.50	1.79	196.27	101.53	0.83	5.15
CKDHL142782	4.52	71.66	1.34	193.95	100.25	0.83	5.15
CKDHL142784	4.66	71.81	1.68	200.81	106.55	0.83	5.15
CKDHL142786	4.00	72.39	1.59	193.39	97.56	0.83	5.15
CKDHL142787	3.62	74.40	1.67	199.44	105.75	0.83	5.15
CKDHL142790	4.32	73.56	1.51	199.09	100.87	0.83	5.15
CKDHL142792	4.08	73.09	2.16	193.29	101.28	0.83	5.15
CKDHL142797	4.55	71.90	1.47	190.24	101.67	0.83	5.15
CKDHL142800	3.78	72.89	1.84	204.76	108.63	0.83	5.15
CKDHL142801	4.46	72.14	1.97	195.12	102.06	0.83	5.15
CKDHL142803	4.74	71.13	1.95	195.61	100.83	0.83	5.15
CKDHL142804	4.73	71.63	1.66	201.01	101.83	0.83	5.15
CKDHL142807	4.14	71.99	1.46	194.96	101.30	0.83	5.15
CKDHL142817	4.78	73.34	1.59	196.73	104.22	0.83	5.15
CKDHL142819	5.05	72.27	1.31	194.11	104.64	0.83	5.15
CKDHL142821	4.51	71.55	2.03	198.93	106.93	0.83	5.15
CKDHL142823	5.28	72.37	1.86	197.90	106.92	0.83	5.15
CKDHL142824	4.68	72.84	1.78	202.81	108.43	0.83	5.15
CKDHL142825	4.33	72.04	1.81	199.81	99.50	0.83	5.15
CKDHL142829	4.66	71.48	1.67	191.13	100.06	0.83	5.15
CKDHL142835	4.49	74.23	1.44	199.85	105.07	0.83	5.15
CKDHL142840	4.71	71.53	1.84	197.53	106.40	0.83	5.15
CKDHL142841	4.83	71.96	1.81	195.81	98.42	0.83	5.15
CKDHL142845	4.93	71.82	1.63	199.48	104.04	0.83	5.15
CKDHL142848	4.48	72.74	1.66	192.29	99.39	0.83	5.15
CKDHL142855	3.94	73.83	1.17	191.65	100.63	0.83	5.15
CKDHL142856	4.32	71.71	1.97	193.77	98.72	0.83	5.15
CKDHL142858	5.45	74.06	1.42	204.91	110.77	0.83	5.15
CKDHL142859	3.97	72.24	1.82	191.88	97.12	0.83	5.15
CKDHL142861	4.69	72.83	1.63	194.42	100.28	0.83	5.15
CKDHL142865	4.35	71.82	1.63	185.90	97.17	0.83	5.15
(CML395/CML444)//CML536	4.16	71.67	2.03	186.53	95.59	0.83	5.15
DH04	3.27	71.93	2.86	184.93	95.34	0.83	5.15
WE1101	3.66	72.11	2.11	189.48	95.48	0.83	5.15
H517	4.39	71.96	2.83	203.57	110.61	0.83	5.15
DK8031	3.42	70.80	2.14	194.97	98.39	0.83	5.15
Duma 43	3.05	70.12	3.54	185.13	88.71	0.83	5.15
WH505	4.50	71.54	2.31	199.75	106.27	0.83	5.15
Pioneer 3253	3.91	71.67	2.53	193.81	99.96	0.83	5.15
Location	**1**	**1**	**1**	**1**	**1**	**1**	**1**
Replication	**2**	**2**	**2**	**2**	**2**	**2**	**2**
Error Variance	**1.08**	**3.01**	**1.60**	**115.54**	**76.45**	**0.02**	**0.15**
Genotypic Variance	**0.41**	**1.67**	**0.44**	**57.35**	**40.37**	**0.00**	**0.00**
Heritability	**0.43**	**0.53**	**0.35**	**0.50**	**0.51**	**0.00**	**0.00**
Grand Mean	**4.33**	**72.62**	**1.74**	**196.22**	**102.26**	**0.83**	**5.15**
LSD	**2.03**	**3.40**	**2.48**	**21.07**	**17.14**	**0.26**	**0.75**
CV	**23.94**	**2.39**	**72.70**	**5.48**	**8.55**	**15.81**	**7.40**

GY, Grain yield; AD, Anthesis date; ASI, Anthesis silking interval; PH, Plant height; EH, ear height; EPP, ears per plant; SEN, leaf scenescence; LSD, least significant difference; CV, coefficient of variation

Appendix Table 15. Mean GY, AD, ASI, PH, EH, EPO and EPP of 120 doubled haploid test cross progenies (CML50/LPS) and ten commercial checks evaluated at one low N stress site in Kenya (Kiboko) during the off season of 2014.

Genotype	GYF	AD	ASI	PH	EH	EPO	EPP
CKDHL140864	1.19	66.50	4.74	136.14	65.70	0.49	0.51
CKDHL140865	1.64	65.56	4.91	143.92	70.69	0.50	0.54
CKDHL140866	1.24	66.03	4.82	137.88	66.80	0.48	0.49
CKDHL140868	0.93	68.02	6.09	143.28	70.40	0.49	0.45
CKDHL140870	1.89	65.48	3.98	144.38	69.63	0.49	0.57
CKDHL140873	1.22	66.22	5.98	146.94	69.20	0.47	0.46
CKDHL140875	1.11	67.08	5.30	137.49	62.51	0.45	0.65
CKDHL140876	1.41	64.98	5.28	142.83	64.36	0.44	0.54
CKDHL140877	1.34	66.15	5.08	145.69	69.06	0.47	0.51
CKDHL140879	1.73	66.20	4.29	144.86	68.40	0.47	0.56
CKDHL140880	1.75	65.15	4.13	144.94	70.27	0.49	0.56
CKDHL140881	1.56	67.35	5.07	148.27	69.03	0.46	0.56
CKDHL140884	1.46	66.40	4.49	142.66	69.35	0.49	0.49
CKDHL140888	1.45	66.25	4.93	141.76	68.85	0.49	0.56
CKDHL140892	1.42	66.10	5.47	141.68	67.64	0.47	0.54
CKDHL140893	1.10	67.41	7.29	136.70	64.40	0.46	0.44
CKDHL140895	1.46	67.90	3.52	140.25	65.23	0.46	0.53
CKDHL140896	0.93	66.58	6.62	136.03	64.96	0.48	0.46
CKDHL140897	1.54	66.04	5.20	140.66	66.73	0.48	0.59
CKDHL140898	1.81	64.64	4.55	145.59	73.68	0.52	0.54
CKDHL140899	1.39	65.82	5.98	142.29	69.77	0.50	0.52
CKDHL140900	0.96	66.93	6.88	141.01	64.36	0.44	0.41
CKDHL140901	1.09	66.07	5.99	142.95	66.13	0.45	0.49
CKDHL140902	1.55	65.61	5.13	148.83	71.34	0.48	0.54
CKDHL140903	1.72	66.27	4.54	149.92	75.91	0.52	0.58
CKDHL140904	1.36	66.90	5.24	142.38	71.04	0.51	0.55
CKDHL140908	1.38	65.25	4.89	145.55	71.70	0.50	0.52
CKDHL140910	1.40	64.87	3.81	142.38	69.50	0.49	0.54
CKDHL140911	1.18	64.70	5.29	152.03	72.25	0.48	0.44
CKDHL140912	1.10	66.55	6.01	142.47	67.82	0.48	0.47
CKDHL140913	1.56	65.86	4.57	143.22	67.44	0.47	0.53
CKDHL140914	1.12	67.52	7.29	147.22	70.13	0.47	0.40
CKDHL140915	1.32	65.78	4.91	139.40	65.47	0.46	0.47
CKDHL140919	0.97	66.83	5.15	132.34	63.10	0.47	0.44
CKDHL140920	1.23	65.75	5.10	142.26	65.52	0.46	0.52
CKDHL140925	1.26	67.53	4.45	138.59	66.16	0.48	0.51
CKDHL140928	1.23	66.46	5.02	139.37	66.29	0.47	0.50
CKDHL140929	1.14	65.92	5.39	140.52	67.44	0.48	0.50
CKDHL140932	1.61	66.20	4.03	143.32	67.39	0.47	0.57
CKDHL140933	1.24	66.86	4.07	136.08	66.01	0.49	0.50
CKDHL142733	0.78	65.94	6.08	124.23	63.19	0.53	0.37
CKDHL140935	1.46	66.05	4.46	137.38	67.07	0.49	0.52
CKDHL140936	1.16	66.12	5.55	142.86	64.06	0.43	0.49
CKDHL140938	0.83	66.77	7.66	136.53	65.06	0.47	0.37
CKDHL140940	1.51	66.48	5.57	142.77	66.43	0.46	0.56
CKDHL140941	1.08	66.88	5.94	147.72	72.56	0.50	0.49
CKDHL140944	0.93	68.00	7.31	139.78	65.87	0.47	0.47
CKDHL140945	0.85	68.10	7.35	136.91	66.05	0.48	0.38
CKDHL140951	1.16	65.99	5.78	147.15	69.71	0.48	0.49
CKDHL140953	1.49	65.63	5.32	148.99	71.57	0.48	0.51
CKDHL140955	1.25	65.42	4.82	138.70	66.01	0.46	0.48
CKDHL140957	1.02	66.80	6.13	135.82	63.98	0.46	0.43
CKDHL140958	1.25	65.72	5.20	146.29	69.81	0.48	0.50
CKDHL140959	1.29	65.47	5.77	146.35	69.79	0.48	0.51
CKDHL140960	1.30	66.78	5.43	142.50	66.89	0.47	0.53
Pioneer 30G19	1.28	64.19	6.74	141.03	65.34	0.46	0.47
WH505	0.98	66.35	6.40	142.05	67.16	0.47	0.54
CKDHL140966	1.44	66.65	5.42	146.64	70.70	0.49	0.49
CKDHL140967	1.05	65.49	5.48	146.42	69.03	0.47	0.45
CKDHL142735	0.98	66.36	5.09	135.89	66.77	0.49	0.46
CKDHL140970	1.33	67.27	5.95	147.35	70.01	0.47	0.50
CKDHL140974	1.36	65.79	4.50	141.45	69.06	0.49	0.56
CKDHL140976	1.35	66.26	5.70	147.11	67.58	0.45	0.53
CKDHL140979	1.55	66.15	5.03	148.96	72.09	0.49	0.51
CKDHL140981	1.06	66.21	6.92	140.45	66.91	0.48	0.42
CKDHL140982	1.30	65.18	3.93	136.28	63.75	0.46	0.62
CKDHL140983	1.20	64.88	5.79	142.07	66.50	0.46	0.52
CKDHL140984	1.46	66.83	4.10	145.29	72.09	0.50	0.47
CKDHL140985	1.35	65.65	4.64	143.61	67.63	0.47	0.57
CKDHL140988	1.31	65.50	5.94	152.89	73.14	0.48	0.52
CKDHL140991	1.26	67.35	5.38	130.45	62.21	0.47	0.40

Genotype	GYF	AD	ASI	PH	EH	EPO	EPP
CKDHL140992	1.26	67.60	5.52	136.50	63.41	0.46	0.48
CKDHL140994	1.14	66.57	4.48	143.60	68.43	0.47	0.44
CKDHL140995	1.22	65.60	5.81	135.36	65.61	0.49	0.45
CKDHL142737	1.13	66.17	6.15	145.48	70.05	0.48	0.53
Pioneer 2859	1.00	65.63	5.32	136.53	64.05	0.46	0.44
CKDHL142743	1.16	65.77	6.32	137.86	66.23	0.49	0.48
CKDHL142745	1.96	64.84	3.70	146.55	70.27	0.49	0.65
CKDHL142746	1.15	67.12	5.27	141.83	67.58	0.47	0.50
CKDHL142750	1.18	67.78	7.81	138.41	66.83	0.49	0.42
CKDHL142754	1.41	66.07	5.06	143.85	70.88	0.51	0.56
CKDHL142755	1.06	66.46	5.95	141.52	69.20	0.49	0.45
CKDHL142757	1.19	67.58	4.94	142.14	64.73	0.44	0.46
CKDHL142758	1.07	66.67	6.11	134.55	66.46	0.49	0.48
CKDHL142759	1.21	65.69	4.00	136.94	67.14	0.50	0.47
CKDHL142762	0.92	68.85	6.80	135.16	64.32	0.47	0.44
CKDHL142764	1.31	66.24	4.23	140.04	68.14	0.49	0.53
CKDHL142767	1.31	66.97	5.85	132.31	63.68	0.48	0.46
CKDHL142772	0.91	67.30	8.05	143.59	65.74	0.46	0.46
CKDHL142773	1.39	66.12	4.15	142.87	69.77	0.50	0.47
CKDHL142774	1.07	66.42	5.82	148.06	69.68	0.47	0.44
CKDHL142777	1.18	66.40	6.60	142.05	66.84	0.47	0.51
CKDHL142782	1.71	65.52	4.19	140.06	67.23	0.48	0.52
CKDHL142784	1.34	64.63	6.24	143.07	68.49	0.47	0.51
CKDHL142786	1.21	67.22	6.50	137.06	65.97	0.48	0.46
CKDHL142787	0.93	68.25	5.68	135.69	64.85	0.48	0.45
CKDHL142790	1.39	65.97	4.86	147.45	67.28	0.45	0.46
CKDHL142792	1.26	66.17	4.45	142.58	67.53	0.48	0.52
CKDHL142797	1.00	66.99	6.25	138.27	67.44	0.50	0.47
CKDHL142800	1.01	65.93	5.78	136.11	66.97	0.50	0.43
CKDHL142801	0.98	67.15	5.72	133.56	64.02	0.48	0.42
CKDHL142803	1.53	65.49	4.56	148.03	67.49	0.46	0.50
CKDHL142804	0.81	67.13	4.58	142.57	65.78	0.45	0.36
CKDHL142807	1.19	65.26	5.23	137.44	65.82	0.48	0.50
CKDHL142817	1.77	65.46	3.63	148.09	71.59	0.49	0.57
CKDHL142819	1.72	66.35	3.49	138.77	67.46	0.50	0.53
CKDHL142821	1.26	64.84	6.25	139.65	68.65	0.50	0.53
CKDHL142823	1.19	68.20	4.44	144.62	69.73	0.49	0.45
CKDHL142824	0.85	67.54	7.54	144.89	68.74	0.48	0.39
CKDHL142825	1.39	65.41	4.26	151.40	71.28	0.47	0.52
CKDHL142829	2.09	64.18	4.25	147.24	71.64	0.49	0.55
CKDHL142835	1.15	66.97	5.99	145.86	66.20	0.45	0.48
CKDHL142840	1.06	66.42	4.74	142.50	68.31	0.48	0.41
CKDHL142841	1.14	66.02	6.31	145.58	63.37	0.42	0.38
CKDHL142845	1.26	66.48	5.81	149.32	70.83	0.48	0.51
CKDHL142848	1.34	66.49	4.81	138.70	65.31	0.46	0.51
CKDHL142855	1.60	65.81	3.59	138.57	67.29	0.49	0.54
CKDHL142856	1.37	65.02	5.39	140.60	65.42	0.46	0.50
CKDHL142858	0.97	67.42	6.74	139.92	67.20	0.48	0.44
CKDHL142859	1.03	66.58	6.58	137.34	63.75	0.46	0.41
CKDHL142861	1.35	67.01	5.37	142.86	66.96	0.47	0.54
CKDHL142865	0.98	64.95	5.14	134.73	66.50	0.50	0.53
(CML395/CML444)//CML536	1.13	64.89	5.87	145.11	65.91	0.44	0.50
DH04	1.05	66.98	5.94	122.73	58.95	0.47	0.41
WE1101	1.13	66.77	7.20	128.47	61.11	0.48	0.45
H517	0.87	67.50	6.82	132.75	68.19	0.51	0.42
DK8031	0.84	65.14	6.39	133.20	63.62	0.47	0.31
Duma 43	0.73	62.71	6.36	136.83	61.66	0.43	0.39
WH505	1.15	67.34	6.46	152.08	73.11	0.48	0.46
Pioneer 3253	0.82	67.03	6.47	136.67	64.69	0.47	0.32
n Locs	**1**	**1**	**1**	**1**	**1**	**1**	**1**
n Reps	**2**	**2**	**2**	**2**	**2**	**2**	**2**
Error Var	**0.20**	**1.28**	**3.21**	**132.04**	**45.08**	**0.00**	**0.01**
Genotypic Var	**0.13**	**1.45**	**1.97**	**64.58**	**19.87**	**0.00**	**0.01**
Heritability	**0.56**	**0.69**	**0.55**	**0.49**	**0.47**	**0.67**	**0.51**
Grand Mean	**1.25**	**66.27**	**5.49**	**141.40**	**67.42**	**0.48**	**0.49**
LSD	**0.87**	**2.22**	**3.51**	**22.52**	**13.16**	**0.05**	**0.23**
CV	**35.70**	**1.71**	**32.64**	**8.13**	**9.96**	**4.90**	**24.25**

GY, Grain yield; AD, Anthesis date; ASI, Anthesis silking interval; PH, Plant height; EH, ear height; EPO, ear position; EPP, ears per plant; LSD, least significant difference; CV, coefficient of variation; GenxEnv; genotype by environment interaction

Appendix Table 16. Mean GY, AD, ASI, PH, EH, EPO and EPP of 120 doubled haploid test cross progenies (CML50/LPS) and ten commercial checks evaluated at three optimum stress sites in Kenya (Kiboko and Kakamega) during the main season of 2014.

Genotype	GYF	AD	ASI	PH	EH	EPO	EPP
CKDHL140864	6.60	72.57	0.62	212.02	110.77	0.52	0.97
CKDHL140865	6.82	73.68	-0.11	209.98	112.63	0.55	0.97
CKDHL140866	5.92	73.41	-0.15	204.65	102.27	0.51	0.98
CKDHL140868	6.60	73.16	0.13	213.81	113.79	0.53	0.97
CKDHL140870	6.95	74.04	-1.46	213.54	113.92	0.53	0.98
CKDHL140873	5.99	73.04	0.88	209.17	103.58	0.50	0.97
CKDHL140875	6.38	73.17	0.90	206.97	102.88	0.50	0.97
CKDHL140876	6.65	73.56	0.26	211.85	108.90	0.51	0.98
CKDHL140877	5.67	74.94	0.46	206.65	101.66	0.49	0.97
CKDHL140879	6.52	74.03	-0.21	215.54	110.89	0.51	0.96
CKDHL140880	6.90	72.75	-0.58	214.98	114.58	0.53	0.98
CKDHL140881	6.20	73.91	-0.20	213.23	107.38	0.50	0.96
CKDHL140884	6.19	72.71	-0.01	213.66	115.59	0.54	0.97
CKDHL140888	6.84	73.68	0.11	214.51	107.71	0.50	0.99
CKDHL140892	6.18	74.51	-0.06	213.88	112.18	0.52	0.97
CKDHL140893	6.01	74.72	0.97	216.21	114.45	0.53	0.96
CKDHL140895	6.43	74.14	0.26	213.72	109.02	0.51	0.98
CKDHL140896	6.63	75.06	-1.37	215.59	112.96	0.52	0.98
CKDHL140897	6.90	73.70	-0.13	213.04	112.94	0.53	0.98
CKDHL140898	6.78	73.49	0.17	211.23	116.46	0.56	0.97
CKDHL140899	6.34	73.95	0.23	213.09	112.45	0.53	0.97
CKDHL140900	5.86	74.11	0.85	215.54	109.95	0.51	0.96
CKDHL140901	6.98	72.84	-0.26	214.78	111.99	0.52	0.98
CKDHL140902	6.37	71.90	1.28	208.33	106.21	0.52	0.96
CKDHL140903	6.36	74.15	0.27	211.48	114.07	0.53	0.98
CKDHL140904	6.72	73.69	0.15	213.76	117.27	0.55	0.97
CKDHL140908	6.69	72.90	0.34	208.83	107.19	0.52	0.99
CKDHL140910	6.42	72.52	-0.64	207.59	105.44	0.51	0.98
CKDHL140911	6.96	73.75	0.66	217.66	116.19	0.53	0.98
CKDHL140912	6.59	74.15	1.22	220.06	115.56	0.52	0.96
CKDHL140913	6.56	73.03	0.34	213.26	109.46	0.51	0.98
CKDHL140914	5.71	74.12	0.44	213.91	109.02	0.51	0.97
CKDHL140915	6.54	72.90	0.61	213.20	111.91	0.53	0.97
CKDHL140919	6.40	73.06	0.70	216.59	112.97	0.52	0.97
CKDHL140920	6.09	75.08	-0.30	211.32	106.46	0.51	0.98
CKDHL140925	6.26	73.61	0.58	207.99	106.64	0.52	0.97
CKDHL140928	6.68	74.62	-0.77	211.46	116.57	0.55	0.96
CKDHL140929	6.75	73.05	0.02	210.83	113.59	0.54	0.97
CKDHL140932	6.71	73.12	0.23	210.73	115.23	0.55	0.99
CKDHL140933	6.05	73.93	0.10	212.55	114.10	0.54	0.99
CKDHL142733	5.77	72.53	0.79	207.78	108.04	0.52	0.97
CKDHL140935	6.19	73.20	0.03	213.13	116.44	0.55	0.98
CKDHL140936	6.62	73.08	0.09	213.71	106.30	0.50	1.00
CKDHL140938	6.17	74.27	1.20	209.93	110.65	0.53	0.97
CKDHL140940	6.70	74.31	0.76	214.96	112.62	0.52	0.97
CKDHL140941	6.54	73.73	0.49	219.60	116.85	0.52	0.98
CKDHL140944	7.09	73.56	1.29	218.58	114.15	0.52	0.99
CKDHL140945	6.07	74.00	1.11	219.27	120.75	0.54	0.99
CKDHL140951	6.54	73.17	0.86	212.76	105.49	0.50	0.97
CKDHL140953	6.74	73.10	-0.12	213.77	110.92	0.51	0.98
CKDHL140955	6.65	73.49	-0.26	208.81	104.59	0.50	0.98
CKDHL140957	6.55	72.87	0.91	209.59	105.13	0.50	0.97
CKDHL140958	6.58	72.79	0.81	206.06	109.08	0.54	0.97
CKDHL140959	7.34	73.79	0.39	218.82	118.51	0.53	1.00
CKDHL140960	6.20	74.56	0.45	213.34	110.43	0.52	0.98
Pioneer 30G19	6.16	70.15	2.93	210.67	98.84	0.47	0.96
WH505	5.15	70.42	2.17	205.10	99.70	0.49	0.96
CKDHL140966	6.58	73.95	0.59	213.51	113.08	0.53	0.99
CKDHL140967	6.44	73.03	0.66	213.84	107.83	0.50	0.97
CKDHL142735	6.42	73.38	0.61	210.88	108.08	0.52	0.96
CKDHL140970	6.30	73.88	0.66	214.99	113.48	0.53	0.99

Genotype	GYF	AD	ASI	PH	EH	EPO	EPP
CKDHL140974	6.48	72.80	0.35	206.68	107.41	0.53	0.98
CKDHL140976	6.68	73.24	-0.47	213.90	109.19	0.51	0.98
CKDHL140979	6.68	74.46	0.39	218.05	119.29	0.54	0.97
CKDHL140981	6.28	73.45	0.46	214.00	111.12	0.52	0.96
CKDHL140982	6.25	72.80	0.48	205.38	106.14	0.53	0.98
CKDHL140983	6.81	72.62	0.48	208.81	102.87	0.50	0.97
CKDHL140984	6.70	73.28	0.03	214.03	112.62	0.52	0.97
CKDHL140985	6.42	72.16	0.70	214.64	112.17	0.52	0.98
CKDHL140988	5.58	73.56	0.22	209.22	107.91	0.52	0.97
CKDHL140991	6.59	72.54	0.87	212.80	110.73	0.52	0.99
CKDHL140992	5.61	74.07	-0.53	216.26	110.90	0.51	0.99
CKDHL140994	6.22	72.97	1.43	210.73	111.65	0.53	0.96
CKDHL140995	5.94	73.90	0.58	212.66	115.10	0.54	0.97
CKDHL142737	6.94	73.36	0.32	220.00	114.83	0.51	0.98
Pioneer 2859	5.95	70.28	2.24	208.01	94.03	0.45	0.98
CKDHL142743	6.40	72.71	0.81	209.20	105.16	0.51	0.97
CKDHL142745	6.92	73.02	0.45	214.35	116.90	0.54	0.98
CKDHL142746	6.63	73.60	0.84	212.18	114.54	0.54	0.98
CKDHL142750	6.10	74.14	0.88	210.29	111.69	0.54	0.97
CKDHL142754	6.62	73.58	1.14	211.16	109.97	0.52	0.97
CKDHL142755	7.28	74.24	0.30	210.58	114.44	0.55	0.98
CKDHL142757	6.84	74.34	-0.08	212.90	105.27	0.49	0.97
CKDHL142758	5.87	72.87	0.73	207.27	107.65	0.52	0.96
CKDHL142759	6.84	73.62	-0.15	211.24	113.08	0.54	0.97
CKDHL142762	7.09	74.46	1.02	216.98	115.19	0.52	0.97
CKDHL142764	6.21	73.70	0.30	211.75	108.76	0.52	0.98
CKDHL142767	6.27	73.97	-0.50	211.63	112.53	0.53	0.98
CKDHL142772	6.50	72.71	1.59	218.22	112.51	0.51	0.99
CKDHL142773	6.32	73.04	-0.59	217.29	118.32	0.54	0.98
CKDHL142774	6.13	73.44	-0.23	218.80	122.01	0.55	0.98
CKDHL142777	6.64	72.95	1.23	215.18	112.12	0.52	0.96
CKDHL142782	6.75	71.83	-0.18	213.77	116.12	0.54	1.00
CKDHL142784	6.28	72.95	0.32	211.55	108.08	0.51	0.96
CKDHL142786	6.52	73.52	1.13	215.38	111.04	0.51	0.97
CKDHL142787	5.99	74.49	0.31	212.32	112.68	0.53	0.97
CKDHL142790	6.73	74.40	-1.09	218.23	112.35	0.51	0.98
CKDHL142792	6.66	73.02	-0.09	214.50	113.49	0.53	0.97
CKDHL142797	6.31	72.85	0.75	208.74	110.95	0.54	0.97
CKDHL142800	6.55	74.06	0.30	214.60	112.30	0.52	0.97
CKDHL142801	6.28	73.13	0.81	211.73	107.10	0.51	0.97
CKDHL142803	7.31	72.17	0.88	219.64	119.31	0.53	0.98
CKDHL142804	6.49	74.35	1.28	213.75	107.10	0.50	0.98
CKDHL142807	6.20	72.38	0.20	210.94	107.39	0.51	0.97
CKDHL142817	6.30	73.36	-0.14	211.01	113.10	0.54	0.99
CKDHL142819	6.92	72.80	-0.27	217.42	117.03	0.53	0.98
CKDHL142821	6.94	72.11	0.39	212.43	114.58	0.54	0.99
CKDHL142823	6.41	73.05	0.67	211.36	114.68	0.55	0.97
CKDHL142824	6.78	74.29	1.26	219.09	116.06	0.52	0.98
CKDHL142825	6.40	71.85	1.41	213.42	108.70	0.51	0.98
CKDHL142829	6.95	71.78	0.32	208.82	105.87	0.51	0.99
CKDHL142835	6.84	73.90	0.88	214.63	114.75	0.53	0.99
CKDHL142840	6.15	73.32	-0.25	211.18	110.26	0.53	0.97
CKDHL142841	6.39	72.32	0.77	208.56	98.77	0.48	0.98
CKDHL142845	6.18	73.36	0.97	216.04	110.73	0.51	0.96
CKDHL142848	6.36	73.94	0.15	208.46	105.35	0.51	0.96
CKDHL142855	6.45	73.29	-0.27	209.95	107.03	0.52	0.98
CKDHL142856	6.77	71.27	-0.26	212.36	109.02	0.51	0.97
CKDHL142858	6.60	75.41	1.28	216.14	119.79	0.55	0.98
CKDHL142859	6.63	73.51	0.65	214.98	110.28	0.51	0.98
CKDHL142861	6.87	73.40	0.53	217.85	112.38	0.51	0.97
CKDHL142865	6.28	73.37	0.93	209.69	110.34	0.53	0.97
(CML395/CML444)//CML536	6.55	71.25	1.24	209.73	103.11	0.49	0.96
DH04	5.07	71.40	1.88	201.40	96.72	0.49	0.96
WE1101	6.30	71.47	1.42	204.46	102.11	0.50	0.97

Genotype	GYF	AD	ASI	PH	EH	EPO	EPP
H517	6.26	74.28	2.05	215.73	116.24	0.54	0.96
DK8031	5.75	71.41	1.02	209.75	103.02	0.49	0.97
Duma 43	5.72	68.10	2.63	206.16	93.36	0.45	0.96
WH505	6.59	73.84	1.04	218.83	110.38	0.50	0.97
Pioneer 3253	5.59	72.17	1.53	211.52	111.47	0.53	0.96
Location	**3**	**3**	**3**	**3**	**3**	**3**	**3**
Replication	**2**	**2**	**2**	**2**	**2**	**2**	**2**
Error Variance	**1.52**	**1.59**	**1.48**	**79.96**	**48.36**	**0.00**	**0.01**
Genotypic Variance	**0.36**	**1.43**	**0.70**	**31.18**	**40.25**	**0.00**	**0.00**
GenxEnv Variance	**0.41**	**0.36**	**0.00**	**26.68**	**7.35**	**0.00**	**0.00**
Location Variance	**3.53**	**71.43**	**1.54**	**74.41**	**124.96**	**0.00**	**0.03**
Heritability	**0.48**	**0.79**	**0.74**	**0.58**	**0.79**	**0.82**	**0.15**
Grand Mean	**6.43**	**73.26**	**0.50**	**212.59**	**110.51**	**0.52**	**0.97**
LSD	**2.42**	**2.47**	**2.38**	**17.53**	**13.63**	**0.04**	**0.23**
CV	**19.18**	**1.72**	**244.61**	**4.21**	**6.29**	**3.86**	**12.29**

GY, Grain yield; AD, Anthesis date; ASI, Anthesis silking interval; PH, Plant height; EH, ear height; EPO, ear position; EPP, ears per plant; LSD, least significant difference; CV, coefficient of variation; GenxEnv; genotype by environment interaction

Appendix Table 17. Result of principal component analysis for 411 inbred lines genotyped with

No	PC1	PC2	PC3
1	-16.243383	48.873330	-26.369300
2	7.389876	52.550327	-70.002820
3	-52.137466	-67.084440	-16.277195
4	-23.263048	-15.055714	-2.087284
5	-50.622856	-59.951523	-15.107074
6	-47.691970	-51.138058	-12.579416
7	-53.437360	-70.762150	-18.019970
8	-62.364346	-92.870060	-27.465094
9	-49.670870	-70.570045	-20.741470
10	-59.049713	-90.487460	-26.043715
11	-27.433796	-22.096296	-5.256089
12	-16.853493	4.434556	6.691100
13	-9.682805	10.254232	4.630742
14	-43.652058	-60.288963	-16.458660
15	-44.916220	-58.259490	-14.089019
16	-16.595210	9.708626	6.623415
17	-19.831638	8.321232	6.255699
18	-15.494673	6.506329	4.941979
19	-20.293053	6.701767	6.648337
20	-22.534050	2.590622	5.139554
21	-17.952208	5.686372	7.052828
22	-21.068731	0.917902	4.262576
23	-22.230703	7.903515	5.978023
24	8.423815	51.925583	-70.270256
25	-15.023202	6.080649	4.418083
26	-31.205410	-6.994476	6.978552
27	-24.001568	0.952518	8.455349
28	-19.605420	6.821126	9.479897
29	-50.184044	-67.448944	-16.703045
30	-11.668084	6.247771	5.474712
31	-13.548815	2.119290	3.518324
32	-17.493065	36.309660	-7.607456
33	-12.483522	10.530193	5.850214
34	-4.597188	6.269019	3.786295
35	-8.838636	12.783917	2.895704
36	-2.360455	5.817859	2.764950
37	-17.227242	9.401948	10.243199
38	-5.706706	11.097036	1.782040
39	-46.247524	-52.879200	-12.860069
40	-37.644120	-50.471302	-12.873481
41	-14.884522	31.233503	-4.695447
42	-16.574066	3.728697	4.586390
43	-17.058462	30.183330	-9.455002
44	17.887741	1.211774	25.145018
45	-16.653740	13.911733	0.572827

No	PC1	PC2	PC3
46	-27.859070	-29.067480	-6.879476
47	-12.859930	14.202847	3.767288
48	-17.214294	44.515186	-19.837955
49	-12.572125	6.813399	5.707744
50	-14.404499	7.795505	2.495768
51	-24.703735	6.270719	7.520614
52	-12.603912	6.930896	3.209535
53	-14.149853	4.638361	5.683487
54	-13.620945	9.289996	1.925229
55	10.981978	8.939809	4.778213
56	-13.292666	5.649009	3.138156
57	-11.600897	4.558088	5.951469
58	-25.071768	16.249350	8.993066
59	-22.733013	9.157388	10.703800
60	-13.623914	3.037699	3.393083
61	-10.844597	5.710959	7.241286
62	-14.089957	8.670948	7.429631
63	-12.712843	7.103751	4.686125
64	-14.799241	6.245054	7.273068
65	-8.049936	8.025227	4.637154
66	25.377625	-2.408567	16.920660
67	-13.178410	8.458851	4.848613
68	-14.737577	3.361867	4.972931
69	-12.537771	4.466522	5.541878
70	-17.477053	6.325963	5.992473
71	-14.969786	9.250690	5.872172
72	-10.491864	6.905998	8.938708
73	-12.188111	10.480496	8.965897
74	-12.872204	8.894132	8.050378
75	-14.608897	8.611940	6.259050
76	-13.860414	8.841633	5.735132
77	21.792046	-0.179033	16.508272
78	-12.752872	6.388236	9.833362
79	-11.045283	3.369491	3.432015
80	-10.971808	6.825589	7.388082
81	-15.062544	7.169719	7.114617
82	-12.419254	5.314645	7.011631
83	-14.069731	8.728868	7.489280
84	-17.647121	8.196420	6.910973
85	-10.553951	6.215406	4.109916
86	-11.729274	8.018117	6.783207
87	-10.857064	8.687329	8.685577
88	3.197033	5.968895	3.974578
89	-12.452273	8.179776	10.092060
90	-9.258780	7.121124	6.226462
91	-9.093021	6.572987	4.484071

No	PC1	PC2	PC3
92	-31.566350	20.147242	22.630280
93	-31.780700	18.991808	21.172531
94	-30.919052	18.180504	21.802158
95	-34.223213	18.317003	24.494524
96	-29.060915	9.612872	20.745094
97	-29.892698	15.724861	22.601028
98	-35.997456	21.587486	22.070007
99	16.682436	2.207050	14.892608
100	-29.992134	15.940281	23.902690
101	-38.717800	18.501070	28.619380
102	-37.080288	19.325018	26.828817
103	-39.080837	19.682253	27.375930
104	-36.704323	20.076508	27.257393
105	-36.640438	20.752987	23.861525
106	-32.948610	17.902308	21.869745
107	-29.760637	16.140985	20.340912
108	-35.215530	21.466665	21.740133
109	-36.370300	21.570915	23.280706
110	-11.476675	57.102985	-31.385437
111	13.230061	6.335561	3.064055
112	-8.834486	5.542555	5.419015
113	-9.031072	5.976809	2.167398
114	-15.415455	5.163619	8.156080
115	-14.783958	7.328516	3.394504
116	-14.407894	9.881993	4.243189
117	-14.189702	6.199713	3.704130
118	-16.868608	6.303293	4.508424
119	-17.095020	5.815637	5.143848
120	-47.910680	-63.691498	-17.315851
121	-53.783150	-69.665810	-16.203909
122	-7.341308	6.930556	7.169917
123	-56.336730	-76.688220	-17.957714
124	-52.916603	-72.831620	-19.594193
125	-42.594460	-45.809746	-9.837740
126	-42.435460	-46.618150	-10.017756
127	-38.372460	-30.763649	-0.621264
128	-22.344652	4.253779	6.655475
129	-22.623060	8.726175	8.443767
130	-27.621038	13.162190	11.977270
131	-15.580428	7.519162	9.887502
132	-17.244278	4.850627	4.604058
133	-4.127794	9.265995	3.736258
134	-14.989118	5.238929	5.392354
135	-19.846079	4.848649	6.924280
136	-13.412946	6.818857	3.710150
137	-15.809544	5.708764	5.225125

No	PC1	PC2	PC3
138	-14.160439	7.578925	3.799427
139	-32.791420	10.610376	16.460900
140	-61.072170	-90.236990	-23.429667
141	-44.183655	-31.681301	1.897009
142	-53.970535	-75.727120	-20.490444
143	-6.583030	11.093995	3.060973
144	-38.787006	-27.901438	1.238525
145	-24.714869	-0.666698	6.335804
146	-22.604550	0.034792	5.297899
147	-22.563350	7.697858	5.798070
148	-21.914670	3.609788	7.269597
149	-46.613407	-68.080246	-16.551746
150	-42.458990	-58.026367	-15.294249
151	-24.400255	-14.228542	-1.665144
152	-13.639504	8.096587	9.411564
153	-21.154007	7.135261	5.869829
154	-13.485365	8.527912	9.169869
155	-17.781181	8.969903	6.962685
156	-15.448792	9.756202	5.916226
157	-20.224192	-7.623990	-0.679128
158	67.271060	-18.969387	-17.627853
159	66.736060	-20.139906	-10.722947
160	62.782913	-14.823294	-31.647022
161	66.037360	-18.100214	-15.666462
162	68.723050	-19.433641	-20.841948
163	67.768616	-19.219927	-19.219296
164	-11.454817	35.758812	-16.025831
165	43.471325	-1.144643	-57.871212
166	64.952670	-16.667751	-24.773132
167	58.384285	-17.297514	-5.797850
168	59.661250	-18.684750	-4.669006
169	70.854996	-18.876411	-27.179533
170	68.033160	-18.482006	-23.409712
171	56.392190	-21.094585	15.320133
172	54.690600	-20.055428	17.163013
173	56.619553	-20.617462	20.007440
174	55.982544	-20.801450	19.628696
175	13.148166	-3.241024	22.464964
176	61.191677	-23.631262	22.317797
177	-15.454728	26.908752	-8.273258
178	85.330240	-37.701970	35.908546
179	73.987724	-30.534664	29.535194
180	76.648340	-32.651283	31.730698
181	80.610040	-35.656906	32.785305
182	79.713715	-34.347275	38.219060
183	84.374670	-35.865345	39.029360

No	PC1	PC2	PC3
184	83.567345	-37.312004	40.690628
185	17.123238	-0.365021	31.424826
186	60.261010	-19.822052	42.664000
187	37.040990	0.555221	-40.332394
188	40.163740	-1.023621	-50.103240
189	38.716396	1.939025	-58.803360
190	33.142708	2.997083	-52.197090
191	63.767982	-11.872390	-27.712053
192	80.323350	-34.472874	38.818203
193	40.080795	0.629995	-46.849390
194	22.872486	0.481047	2.128347
195	24.496515	-0.264401	5.056400
196	-13.818665	8.889195	7.893156
197	40.468754	-0.037787	-43.393000
198	46.017090	-9.025153	27.477388
199	51.248753	-11.329862	31.690413
200	38.630790	-5.450174	19.732162
201	48.156070	-3.533975	-43.282500
202	60.530804	-3.812833	-60.331160
203	54.209890	-3.255326	-47.806820
204	55.968540	-4.607383	-50.725280
205	54.546173	-3.860223	-60.551136
206	43.003887	-3.562097	-30.220303
207	3.498748	7.349884	7.163178
208	63.314630	-1.379956	-97.431694
209	28.390766	1.067341	-19.147417
210	51.679790	-9.903675	-2.433519
211	37.925240	-0.155926	-29.156746
212	-15.127428	19.359135	-0.256712
213	41.481550	0.790760	-49.190926
214	33.922634	-0.787025	-23.307493
215	65.285300	-16.928963	-27.253107
216	39.106133	-3.111575	-6.999508
217	26.469790	1.805865	-3.425015
218	-11.842079	55.081120	-30.432749
219	38.402466	-3.221863	-30.029032
220	39.992430	-1.180609	-34.308440
221	46.429210	-5.026852	-27.830526
222	46.472443	-5.486017	-20.706362
223	36.310825	-4.004492	-1.132587
224	32.028168	-2.279357	4.944140
225	36.776897	-5.401888	7.194359
226	38.302128	-0.018148	-32.369583
227	36.959595	0.473393	-31.620400
228	33.704845	0.063380	-25.218735
229	-11.666175	6.995253	5.031874

No	PC1	PC2	PC3
230	44.970932	-6.515424	-7.306543
231	47.061947	-6.809609	-9.475068
232	29.281546	-0.966787	-15.595231
233	59.797530	-3.508132	-62.321857
234	42.722200	-5.093417	-24.987864
235	54.688038	1.247413	-83.694275
236	29.635500	0.412849	-1.852599
237	43.443623	1.331758	-37.874928
238	56.703690	-14.176954	32.205444
239	44.711510	-7.265095	-2.793298
240	-0.364388	8.649075	1.227060
241	67.345880	-0.714605	-103.636460
242	42.574932	-10.295965	12.835671
243	25.552284	-6.554469	6.170622
244	65.153550	-20.163230	56.539288
245	22.233980	0.480100	1.002505
246	9.063057	1.632889	13.174299
247	71.288410	-22.660707	62.162502
248	67.328810	-21.604504	57.633366
249	79.149450	-25.888266	69.888320
250	32.516260	-5.094782	7.601838
251	-12.410739	36.884872	-24.078197
252	20.817904	2.339456	-0.680770
253	23.278630	-1.394234	10.173285
254	20.809736	3.911487	4.110687
255	34.383446	-5.976728	11.541938
256	46.949314	-10.262035	36.064380
257	19.905981	1.354673	-0.266712
258	66.313416	-20.374731	55.204200
259	78.386410	-25.608053	69.287370
260	76.694885	-25.322035	64.808500
261	6.106368	8.008801	-10.707316
262	23.203938	0.060775	2.061063
263	78.815575	-25.179317	66.975850
264	18.299244	2.317394	3.406637
265	63.495100	-19.093224	53.445820
266	18.616064	6.876793	0.307980
267	3.228830	7.367866	-0.977756
268	11.324665	5.416506	-1.718621
269	17.149303	7.538076	-5.258831
270	15.867407	5.568116	-1.693711
271	17.222760	6.820682	-2.137176
272	-2.550443	6.599286	3.506983
273	16.145014	6.503809	-2.990396
274	12.610364	6.610253	-2.204240
275	17.953382	7.563151	-7.976736

No	PC1	PC2	PC3
276	1.029319	9.874389	0.259546
277	-3.458332	19.100336	-2.658013
278	1.876881	7.341763	2.726399
279	-0.376328	11.666719	-0.196384
280	79.542854	-34.068130	38.689663
281	6.090427	7.950822	1.502499
282	0.941723	8.529410	0.626163
283	1.797354	6.067828	-2.602703
284	-0.031232	9.150204	-0.349473
285	1.964184	8.293264	-0.669680
286	2.097628	6.588617	0.507299
287	-9.923276	33.349735	-17.573742
288	-19.881413	35.455364	-16.570670
289	1.408119	6.512645	-2.919854
290	2.798255	6.802231	0.956606
291	-3.560033	10.177031	3.114764
292	1.222721	6.970252	-0.967194
293	-6.744124	8.196758	1.141991
294	-5.731595	6.434172	3.216961
295	-1.489846	7.076368	-0.546207
296	8.906957	8.488194	-4.381631
297	-5.653339	9.496784	3.400419
298	0.507132	5.638680	2.292961
299	-9.045040	10.847008	9.582475
300	10.482154	10.134285	-4.453057
301	9.642318	9.883559	-4.424238
302	10.692192	8.301210	-3.228479
303	14.160154	5.702766	-1.110165
304	22.166500	5.048202	-29.197730
305	4.807844	23.151514	-24.936880
306	7.831222	5.850400	9.899175
307	41.567043	-5.938552	27.333630
308	-1.882045	11.350819	2.179997
309	-6.127422	22.256102	-9.808734
310	-12.996940	16.484556	-1.942409
311	-2.815762	12.118695	-0.510474
312	4.910111	13.347812	-2.300071
313	1.380109	7.889229	-4.344368
314	-11.780241	52.762840	-33.116547
315	4.553722	4.928127	2.507404
316	2.352872	15.238684	-0.804850
317	5.738178	6.111350	0.560412
318	-14.321651	11.020443	6.946956
319	-20.044136	11.222850	5.481003
320	-2.143755	20.050220	-4.443878
321	2.500321	16.674072	-5.974592

No	PC1	PC2	PC3
322	1.416956	18.124771	-5.408719
323	-5.496597	9.290991	-0.925536
324	-6.139142	8.582906	3.369519
325	-13.827488	46.181797	-23.437618
326	-2.070604	17.782433	-2.545512
327	-10.378391	8.966937	6.057617
328	0.791998	4.144553	0.873675
329	-19.897543	39.288090	-21.036957
330	-12.531514	44.652596	-22.480469
331	-10.014281	6.883248	5.178026
332	-14.784730	4.819964	5.672903
333	-16.025732	5.449589	4.814080
334	-8.742647	5.132654	2.722098
335	-5.779951	10.654720	1.872205
336	-10.984761	9.368104	7.207510
337	-11.932843	6.397111	3.682736
338	-4.201977	6.349808	-0.065646
339	-12.791472	10.229620	6.058208
340	-9.125365	10.757843	9.654110
341	-10.174750	8.582414	6.961723
342	-7.905011	7.820471	7.103775
343	-14.087025	5.223173	5.005244
344	4.347150	18.215714	3.241508
345	-23.182804	6.601310	11.589778
346	-18.683962	5.213479	4.609398
347	0.091750	10.606695	4.968480
348	-31.173662	16.843540	13.511161
349	-5.538593	10.926230	-1.249773
350	-25.098492	7.795195	9.756064
351	-32.955250	17.474543	20.699291
352	-19.676210	7.858041	7.591897
353	-19.218020	11.165469	9.466269
354	-12.686352	7.629545	5.437931
355	-3.490756	3.731282	1.552165
356	-23.882118	11.134024	10.771655
357	-15.819973	14.622705	6.909565
358	-20.693485	11.411490	5.592914
359	-24.520061	4.531214	6.800470
360	-13.399209	4.907741	7.354970
361	-20.514520	-2.129590	6.043839
362	-4.040606	6.092255	0.744305
363	-4.278945	6.259499	0.547567
364	-6.879919	13.747049	2.325210
365	-24.319298	-0.308566	5.957607
366	-32.521040	-12.587218	2.351446
367	-23.821745	-4.465890	3.819689

No	PC1	PC2	PC3
368	-9.867004	48.903180	-24.900177
369	-67.968060	-110.466370	-31.190443
370	-20.253530	6.835714	8.993275
371	-28.710222	17.791805	12.462839
372	-32.338318	14.513310	19.491400
373	-34.357647	19.567331	19.904871
374	-34.275790	19.558685	20.749985
375	-32.387110	18.037912	19.712523
376	-24.759186	1.167103	6.245953
377	-16.920761	7.486058	5.807246
378	1.185692	7.013800	0.104394
379	-8.963135	60.058240	-34.878265
380	-20.688606	2.978667	2.547179
381	-6.909079	8.066040	4.073292
382	-7.333234	45.233890	-19.273180
383	-10.900648	5.681895	4.196868
384	29.876003	-7.021388	39.554176
385	-30.900215	16.046305	13.146537
386	-4.786295	6.939579	4.984197
387	-58.769592	-89.112050	-23.926840
388	-24.908266	45.706450	-18.459076
389	-18.217432	10.068589	7.080101
390	-10.951520	59.391514	-35.737717
391	-24.873104	7.579076	9.635488
392	-35.734715	16.334883	20.716696
393	-61.589684	-95.957085	-28.058752
394	-19.917088	4.031317	4.910327
395	-50.190453	-69.474525	-17.322716
396	-59.390705	-94.174400	-27.485025
397	-19.005419	10.962839	9.386340
398	-9.053308	12.067443	5.568955
399	-20.957388	7.113495	5.818850
400	-23.156982	3.434444	7.478922
401	5.801232	54.354496	-70.021034
402	-24.280207	9.604489	9.274888
403	-24.034780	6.434568	8.426351
404	-23.777578	4.778902	9.874414
405	-24.241577	11.401786	11.955909
406	-25.577900	3.689748	7.647245
407	-26.321493	4.435512	6.997468
408	-31.351027	-20.784353	-1.578520
409	-25.733477	-3.792861	5.083935
410	-29.689460	9.754638	14.927705
411	-48.811775	-64.509766	-16.019838