USE OF INBRED LINESECONDARY TRAITS IN PREDICTING PERFORMANCE IN MAIZE (*ZEA MAYS L*) HYBRIDS UNDER LOW NITROGEN

BY

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Declaration

I KanyantaMusonda hereby declare that this thesis represents my original work and has never been previously submitted nor will it be submitted to any other University for any award of degree, diploma or certificate. Where sources of information from work by other authors have been cited, due acknowledgement has made in the text.

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Approval

This dissertation of MusondaKanyanta was approved as fulfilling part of the requirements of the award of Master of Science in Plant Breeding and Seed Systems by the University of Zambia

Examiner's Name and signature Date

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Dedication

To my wife Chikondi and my children Emmanuel, Kanyanta and Chikondi

Abstract

Maize (*Zea mays L)* is an important multi-purpose staple cereal crop in Zambia.More than 70% of people derive their livelihood from agriculture of which maize production is the major enterprise. Maize production is affected by biotic and abiotic stresses. Most varieties developed in the Zambian National Maize Breeding Programmes have no tolerance to low nitrogen stress. This is so because variety selection is done under optimum N conditions and thereafter released to farmer conditions that are mostly Nitrogen stressed. To predict performance of hybrids on the basis of their inbred lines', secondary traits of inbred lines were investigated to establish their relationships with grain yieldof their respectivehybrids under optimum and Low nitrogen levels. Therelationships between hybrid grain yield and yield secondary traits of inbred lines under optimum and Low nitrogen levelswere determined. Yield secondary traits studied included Ears per plant (EPP), Chlorophyll content (CC), Plant height (PH) and AnthesisSilking Interval (ASI).Thirty seven (37) Hybrids, their parents and three local checks (MRI 624, MRI 514 and ZMS 606) were evaluated in a randomised complete block design (RCBD) under optimum and low N conditions at Golden Valley Agricultural Research Trust (GART) Chisamba farm.Under optimum N plant height and Chlorophyll content significantly (45% and 26%, respectively) affected grain yield of inbred lines.For hybrids, plant height and ears per plant had significant influenceof 11.4% and 37%, respectively on GY under low N conditions. Also, Plant height had significant influence (35%) on GY under optimum conditions. Plant height and Chlorophyll content of inbred lines had a significant influence of 28 % and 7% respectively on hybrid grain yield. Plant height of inbred lines had a significant influence of 28 % on hybrid grain yield. Indeed,Chlorophyll content showed a significant influence of 7 % of the total variation in Hybrid grain yield. Conclusions were that low N depresses performance, in terms of yield, of both inbred lines and hybrids. The reduction in yield is associated with a reduction in EPP, PH and CC and an increase in ASI. Plant height influenced grain yield of the inbred lines under low N stress conditions. Under optimum conditions it was plant height and CCthat positively influenced grain yield. For hybrids under low N conditions, plant height and ears per plant positively influenced grain yield of the hybrids, while under optimum conditions, only plant height positively influenced grain yield. Under low N conditions, plant height and CCof inbred lines positively influenced hybrid grain yield. Thus,if inbred lines with increased chlorophyll content and plant height under low N are selected, they would give Hybrids with higher yields under small holder farmer conditions which are generally low N conditions in Zambia.

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CHAPTER 1: INTRODUCTION

Maize (*Zea mays L)* is an important multi-purpose cereal crop used for food, fodder, and bio fuels all over the world and it accounts for 15% to 56% of total calories intake of people in developing countries (FAO 2011). Total world maize production was 817 million tons in 2009 and this was grown on about 6 million hectares which is higher than cultivated land area under wheat or rice. About 62 million or one third of all malnourished children live in farming systems where maize is the most important crop. The relevance of maize for poor farmers is only surpassed by rice, (Hyman et al., 2008).

Maize is a staple crop in Zambia and many other African countries. It is a cornerstone of the Zambian agricultural economy and government agricultural policy as it accounts for 25-30% of the gross value of smallholder crop output and 40% of the country's calorie intake (Zulu et al., 2006). More than 70% of people derive their livelihood from agriculture of which maize production is the major enterprise (Govereh et al., 2008). Maize is Zambia's number one commodity in terms of value, second after sugarcane in production and fourth in exports after sugar, cotton, and tobacco (FAO 2011). It represents an estimated 41% of gross farm household income and 33% of total household crop sales (Chapoto et al., 2011).

The average yield of maize in Southern Africa in 2011 was 1.2 tons/ha while the overall picture in Africa was 1.4 tons/ha (FAO 2011). Average maize yield in Zambia was 1.3 t/ha (CSO/MACO/FSRP 2011). There are three categories of farmers in Zambia that grow maize. They are categorised on the basis of their area under production, amount of inputs used and extent of mechanisation (CSO/MACO/FSRP 2011). The first category is that of commercial farmers who on average cultivate more than 10 ha of land and their production systems are highly mechanized. The second category of maize growers is that of emergent farmers that mostly utilize ox- drawn power. Their average farm size ranges between 5 and 10 ha of land under production and contribute 20% to the total production. The third category is that of small holder farmers who contribute 75% to the total national basket in terms of Maize production. They use less than 5 hectares of land under production. In most cases small holder farmers use land that is under customary tenure system. They usually employ manual family labour and to a lesser extent animal draft power at most stages of production.

The system of production is characterised by use of little or no fertilisers at all due to financial constraints. Yields under this category are less than the national average, 1.3 tons/ha, largely due to low Nitrogen stress and weed infestation. Jayne et al (2009) reported that the average application rate of nitrogen fertilizer in Zambia was 25 kg N/ha among small holder farmers. Most of the smallholder farmers that applied fertilisers in maize depended on government farmer inputs subsidy programme (GRZ-MOF 2005).

Out of the three main cereal-grain crops of sub-humid to semi-arid tropics (maize, sorghum, pearl millet), maize produces the highest yields when soil fertility is adequate and moisture is in sufficient quantities, but maize is the least tolerant to low nitrogen stress (Kumar et al., 2007). The savannas of Africa where Zambia lies have great potential for maize production due to their adequate rainfall status for the relatively long growing season, sufficient radiation levels, lower night temperatures and reduced incidence of pests and diseases (Ple'net andLemaire 1999).

Soils in most parts of West, Central and Southern Africa are mainly kaoliliniticAlfisols which are generally low in organic matter, cation exchange capacity and in nitrogen (Miti et al., 2010). Soils in Zambia are generally deficient in nitrogen (Ple'net andLemaire 1999). Beyond this inherent poor soil nitrogen status extensive land-use contributes to the poor soil fertility as a result of serious land degradation and nutrient depletion with nitrogen being the most depleted nutrient. This coupled with climate change in the tropics has led to poor availability of N for plant uptake hence seriously limiting productivity (Kling et al., 1996). Nitrogen deficiency has been identified as the primary constraint to maize production in the mid-elevation and tropical lowlands worldwide (Muruli and Paulsen 1981). These regions characterise the predominant maize production zones in Sub-Saharan Africa, where Zambia lies. To increase maize yields, farmers typically become dependent on chemical fertilizers, mainly nitrogen whose price keep on rising (Pandey et al., 2000).

Whereas there are many challenges in breeding for abiotic stresses such as low N stress tolerance, studies have indicated that there is sufficient genetic variation in maize germplasm to breed for superior nitrogen use efficiency in maize (Masclaux et al., 2000; Bänziger et al., 2000;Bänziger, 1999). It was reported that newly developed maize hybrids outperformed old ones even at reduced N rates (Bänziger, 1999) .This was because new maize hybrids had higher Harvest Index (HI), large and efficient root system during the growth period and a large proportion of N accumulated in roots at early growth stages was remobilized for grain growth in the late grain filling stage (Bänziger, 1999;Zeiger and Taiz, 2006).

When breeding for nitrogen stress tolerance, breeders typically evaluate hybrids under low N conditions. However, this process is lengthy and costly as breeders must form separate crossing blocks and evaluate all lines being developed. Also heritability and genetic variances for grain yield usually decrease under abiotic stress as yield levels fall. In addition, there is high genotype x environment interactions involved in stressed experiments thus making it difficult to identify the best germplasm under conventional breeding for low Nitrogen stress (Bolan˜os, and Edmeades. 1996). To overcome this challenge, Bänziger(1999) indicated that any yield component or pathway (secondary trait) that can be measured with less work and error than a final character (yield), and which is highly correlated with the final character (yield) under field conditions, would be useful as an aid to plant improvement. Thus if secondary traits affecting yield (i.e. Leaf Chlorophyll content, Anthesissilking interval, plant height and number of ears per plant) of inbred lines under low N, or optimal conditions are strongly correlated to hybrid performance, it is then possible to select lines early without subjecting them to crossing blocks and thereby reducing on costs and time for breeding for low N stress tolerance (Bänziger et al., 2000).

The overall objective of this study was to establish the basis of identifying superior maize inbred lines in hybrid combinations under low and optimum nitrogen levels.

Specific objectives were to:

1. Establish the relationships between grain weight and secondary traits of inbred lines and hybrids under optimum and low nitrogen levels

2. Determine the relationships between hybrid grain weight and yield secondary traits of inbred lines under optimum and low nitrogen levels.

CHAPTER 2: LITERATURE REVIEW

2.1 Overview of the maize plant

Maize is a monoecious diclinous species where individuals have separate unisexual florets (staminate and pistillate) which produce gametes of both sexes in physically separated parts of the same plant (Cheng and Paredy, 1994).The male inflorescence is a broad panicle consisting of a central spike and basal lateral branches whereas the unbranched grain bearing female inflorescence is produced several nodes below the tassel by an auxiliary bud (Cheng and Paredy, 1994). Generally maize plants have a single stem, called a stalk, which grows vertically upward from the ground. The height of the stalk depends both on the variety of the maize and the environment in which it is grown. As the stalk grows, leaves emerge. A typical maize plant grown in Zambia will have a stalk that is 1 to 3 metres tall and 16 to 22 leaves (Miti et al., 2010). The lower part of each leaf, the leaf sheath, wraps around the stalk and is attached to the stalk at a juncture called a node. Typically the lowest four nodes are below ground. Roots develop from each of these nodes. Sometimes, roots develop from the first aboveground node, and these are known as brace roots. Some varieties of Maize in certain environments produce secondary stalks, known as tillers, which grow outward from near the base of the main stalk (Tollenaar et al., 1999).

2.2. Nitrogen uptake and utilization

2.2.1 Maize nutrient requirements

Maize nutrients requirements depend on the yield potential of a particular variety and also the target yield that the farmer wishes to realize. In general, the fertilizerrequirements for maize in tropical conditions is about 100-120 kg N /ha, 40 kg P/ha and 50 kg K/ha (Yayock et al., 1988). Nitrogen rates of yield 170 kg N/ha, 45 kgN/ha and 20 kg N/ha N are recommended to achieve yield levels of 8 t/ha, 3 t/ha and 2 t/ha respectively. Nitrogen is applied within 8 weeks after planting, depending on rainfall distribution (Miti et al., 2010). Nitrogen is available to the crop as nitrate or ammonium in the soil over the life-cycle of the crop. Grain yield of a Maize plant is determined by Available Nitrogen (Bänziger et al., 2000).

2.2.2 Effects of Nitrogen Stress on Maize

Nitrogen in the plant is a component of chlorophyll the light capturing pigment. It plays a vital role in photosynthesis. Also, it is a part of proteins and enzyme systems in plants (Zeiger, E and Taiz, L 2006). Nitrogen stress reduces crop photosynthesis by reducing leaf area development and leaf photosynthesis rate and by accelerating leaf senescence. The number of rows per maize kernel is set by the time most tropical maize plants have 12-14 visible leaves and the number of kernels per row by the time 16-18 leaves are visible (Banziger 1999). The number of ovules that ultimately develop into mature kernels is affected by the extent of kernel abortion in the two weeks bracketing flowering (Below, 1997). Severe N stress delays both pollen shed and silking, but the delay in silking is relatively more, so that the ASI becomes greater under N stress at flowering. Also, the number of roots that develop under low N is less. (Bänziger et al., 2000).Scharf et al., (2002)reported that nitrogen stress affects different yield-determining factors depending on stage of development when the stress occurs, some of these factors such as leaf area development before flowering, number of ear spikelets and kernel and ear abortion during flowering may not recover when affected by the stress while others such as photosynthetic rate may recover when conditions became favourable.

2.2.2.1 Morphological responses to nitrogen in maize

Sufficient N fertiliser application increases grain yield because the number of spikelets per ear is increased unlike under N stress (Lemcoff and Loomis, 1986). The number of grains that develop from spikelets of fertilized plants is higher and grain weight increases as the level of N fertilization increase (Oikeh et al., 1998; Zeiger and Taiz, 2006; Muruli and Paulsen, 1981). Application of N fertilizer increases the length and diameter of maize ears (Pandey et al., 2000).Because maize evolved in the tropics where N is inherently deficient, natural selection has favoured the early uptake of N from the soil, its storage in leaves as photosynthetic enzymes, and its subsequent remobilisation to the developing grain during grain filling (Bruce et al., 2002). Nitrogen accumulation in the grain decreases under N stress (Bennett et al., 1989), thus, the demand for N by the growing ear is met by the remobilisation of N from the leaves and stem and delayed leaf senescence contributes to larger biomass gains under severe N stress (Zeiger and Taiz, 2006) .

Grain yield was found to be positively associated with shoot biomass and N content at anthesis under conditions of N deficiency (Muchow, and Carberry 1989). It was shown that differences in leaf N affected radiation use efficiency and, thus, biomass production. Plant height of N stressed plants has been shown to be a good indicator of biomass at anthesis (Lafitte and Edmeades, 1994). In contrast,Eghball and Maranville (1993) reported that nitrogen fertilization slowed down root growth and a smaller root mass, was associated with greater N availability, limited root penetration and, hence, reduces nutrient and water extraction from the soil.

2.2.2.2 Physiological responses to nitrogen in maize

The morphological and physiological responses of maize to low Nitrogen include smaller plants, reduced radiation use efficiency, accelerated leaf senescence after anthesis, increased mobilization of vegetative N to the grain, and a lower plant N concentration (Muchow and Carberry, 1989). Low Nitrogen stress delays shoot elongation and leaf growth, but increases root growth (Moll et al., 1994). Nitrogen deficiency inhibits photosynthetic capacity through decreased stromal and thylakoid proteins synthesis, including several key enzymes of the Calvin cycle such as phosphoenolpyruvate enzyme (PEPCase) and RibuloseBisphosphate Carboxylase enzyme (RuBPCase) which are major carbon-assimilating enzymes in C4 plants (Ding et al 2005. The capacity of carbon assimilation during photosynthesis is impaired by N deficiency (Ding et al 2005). Under high N supply, uptake of N depends mainly on the growth-related demand for N, whereas under low N, uptake of Ndepends on morphological and physiological characteristics of the plant (Presterl et al., 2002). Much of the effect of nitrogen deficiency on crops can be explained through radiation interception and radiation use efficiency that relate directly to photosynthesis, plant growth and carbon assimilation (Bänziger et al., 2000). Photosynthetic efficiency depends on many physiological and biochemical processes, such as stomatal and mesophyll conductance, photochemical capacity of PS II, and amounts and activities of carbon fixation enzymes. Chloroplasts contain 70–80% of the cell nitrogen (Lange et al.,1981). Photosynthetic performance requires proteins for all steps of the process including formation of the light-harvesting chlorophyll – protein complexes of the antenna (Bungard et al., 1997), so N deficiency affects these processes, depending on the severity.

The response of grain yield to N deficiency is mostly due to their different rates of accumulation of dry matter after anthesis. Consequently nitrogen deficiency reduces efficient function of each parameter and finally photo assimilates production and development (Chen et al., 2003).

2.3. Maize Yield components

2.3.1 Crop System Formation theory

Maize undergoes different stages to complete its life cycle. Maize grows in two main stages,that is vegetative and reproductive (Roth et al., 2013). Within each stage different growth stages are designated by different scales (Subedi and Ma 2009). Yield of any crop refers to the total amount of the part of plant harvested on a given area. In Maize, yield is usually referred to as the grain yield and folder yield (Subedi and Ma 2009). Yield can be divided into components of plants per ha, ears per plant (EPP), grains per ear (GPE) and grain weight per unit area (GW)(Bänziger et al., 2000).

2.3.2 Environmental stress effects on Source and sink relationships.

Grain yield is determined by the degree to which structures such as ears, kernels, and endosperm cells, which serve as repositories, or sinks, for assimilates are established. During the pre-flowering stage, maize establishes many ears and florets than can finally be filled. In two weeks bracketing flowering, the number of ears, kernels, and endosperm cells that are filled is determined (Bänziger et al., 2000). During grain filling, the supply of assimilates determines the extent to which ears, kernels, and endosperm cells established during flowering are filled. The timing and intensity of stress determine the extent to which the source or the sink limits yield. Low N stress occurring after flowering causes the leaves to senesce early affecting the supply of assimilates resulting in the plant having many small kernels. Because of the many ways stress can affect a maize crop, genotype x environment interactions are frequent (Bänziger, 1999).

2.3.3 Source

The total supply of assimilates (or nutrients or water) is determined by: The amount of a growth factor taken up by the plant, such as radiation, water, nitrogen and the efficiency with which that factor is converted by the plant into carbohydrates, proteins and lipids and the time available for acquiring the growth factor. If crop development is rapid, the time available for radiation capture is less than if crop development is slow. So in radiation limited situations, early maturing maize will yield less than late maturing maize. Under low N conditions, a considerable part of available Nitrogen to the plant (NA) is supplied by mineralization of organic soils, which proceeds at a rate determined by soil moisture, temperature, and the biological activity of the soil. Thus the time available to the crop to capture N released by mineralization will govern NA, and late maturing cultivars will therefore take up more N than early maturing cultivars. Stress from low N reduces leaf area (%RI) which is the fraction of incident radiation intercepted by green leaves (e.g., 45% overthe crop life cycle), if the stress occurs before flowering. At any time of crop development, stress reduces crop photosynthetic rate and therefore total assimilates available to the crop (Bänziger et al., 2000).

2.4. Environmental and climatic requirements of maize

Maize is a short-day plant with 12.5 hours/day being the critical photoperiod. Photoperiods greater than this may increase the total number of leaves produced prior to initiation of tasselling, and may increase the time taken from emergence to tassel initiation (Tollenaar et al.,, 1999). Maize requires well‐drained loamy soils and is relatively well adapted to a wide range of soils with pH 5.0 to 8.0. It is not as acid tolerant, but is more tolerant to low phosphorus (P) than other crops such as soybean. Aluminium toxicity could become a problem on soils with pH less than 5.0 (Al > 40%), which includes sandy soils. Maize is moderately sensitive to salinity, which reduces uptake of nutrients and decreases total dry matter production (Bänziger et al., 2000).

2.5. Breeding for low N stress tolerance in maize.

Uptake and translocation of N in maize are under polygenic control (Pollmer et al., 1979). A number of studies (Muruli and Paulsen, 1981; Ding et al., 2005; Gallais and Coque1, 2005), have shown that there is genotypic variation in Nitrogen use efficiency (NUE), thus enabling breeders to improve this trait. Indeed evidence has been established that maize grain yield can be affected by interactions between the genotype and the level of N fertilization. Generally, higher-yielding hybrids are expected to exhibit a higher average NUE, save for the differential responses when significant interactions are realized. Nitrogen use efficiency of a cultivar is generally determined by N- uptake efficiency which is the efficiency of a plant in recovery of N from the soil and N-utilization efficiency, which is the efficiency of a plant in the utilization of

N to produce grain yield. Grain yield is ultimately limited by N uptake and N-utilisation (Edmeades et al., 1999). Significant positive correlation were established between N accumulation and NUE suggesting that total N accumulation is more important than N utilization in plants for NUE (Hirel et al., 2001). Other reports suggested that N utilization could be more important in determining NUE at low N input (Gallais and Coque1 2005). In comparison with N-inefficient hybrids, N-efficient maize hybrids had a higher net photosynthetic rate at the kernel filling stage, although their N concentrations in the vegetative organs were similar (Chen et al., 2003). Nitrogen use efficiency has been defined as the production of grain yield per unit of N from soil and fertilizers (Hirel et al., 2001). Numerous studies (Pollmer, et al., 1979; Hirel et al., 2001;Perez-Velasquez et al., 1995)suggest that NUE and its related physiological traits such as N accumulation and re-translocation are mainly controlled by additive gene effects.Quantitative genetic approach by associating metabolic functions and agronomic traits to DNA markers study suggested that increased productivity in maize genotypes was due to their ability to accumulate nitrate in their leaves during vegetative growth and to efficiently remobilize this stored nitrogen during grain filling (Hirel et al., 2001).

2.6 Use of secondary traits to develop Low N stress-tolerant Maize

Reviews of (Bänziger and Lafitte 1997; Bolaños and Edmeades 1993; Bolaños et al. 1993; Edmeades et al., 1993; Lafitte and Edmeades, 1994) indicated that secondary traits were related to low N stress tolerance in Maize. Monneveux et al., (2005) reported that identification of low N tolerance related traits aid in the development of indirect selection for yield and marker assisted selection under low N. In addition, tolerance to high plant population density was proposed as an alternative breeding strategy to improve low N stress tolerance in maize. Grain yield under stress was strongly correlated with a high harvest index, more ears per plant, and a short anthesis-silking interval (ASI) and moderately correlated with delayed leaf senescence, high leaf chlorophyll content, and plant height (Edmeades et al., 1999). Banziger et al., 2000 reported that CIMMYT evaluated many secondary traits for their value in a low N breeding program under low N and estimated that selection gains were increased by use of secondary traits. Secondary traits that were found to be important in low N stress tolerance were ears per plant (EPP) which accounted for 20% N stress tolerance, its heritability was high that is to say, traits can be assessed precisely in the genotypes evaluated and are transmitted to the offspring of these genotypes (Falconer 1989), and its relationship with grain yield under severe N stress was high. Heritability and genetic variance of EPP increased as the severity of the N stress increased and the trait was measured by counting the number of ears with at least one fully developed grain and then dividing by the number of harvested plants. Another trait was leaf senescence which accounted for 20% of N stress tolerance, its heritability was high and the relationship with grain yield was medium to high. Selection for this trait was on the basis of delayed leaf senescence (stay-green) although a SPAD meter that measures the amount of Chlorophyll content in the leaves of the plant can be used to measure this trait. Anthesis-silking interval (ASI) was another secondary trait which accounted for 10% of N stress tolerance, it heritability was medium and the relationship with grain yield was also medium under severe N stress. Selection for this trait was on the basis of r reduced or negative ASI. Heritability and genetic variance for ASI increased as N stress became more severe.

2.7 Evaluation of Land races and Hybrids for low N tolerance

CIMMYT evaluated a wide range of landraces for low N tolerance. About 3% of land races compared favourably with eliteadapted germplasm for low N tolerance. Thus they suggested that, improving adapted elite germplasm for low N tolerance was better than working with landraces (Sallah et al., 1996). This was similar to the finding of Ajala et al., (2007) who reported thatattempts at developing N-efficient maize genotypes resulted in the identification of hybrids as having better Nitrogen use efficiency than open pollinated cultivars. In another study, D'Andrea et al., (2009) reported that Grain yield was always larger for hybrids than for inbred lines, but N deficiency affected the former more than the latter (average reduction in grain yield of 40% for hybrids and of 24% for inbred lines). They found that larger grain yield of hybrids than of inbred lines at zero N was associated to enhanced dry matter accumulation as a result of improved light interception during the life cycle of the crop andimproved biomass partitioning to the grain of the maize crop.

CHAPTER 3: MATERIALS AND METHODS

3.1 Location

The study was conducted at Golden Valley Agricultural Research Trust (GART) in Chisamba in the 2011/12 agricultural season. The research station is situated at 14.97° south, 28.10° East and altitude of 1148m above sea level. The Rainfall season at GART is generally from November to March with average rainfall amount being 800 -1000 mm.The land used for the research was used for Conservation Agriculture research previously. According to the cropping history, the previous crops grown on the experimental site was Maize under Conservation agriculture research. Soil samples were collected for analysis from experimental site prior to planting. The soils were classified as Clay loams other soil parameters were as indicated in table 3.

3.2 Materials used in the Study

Thirty seven (37) Hybrids and their parents including three checks (MRI 624, MRI 514 and ZMS 606) were used in the study (Table 1) giving a total of 40 treatments. These lines consisted of a diverse range(white, yellow, Quality Protein Maize, normal Maize) of improved and elite germplasm for low N stress tolerance tested over a wide range of environments from CIMMYT.

Table 1: Inbred lines and crosses evaluated at GART during the 2011/2012 season

INBRED LINE	HYBRID PEDIGREE
CML491	(CML491)/(CML444/CML395)
CLQRCWQ26	(CLQRCWQ26)/(CML444/CML395)
CLWN273	(CLWN273)/(CML444/CML395)
CML495	(CML495)/(CML444/CML395)
CLRCW96	(CLRCW96)/(CML444/CML395)
CLRCW98	(CLRCW98)/(CML444/CML395)
CLRCY030	(CLRCY030)/(CML444/CML395)
CLRCW106	(CLRCW106)/(CML444/CML395)
CLRCW105	(CLRCW105)/(CML444/CML395)
CLWN234	(CLWN234)/(CML444/CML395)
CLYN251	(CLYN251)/(CML444/CML395)
La Posta Seq C7-F64-2-6-2-2-B-B-B	(La Posta Seq C7-F64-2-6-2-2-B-B)/(CML444/CML395)
CLYN255	(CLYN255)/(CML442/CML312)
CLYN256	(CLYN256)/(CML442/CML312)
CLYN259	(CLYN259)/(CML442/CML312)
CKL5003	(CKL5003)/(CML442/CML312)
CLWQ212	(CLWQ212)/(CML442/CML312)
CLWN318	(CLWN318)/(CML442/CML312)
CLWN243	(CLWN243)/(CML442/CML312)
CLQRCWQ123	(CLQRCWQ123)/(CML442/CML312)
CLYQ216	(CLYQ216)/(CML442/CML312)
CLYQ231	(CLYQ231)/(CML442/CML312)
CLYQ296	(CLYQ296)/(CML442/CML312)
CLQRCWQ133	(CLQRCWQ133)/(CML442/CML312)
CLQRCWQ48	(CLQRCWQ48)/(CML442/CML312)
P30 C9 -119-1-1-2-1-1-B	(P30 C9 -119-1-1-2-1-1-B)/(CML442/CML312)
P30 HSRS C10-160-1-2-1-B	(P30 HSRS C10-160-1-2-1-B)/(CML442/CML312)
P30 HSRS C10-191-1-1-1-B	(P30 HSRS C10-191-1-1-1-B)/(CML442/CML312)
P30 HSRS C10-58-2-1-1-B	(P30 HSRS C10-58-2-1-1-B)/(CML442/CML312)
CLRCY039	(CLRCY039)/(CML442/CML312)
CLQRCWQ117	(CLQRCWQ117)/(CML442/CML312)
CLRCY040	(CLRCY040)/(CML442/CML312)
CLRCY044	(CLRCY044)/(CML442/CML312)
CLWN228	(CLWN228)/(CML442/CML312)
CLWN240	(CLWN240)/(CML442/CML312)
CLWN237	(CLWN237)/(CML442/CML312)
CLWN236	(CLWN236)/(CML442/CML312)
	MRI 624 (check 1)
	MRI 514 (check 2)
	ZMS 606 (check 3)

3.3 Crop management

Two planting conditions were used in this study involving optimal nitrogen and low nitrogen fields. Trials were planted under rain fed conditions on December 10, 2011.

Trials under optimal conditions were given basal and top dressing fertiliser on the basis of soil as shown in table 2. Basal fertiliser was applied at planting (at 400kg/ha compound D) while top dressing fertiliser (400 Kg/Ha of 60% Urea) was applied 30 days after planting. Both basal and top dressing fertilisers were manually applied. Land was ploughed conventionally by tractor and crop debris from previous seasons was removed.

Trials under low N were on a block which was depleted of N using a uniform crop of sorghum during the winter season of 2011/12. The sorghum was removed prior to land preparation. Neither basal nor top dressing fertiliser was applied to the trials.
Standard crop management practices such as weeding by hands. No pests of economic importance were noticed in all trials.

3.4 Experimental design

Trials were arranged in a Randomised complete block design with three replications. Each plot consisted of 1 x 5m rows. Each row had 21 planting stations (25 cm apart) with 75 cm between rows. Planting stations were double planted and later thinned to one plant per station to give an approximate population of 44,000 plants/ha. Two border rows were planted round about all the trials.

3.5 Data collection

Data was collected on parameters that were related to Nitrogen stress tolerance and included: Grain yield (GY), Ears per plant (EPP), Chlorophyll content (CC), Plant height (PH) and AnthesisSilking Interval (ASI). These parameters were as described by Bänziger et al (2000).

i. Grain yield

Harvested ears were shelled to obtain grain weight per unit area in kilograms. Grain weights were then adjusted to12.5% moisture and converted to tons per hectare using the formula:
GY (t/ha) = [grain weight (per plot) X (100- moisture content) X 10000]/ (87.5 x plot size x 1000)
Moisture content of a sample of kernels for each sample plot was measured using a hand held moisture meter (Dickey John Mult grain model 46233-12223A)

ii. Ears per plant (EPP)

At harvest, the total number of plants harvested and Number of ears harvested in each plot were recorded. Then an average number of ears per plant was derived by dividing the total number of ears harvested by the total number of plants harvested in each plot.

iii. Anthesissilking interval (ASI).

Days to male flowering (anthesis) were recorded when approximately 50% of the plants in the plot were shedding pollen, and days to female flowering (silking) were recorded when approximately 50% of the plants in the plot had extruded silks. The ASI was calculated as the difference between days to silking and days to anthesis.

i. Chlorophyll Content (CC).

At the first sign of anthesis in each experiment, chlorophyll content readings were taken using hand-held MINOLTA 502 SPAD (Soil Plant Analysis Development) meter (Minolta Camera, 1989) and recorded in SPAD units. The metre calculates the ratio of absorbance at 650 nm (chlorophyll absorbance peak) and at 940 nm (non-chlorophyll absorbance). Readings were taken on the third leaf from the tassel and on the ear leaf during flowering. The measurements were taken on five alternating plants from each plot, midway between the stalk and leaf tip and between the midrib and the edge of the leaf and the average reading was recorded.

Figure 1 : Chlorophyl content data collection using SPAD meter at GART- Chisamba in 2011/2012 season.

Figure 2: Plant height data collection at GART – Chisamba in 2011/12 season

Plant height was measured using a 5m ruler from the soil surface to the tallest tassel branch as shown in picture 2 above.

3.6 Data Analysis

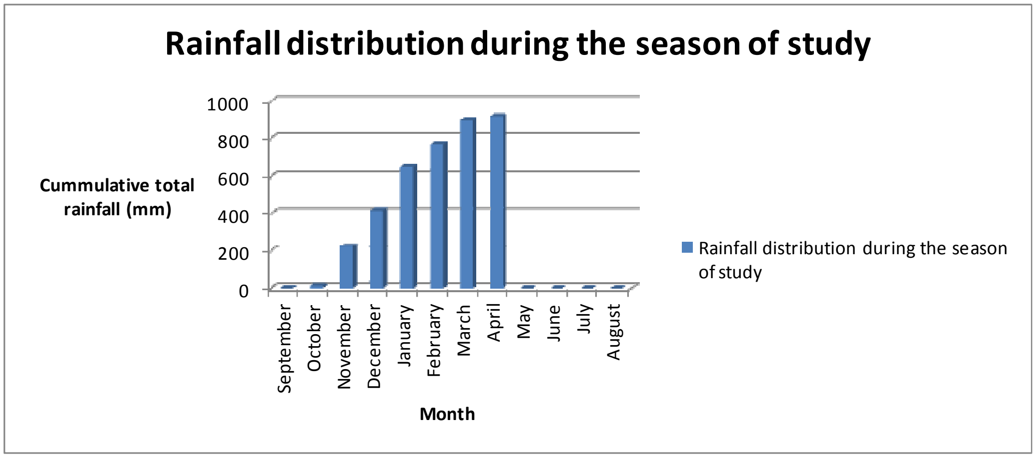
The collected data on different components were compiled and analysed statistically using the GENSTAT 13^{th} Edition and IBM-SPSS 20^{th} Edition computer statistical packages. Data were subjected to analysis of variance (ANOVA) using General treatment structure in Randomised Block Model procedure. Means were separated using Fisher level of significance(LSD). Simple linear regression analysis was used to measure character associations. In order to study the cause and effect relationship between variables measured on grain yield, a stepwise multiple regression analysis was carried out.

CHAPTER 4: RESULTS

4.1. General

Soil type, Climate (temperature, rainfall, relative humidity) data were collected during the research period as shown below.

Table 2: Weather Parameters during the study period



Soil pH was within the acceptable range for maize and therefore acidity could not have had an effect on Nitrogen uptake on the Maize plant. Equally other soil parameters as indicated in Table 4.2 below could not significantly affect uptake of Nitrogen from the soil.

Table 3: Soil Diagnosis Results for Low N Trial

Test identification (Unit)	Test Used	Reference	Amount	Limit
Ph			6.53	
OM %	Walkley& Black		5.28	Very high
N %	Kjeldal		0.24	Below opt
P (mg/kg)	Bray1		7.46	Below opt
K (meq/100g)	Ammonium Acetate		0.36	
Na(meq/100g)	Ammonium Acetate		0.27	
Ca(meq/100g)	Ammonium Acetate		15.71	
Mg (meq/100g)	Ammonium Acetate		3.35	
Cu (mg/Kg)	DPTA		8.26	
Fe (mg/Kg)	DPTA		3.58	
Mn (mg/Kg)	DPTA		0.48	
Zn (mg/Kg)	DPTA		5.18	
S (mg/Kg)	DPTA		18.52	
Sand (%)	Hygrometer		33	
Clay (%)	Hygrometer		30	
Silt (%)	Hygrometer		16	
Texture (USDA)	Soil class		Clay Loam	

4.2 Performance of inbred lines under low N

Table 4 presents the analysis of variance for GY, EPP, ASI, PH and Chlorophyll contentunder low N. Significant differences were detected for EPP only.

Table 4: Mean squares for variables measured and derived for maize inbred lines under low N at GART during the 2011/12 season

Source	Df	GY	EPP	ASI	PH	CC
Rep	2	9.16	0.03	2.42	259.40	394.72
Inbred lines	36	2.26ns	0.07*	7.12ns	855.50 ns	82.46ns
Error	78	1.92	0.04	4.99	899.20	72.74
Total	116					

Key: GY – Grain yield , EPP – Ears per plant, ASI- Anthesissilking interval, PH- Plant height, Chlorophyll content, ns – not significant, * Significant, ** Highly significant

Ears per Plant

Significant differences (P< 0.05) among inbred lines were observed for EPP. The mean number of EPP was 0.84.The best three performing inbred lines were La Posta Seq C7-F64-2-6-2-2-B-B-B, P30 HSRS C10-58-2-1-1-B and CLQRCWQ123 with 1.30, 1.15 and 1.09 number of ears per plant respectively. Lowest numbers were obtained from inbred lines CLRCY044, CLWN237 and CLYN251 which had 0.64, 0.57 and 0.47 EPP respectively as shown in Table 5.

Table 5: Means for variables measured and derived for maize inbred lines under low N at GART during the 2011/12 season

ENTRY	GY (t/ha)	EPP (#)	ASI (days)	PH (cm)	CC (SPAD Readings)
La Posta Seq C7-F64-2-6-2-2-B-B-B	1.81	1.30	3.00	112.00	4.75
P30 HSRS C10-58-2-1-1-B	1.13	1.15	0.67	97.10	6.42
CLQRCWQ123	1.56	1.09	2.33	136.70	4.00
CLQRCWQ117	1.65	1.09	3.33	150.30	4.67
CLRCY030	2.57	1.01	4.67	149.80	3.67
CML495	3.05	1.01	2.00	154.50	4.17
CLYQ216	2.23	0.98	3.33	130.20	4.58
CLQRCWQ48	1.58	0.93	1.33	133.60	5.42
CLWN236	1.15	0.93	5.67	143.30	5.25
CML491	1.55	0.92	0.33	119.70	6.08
CLRCW106	1.92	0.91	3.67	135.30	5.67
CLRCW96	1.07	0.89	2.00	104.10	4.42
CLRCY039	1.49	0.89	3.33	132.70	7.00
CLYN259	1.36	0.88	6.33	106.70	3.92

CLWQ212	1.63	0.85	3.33	137.20	5.83
P30 HSRS C10-191-1-1-1-B	1.27	0.85	3.67	137.10	5.75
CLWN228	1.66	0.85	3.00	137.90	4.92
CLYN256	1.77	0.85	6.00	129.80	5.00
CLRCW105	1.21	0.83	5.67	134.90	5.00
CLRCW98	2.12	0.81	2.33	142.10	6.00
CLYQ296	1.47	0.81	1.67	128.20	5.00
CLWN273	2.69	0.80	4.67	146.00	4.50
CLWN234	0.85	0.80	3.67	102.80	5.58
CLQRCWQ133	1.52	0.79	2.00	156.90	3.83
CLYQ231	1.20	0.78	2.00	110.70	6.50
CLRCY040	1.61	0.78	3.00	120.30	4.08
CKL5003	1.67	0.75	3.33	145.60	3.67
CLWN243	2.73	0.74	2.67	116.50	4.00
P30 C9 -119-1-1-2-1-1-B	1.25	0.74	4.00	124.60	5.75
CLQRCWQ26	1.76	0.73	3.00	177.30	4.08
CLWN318	1.27	0.72	5.00	138.10	5.67
P30 HSRS C10-160-1-2-1-B	3.22	0.72	4.00	159.10	4.42
CLYN255	1.35	0.69	3.67	126.50	4.75
CLWN240	1.17	0.67	3.67	118.30	4.67
CLRCY044	1.41	0.64	5.67	138.70	4.58
CLWN237	0.12	0.57	3.33	115.20	4.50
CLYN251	0.92	0.47	7.67	118.20	4.92
Mean	**1.62**	**0.84**	**3.49**	**131.57**	**4.95**
LSD	**2.25**	**0.33**	**3.63**	**48.75**	**13.86**
CV%	**26.7**	**3.2**	**7**	**1.9**	**13.1**

4.3 Relationship of grain weight to secondary traits of inbred lines and hybrids.

Step wise regression analysis of GY on other secondary traits of inbred lines under low N (Table 6) showed that, only PH had a significant influence of 15.8 % on GY of inbred lines. Other traits showed no significant influence on the total variation in grain yield of hybrids. Most of the variation (74%) in yield of inbred lines could not be explained by the parameters measured.

Table 6:Stepwise regression of grain yield of inbred lines secondary traits under low N

Inbred line variable	Partial Square	R-Model square	R – F – Value	Pr> F
EPP	0.029	0.029	1.136	0.293
ASI	0.029	0.000	0.000	0.985
PH	0.187	0.158	6.984	0.012
CC	0.251	0.064	2.995	0.092

Dependent Variable: GY of inbred lines

4.4 Performance of inbred lines under optimum N

Table 7 presents the analysis of variance for GY, EPP, ASI, PH and CC under optimum N. Significant differences were detected among inbred lines for all the parameters. Table 8 presents the means for all parameters of inbred lines evaluated.

Table 7: Mean squares for variables measured and derived for maize inbred lines under Optimum N at GART during the 2011/12 season

Source	d.f.	GY	EPP	ASI	PH	CC
Rep	2	3.67	0.04	11.01	124.62	45.10
Inbred Lines	36	26.50***	0.11***	7.86***	2105.41***	365.50***
Error	78	0.93	0.04	2.89	70.68	132.80
Total	116					

Table 8: Means for measured and derived variables of inbred lines evaluated under Optimum Nitrogen at GART during the 2011/12 rain season

INBRED LINE	GY (t/ha)	EPP (#)	ASI (interval)	PH (cm)	CC (SPAD readings)
CML491	4.98	1.33	2.00	171.43	40.90
CLQRCWQ26	4.18	1.16	4.00	183.23	36.30
CLWN273	2.82	1.20	2.67	171.70	34.80
CML495	3.91	0.98	2.00	169.47	39.50
CLRCW96	4.25	1.09	3.33	194.93	32.70
CLRCW98	5.58	1.07	5.33	203.40	36.70
CLRCY030	5.25	1.06	2.00	168.27	45.80
CLRCW106	3.15	0.71	4.33	216.50	35.80
CLRCW105	4.39	1.08	2.67	209.10	29.40
CLWN234	3.55	0.86	3.33	192.37	29.80
CLYN251	4.02	1.47	1.00	134.47	56.80
La Posta Seq C7-F64-2-6-2-2-B-B-B	2.85	1.26	-0.67	151.87	22.20
CLYN255	3.46	1.19	3.67	159.23	39.10
CLYN256	3.45	0.93	4.67	162.57	24.50
CLYN259	4.08	1.13	2.67	168.57	42.20
CKL5003	5.79	1.01	5.00	181.57	42.30
CLWQ212	1.64	1.11	6.67	189.73	40.20
CLWN318	5.28	1.25	1.67	170.57	37.20
CLWN243	5.40	1.10	0.67	169.00	39.00
CLQRCWQ123	5.53	1.41	4.67	214.53	47.50
CLYQ216	2.95	0.82	2.33	201.23	35.60
CLYQ231	4.17	1.08	1.67	160.37	44.50
CLYQ296	4.24	1.29	3.00	145.03	30.80
CLQRCWQ133	4.00	1.12	5.33	198.60	37.60
CLQRCWQ48	2.68	1.01	2.33	209.57	23.70
P30 C9 -119-1-1-2-1-1-B	2.82	1.26	2.33	165.90	38.70
P30 HSRS C10-160-1-2-1-B	3.27	1.51	1.33	157.13	25.90
P30 HSRS C10-191-1-1-1-B	3.45	1.23	2.67	157.87	25.10
P30 HSRS C10-58-2-1-1-B	2.54	1.51	4.67	131.87	24.20
CLRCY039	4.79	1.16	4.67	170.30	45.80
CLQRCWQ117	5.27	1.20	2.33	185.53	41.20
CLRCY040	4.35	0.87	4.33	150.77	32.90
CLRCY044	3.27	1.05	0.67	150.33	29.40
CLWN228	5.91	1.56	2.00	186.57	33.80
CLWN240	5.26	1.20	4.67	206.30	35.90
CLWN237	3.87	1.01	0.33	171.47	30.50
CLWN236	4.02	1.12	1.33	179.43	23.50
Mean	**4.06**	**1.15**	**2.91**	**176.00**	**35.45**
$LSD_{0.05}$	**1.57**	**0.34**	**2.76**	**13.67**	**18.73**
CV%	**6.2**	**2.9**	**17.7**	**1**	**2.8**

Grain yield

As seen in ANOVA table 5.2a, differences in grain yield among inbred lines under Optimum N were significant ($p<0.05$). The average GY was 4.06 t/ha. The best three inbred lines under optimum N regime were CLWN228, CKL5003 and CLRCW98 with 5.91 t/ha, 5.79 t/ha and 5.58 t/ha respectively. Lowest performing inbred lines were CLQRCWQ48, P30 HSRS C10-58-2-1-1-B and CLWQ212 with 2.68 t/ha, 2.54 t/ha and 1.64 t/ha respectively.

Ears per plant

Ears per plant showed highly significant differences ($p< 0.001$) among inbred lines. The average EPP was 1.15. Inbred lines CLWN228, P30 HSRS C10-58-2-1-1-B and P30 HSRS C10-160-1-2-1-B had 1.56, 1.51 and 1.51 derived number of ears per plant respectively while CLWN234, CLYQ216 and CLRCW106 were the least performing with 0.86, 0.82 and 0.71 ears per plant respectively.

Anthesissilking interval

Anthesissilking interval of inbred lines under optimum N showed highly significant differences ($p<0.001$). Average ASI was 2.91. La Posta Seq C7-F64-2-6-2-2-B-B-B had the most desirable and least ASI of - 0.67, seconded by CLWN237 with 0.33 and then CLRCY44 with 0.67 ASI. Inbred lines with larger ASI were CLWQ212, CLQRCWQ133 and CLRCW98 which had 6.67, 5.33 and 5.33 in that order.

Plant height

Plant height showed highly significant differences at ($p<0.001$). The average PH was 176 cm. Among Inbred lines, CLRCW106, CLQRCWQ123 and CLQRCWQ48 were the tallest with 216.50cm, 214.53 cm and 209.57cm correspondingly while the shortest were CLYQ296, CLYN251 and P30 HSRS C10-58-2-1-1-B with 145.03 cm, 134.47cm and 131.87 cm heights respectively.

Chlorophyll Content

Differences with respect to Chlorophyll content were also highly significant at ($p< 0.001$). Average Chlorophyll content was 35.45. Among inbred lines, CLYN251, CLQRCWQ123 and CLRCY039 showed the highest Chlorophyll content with 56.8, 47.5 and 45.8 while inbred lines CLQRCWQ48, CLWN236 and La Posta Seq C7-F64-2-6-2-2-B-B-B had the least Chlorophyll content of 23.70, 23.50 and 22.20 respectively as shown in table 9 below.

4.5 Relationship of grain weight to secondary traits of inbred lines and hybrids.

Under optimum conditions PH and CC had significant influence on the GY of hybrids of 45 % and 26 % respectively. The measured and derived parameters explained a substantial amount of (70.8 %) of variation of the GY as shown in Table 9

Table 9: Stepwise GY on inbred under Optimum

Inbred line variable	Partial Square	R-Model square	R – F - Value	Pr> F
EPP	0.001	0.001	0.050	0.824
ASI	0.019	0.018	0.669	0.419
PH	0.449	0.430	28.083	0.000
CC	0.708	0.259	31.077	0.000

Dependent Variable: GY Line

4.6 Performance of Hybrids under low N

Table 10 presents the analysis of variance for GY, EPP, ASI, PH and Chlorophyll Contentunder low N. Significant differences were detected among hybrids for ASI, EPP and PH. Table 11 presents the means for all parameters of Hybrids evaluated. .

Table 10: Mean squares for variables measured and derived for maize Hybrids under low N at GART during the 2011/12 season

Source	d.f.	GY	ASI	EPP	PH	CC
Rep	2	0.14	20.25	0.00	634.60	25.15
Hybrids	39	1.676ns	7.65**	0.03***	648.00***	64.24ns
Error	78	1.49	4.04	0.01	208.70	84.20
Total	119					

Table 11: Mean squares for variables measured and derived for maize Hybrids under low N at GART during the 2011/12 season

HYBRID	GY (t/ha)	ASI (interval)	EPP (#)	PH (cm)	CC (SPAD Readings)
(CML491)/(CML444/CML395)	3.38	2.00	0.98	201.10	26.63
(CLQRCWQ26)/(CML444/CML395)	4.36	3.00	0.90	218.80	31.43
(CLWN273)/(CML444/CML395)	3.16	2.33	0.89	223.20	21.43
(CML495)/(CML444/CML395)	4.91	4.00	0.94	200.00	35.07
(CLRCW96)/(CML444/CML395)	2.78	7.00	0.69	209.80	21.97
(CLRCW98)/(CML444/CML395)	3.17	3.00	0.90	209.00	26.17
(CLRCY030)/(CML444/CML395)	4.52	3.33	0.96	204.70	21.00
(CLRCW106)/(CML444/CML395)	2.44	2.33	0.77	200.10	22.93
(CLRCW105)/(CML444/CML395)	4.15	1.33	0.88	231.90	28.80
(CLWN234)/(CML444/CML395)	2.25	0.09	0.85	175.20	22.37
(CLYN251)/(CML444/CML395)	3.29	1.33	0.63	180.70	27.03
(La Posta Seq C7-F64-2-6-2-2-B-B)/(CML444/CML395)	3.94	5.00	0.90	206.70	26.23
(CLYN255)/(CML442/CML312)	2.97	3.33	0.98	187.70	26.60
(CLYN256)/(CML442/CML312)	3.06	2.00	0.84	177.40	24.67
(CLYN259)/(CML442/CML312)	2.67	3.00	0.89	200.70	31.83
(CKL5003)/(CML442/CML312)	2.51	3.67	0.74	189.60	28.77
(CLWQ212)/(CML442/CML312)	4.26	3.00	0.91	217.30	30.27
(CLWN318)/(CML442/CML312)	3.60	2.67	0.85	193.00	34.50
(CLWN243)/(CML442/CML312)	3.01	3.00	0.82	197.00	35.80
(CLQRCWQ123)/(CML442/CML312)	3.24	3.67	0.85	210.60	28.93
(CLYQ216)/(CML442/CML312)	3.06	4.00	0.86	202.60	31.43
(CLYQ231)/(CML442/CML312)	3.27	3.67	0.79	191.00	24.10
(CLYQ296)/(CML442/CML312)	4.30	5.00	1.05	189.60	18.83
(CLQRCWQ133)/(CML442/CML312)	3.90	4.33	1.03	212.50	23.17
(CLQRCWQ48)/(CML442/CML312)	4.13	2.67	0.94	210.40	28.40
(P30 C9 -119-1-1-2-1-1-B)/(CML442/CML312)	2.65	4.33	0.84	189.90	21.73
(P30 HSRS C10-160-1-2-1-B)/(CML442/CML312)	4.49	3.67	0.98	181.90	26.60
(P30 HSRS C10-191-1-1-1-B)/(CML442/CML312)	3.39	5.00	0.86	184.00	22.83
(P30 HSRS C10-58-2-1-1-B)/(CML442/CML312)	2.46	5.67	0.76	164.10	29.53
(CLRCY039)/(CML442/CML312)	3.64	3.00	0.89	205.40	34.03
(CLQRCWQ117)/(CML442/CML312)	3.23	2.67	0.78	204.20	25.73
(CLRCY040)/(CML442/CML312)	1.87	7.33	0.65	173.90	24.47
(CLRCY044)/(CML442/CML312)	3.71	1.00	0.95	186.70	25.47
(CLWN228)/(CML442/CML312)	3.66	4.33	0.98	201.60	20.43
(CLWN240)/(CML442/CML312)	2.46	5.67	0.78	192.70	22.23
(CLWN237)/(CML442/CML312)	3.48	1.67	0.88	195.50	29.97
(CLWN236)/(CML442/CML312)	4.28	2.33	0.88	195.50	34.13
MRI 694 Local check 1	2.67	4.33	0.67	199.20	21.63
MRI 514 Local check 2	4.15	3.67	0.79	218.00	33.90
ZMS 606 Local check 3	4.62	6.67	0.86	218.30	23.67
Mean	3.43	3.50	0.86	198.79	26.87
LSD0.05	1.99	3.27	0.19	23.49	14.92
CV%	1.7	20.3	0.7	2	3

Anthesissilking interval (ASI)

Differences among means for ASI were significant at 0.01 confidence level (p<0.01). Average ASI was 3.50. Hybrids (CLWN234)/(CML444/CML395), (CLYN251)/(CML444/CML395) and (CLRCY044)/(CML442/CML312) had the least ASI of 0.09, 1 and 1.33 respectively while ZMS 606, (CLRCW96)/(CML444/CML395), and (CLRCY040)/(CML442/CML312) had the highest ASI of 6.67, 7.00 and 7.33 respectively.

Ears per plant (EPP)

Ears per plant (EPP) was highly significant (P<0.001). The mean EPP was 0.86. Hybrids (CLYQ296)/(CML442/CML312), (CLQRCWQ133)/(CML442/CML312), and (CLWN228)/(CML442/CML312) had the highest EPP of 1.05, 1.03 and 0.98 respectively while the lowest inbred lines with respect to EPP were (CLRCY040)/(CML442/CML312) and (CLYN251)/(CML444/CML395) with 0.65 and 0.63 respectively.

Plant Height (PH)

Plant height (PH) was highly significant (P<0.001). The mean PH was 198.79cm. Hybrids (CLRCW105)/(CML444/CML395), (CLWN273)/(CML444/CML395) and (CLQRCWQ26)/(CML444/CML395) had the highest PH of 231.9cm, 223.20cm and 218.80cm respectively. While (CLWN234)/(CML444/CML395), (CLRCY040)/(CML442/CML312) and (P30 HSRS C10-58-2-1-B)/(CML442/CML312) had the lowest PH of 175.2cm,173.9cm and ,164.1cm respectively.

4.7 Relationship of grain weight to secondary traits of inbred lines and hybrids.

With regards to hybrids under low N, it was EPP and PH only had significant influence of 37 % and 11 % respectively on the GY. Chlorophyll content had a strong tendency (P = 0.068) of influencing GY. A substantial (53%) amount of variation in GY was explained by the parameters measured as indicated in Table 12 below.

Table 12: Stepwise regression of grain yield of Hybrids secondary traits under low N

Hybrid Variable	Partial Square	R-Model square	R – F – Value	Pr> F
EPP	0.370	0.370	22.303	0.000
ASI	0.371	0.001	0.071	0.792
PH	0.485	0.114	7.998	0.008
CHLOROPHYL CONTENT	0.533	0.047	3.534	0.068

Dependent Variable: GY Hybrids

4.8 Performance of Hybrids under optimum N

Table 13: presents the analysis of variance for GY, EPP, ASI, PH and CHLOROPHYL CONTENT under optimum N. Significant differences were detected among hybrids for GY, ASI, and PH. Table 14: presents the means for all parameters of inbred lines evaluated. .

Table 13: Mean squares for variables measured and derived for maize Hybrids under Optimum N at GART during the 2011/12 season

Source	d.f.	GY	ASI	EPP	PH	SEN	CC
Rep	2	0.16	0.41	0.00	892.77	0.42	305.90
Hybrids	39	3.78***	2.53**	0.02ns	361.95***	0.26ns	276.50 ns
Error	78	1.55	1.27	0.01	89.77	0.20	345.10
Total	119						

Table 14: Means for measured and derived variables of hybrids evaluated under Optimum Nitrogen at GART during the 2011/12 rain season

HYBRID	GY (t/ha)	EPP (#)	ASI (interval)	PH (cm)	CC (SPAD readings)
(CML491)/(CML444/CML395)	10.79	1.33	1.00	246.00	49.90
(CLQRCWQ26)/(CML444/CML395)	10.03	1.33	1.04	251.00	54.30
(CLWN273)/(CML444/CML395)	9.93	1.33	1.12	257.37	58.70
(CML495)/(CML444/CML395)	10.07	2.00	0.99	246.10	47.00
(CLRCW96)/(CML444/CML395)	10.76	2.67	1.04	251.53	44.30
(CLRCW98)/(CML444/CML395)	9.19	2.33	0.91	265.30	46.20
(CLRCY030)/(CML444/CML395)	11.20	0.33	0.97	250.00	53.10
(CLRCW106)/(CML444/CML395)	10.28	2.33	0.95	249.13	54.30
(CLRCW105)/(CML444/CML395)	9.94	1.33	1.00	258.77	49.10
(CLWN234)/(CML444/CML395)	9.62	0.67	1.01	235.57	48.30
(CLYN251)/(CML444/CML395)	10.19	0.67	1.11	228.20	55.30
(La Posta Seq C7-F64-2-6-2-2-B-B)/(CML444/CML395)	9.81	2.33	1.00	253.63	42.40
(CLYN255)/(CML442/CML312)	8.33	2.00	0.94	233.63	51.90
(CLYN256)/(CML442/CML312)	9.95	3.00	1.06	246.60	42.90
(CLYN259)/(CML442/CML312)	7.86	2.33	0.98	241.73	47.50
(CKL5003)/(CML442/CML312)	9.32	3.33	1.07	236.60	69.00
(CLWQ212)/(CML442/CML312)	9.94	4.00	1.15	252.30	34.30
(CLWN318)/(CML442/CML312)	8.74	1.00	0.95	225.90	34.60
(CLWN243)/(CML442/CML312)	9.10	1.67	0.95	237.97	39.10
(CLQRCWQ123)/(CML442/CML312)	11.53	2.33	1.14	250.27	48.80
(CLYQ216)/(CML442/CML312)	8.79	2.33	1.04	247.63	52.60
(CLYQ231)/(CML442/CML312)	8.38	1.67	1.01	220.00	49.40
(CLYQ296)/(CML442/CML312)	9.31	2.00	1.14	232.73	51.10
(CLQRCWQ133)/(CML442/CML312)	11.25	3.00	1.11	250.57	44.90
(CLQRCWQ48)/(CML442/CML312)	8.38	2.33	0.93	254.50	41.40
(P30 C9 -119-1-1-2-1-1-B)/(CML442/CML312)	7.50	3.67	1.10	239.97	47.90
(P30 HSRS C10-160-1-2-1-B)/(CML442/CML312)	7.35	1.67	1.11	225.43	40.90
(P30 HSRS C10-191-1-1-1-B)/(CML442/CML312)	9.03	2.00	1.21	223.43	56.40
(P30 HSRS C10-58-2-1-1-B)/(CML442/CML312)	7.67	2.67	1.08	220.20	41.60
(CLRCY039)/(CML442/CML312)	10.98	2.67	1.09	242.10	31.20
(CLQRCWQ117)/(CML442/CML312)	9.91	1.67	0.98	243.33	57.90
(CLRCY040)/(CML442/CML312)	8.99	2.00	1.11	239.90	43.50
(CLRCY044)/(CML442/CML312)	7.87	1.00	1.13	233.53	24.50
(CLWN228)/(CML442/CML312)	10.35	1.33	1.05	252.10	44.90
(CLWN240)/(CML442/CML312)	8.97	4.00	1.05	244.70	63.30
(CLWN237)/(CML442/CML312)	8.35	2.00	1.09	231.60	52.20
(CLWN236)/(CML442/CML312)	11.31	2.00	1.04	245.73	49.40
MRI 694	9.54	4.33	1.00	245.43	56.40
MRI 514	10.95	2.33	1.00	249.03	47.10
ZMS 606	9.16	2.67	1.02	252.67	76.60
Mean	9.52	2.14	1.04	242.80	48.61
LSD0.05	2.03	1.83	0.18	15.40	30.20
CV%	0.70	4.70	0.50	1.90	5.70

Grain Yield

Grain yield means among Hybrids were highly significant ($p < 0.001$) under optimum N regimes. The mean GY was 9.52 t/ha. Best performing Hybrids were (CLQRCWQ123)/(CML442/CML312), (CLWN236)/(CML442/CML312) and (CLQRCWQ133)/(CML442/CML312) with 11.53 t/ha, 11.31 t/ha and 11.25 t/ha respectively. The Least performing Hybrids were (P30 HSRS C10-58-2-1-1-B)/(CML442/CML312), (P30 C9 -119-1-1-2-1-1-B)/(CML442/CML312) and (P30 C9 -119-1-1-2-1-1-B)/(CML442/CML312) which had 7.67 t/ha, 7.50 t/ha and 7.35 t/ha respectively.

Anthesissilking interval (ASI)

Differences among means for ASI were highly significant ($p<0.001$). Average ASI was 2.14. Hybrids(CLRCY030)/(CML444/CML395), (CLWN234)/(CML444/CML395) and (CLYN251)/(CML444/CML395) had the least ASI of 0.33,0.67 and 0.67 in that order while (CLWN240)/(CML442/CML312), (CLWQ212)/(CML442/CML312), MRI 694 and had highest intervals of 4.00, 4.00 and 4.33 respectively.

Plant Height (PH)

Plant height (PH) was highly significant ($P<0.001$). The mean PH was 242.80 cm. Hybrids (CLRCW98)/(CML444/CML395), (CLRCW105)/(CML444/CML395) and (CLWN273)/(CML444/CML395) had 265.30cm, 258.77cm and 257.37 cm correspondingly and were the highest. Crosses least in plant height were (P30 HSRS C10-191-1-1-1-B)/(CML442/CML312), (P30 HSRS C10-58-2-1-1-B)/(CML442/CML312), (CLYQ231)/(CML442/CML312) and had 223.43 cm, 220.20cm and 220.00 cm respectively

4.9 Relationship of grain weight to secondary traits of inbred lines and hybrids.

For hybrids, only PH had a significant influence of 35 % on GY and explanation of the total variation in GY was relatively less (37.7%) attributed to the measured and derived parameters as indicated in Table 15 below.

Table 15:Stepwise regression of GY on Hybrids under optimum

Hybrid Variable	Partial Square	R-Model square	R – F - Value	Pr> F
EPP	0.009	0.009	0.331	0.569
ASI	0.009	0.000	0.005	0.946
PH	0.353	0.344	19.133	0.000
CC	0.357	0.004	0.205	0.653

Dependent Variable: G Hybrid

Stepwise multiple regression

Stepwise regression showed that small and significant contributions to total variations were observed as common factors among the four traits considered on Grain yield of hybrids as shown in Table 17 and Table 18.

Under low N, Plant height showed the highest significant influence of 28 % on Hybrid grain yield. Also, Chlorophyll Contentdemonstrated a significant influence of 7 % on the total variation in hybrid grain yield. Ears per plant, GY and ASI of inbred lines showed no significant influence on the total variation in grain yield of hybrids. The total variation on yield was therefore 43 % as show in Table 17

Table 16: Stepwise regression of Hybrid maize grain yield on variables across inbred lines and Hybrids under low N

Inbred lines Variable	Partial Square	R Model Square	R - F –Value	Pr> F
GY	0.065	0.065	2.662	0.111
EPP	0.068	0.003	0.106	0.746
ASI	0.075	0.007	0.286	0.596
PH	0.355	0.279	15.148	0.000
CC	0.425	0.070	4.144	0.050

Dependant variable: Grain yield of Hybrids

Under optimum N regime, GY of inbred lines had a significant influence on Hybrid yield explaining 4.4 % of the total variations in Hybrid grain yield (Table 17). Plant height showed the highest significant influence of 28 %. Also, ASI showed a significant influence of 4.7% on Hybrid grain yield. Plant height was equally significant with 5.2 % influence on Hybrid grain yield. Moreover, Chlorophyll Content indicated a significant influence of 3.4 % on the total variation in Hybrid grain yield. Ears per plant had no significant influence on the total variation in Hybrid grain yield. The total variation on Hybrid grain yield was 43 %.

Table 17: Stepwise regression of maize grain yield on variables across inbred lines and Hybrids under Optimum N

Inbred lines Variable	Partial Square	R Model Square	R - F –Value	Pr> F
GY	0.044	0.044	1.742	0.195
EPP	0.045	0.001	0.057	0.812
ASI	0.093	0.047	1.878	0.179
PH	0.145	0.052	2.144	0.152
CC	0.179	0.034	1.408	0.244

CHAPTER 5: DISCUSSION

5.1 Effects of Nitrogen on genotype performance

The change in inbred lines performance due to increase in Nitrogen level was 60%, 26%, -20%, 25% and 32 % for GY, EPP, ASI, PH and CC respectively while that due to in hybrid performance was 64%, 47%, -236%, 18% and 42% for GY,EPP, ASI, PH and CC in that order. This showed that as N increased, yield also increased (60 %).The increase in yield due to increase in N among hybrids was 64%.

The increase in yield among inbred lines and hybrids was associated with an increase in EPP, PH and CC and a decrease in ASI. This was so because Nitrogen enhances growth and development of genotypes as it is part of every protein in the plant thus it is required for every process of plant growth and development (Ding et al., 2005). Nitrogen is part of the chlorophyll molecule, which is involved in the manufacture of energy through photosynthesis (Lawler 2002 and Toth et al., 2002) Similar findings were reported byIbrahim and Hala(2007) and Kesi and Pawel(2011) who showed that increasing N level increased GY, PH, EPP and plant leaf chlorophyll content. Also, Pandey et al (2000) reported thatincreasing N application increased chlorophyll content in maize.

5.2 Relationship of grain weight to secondary traits of inbred lines and hybrids.

The influence of secondary traits on GY in inbred lines under low N conditions showed that only plant height explained significant proportion(15.8%) of the total variation observed in GY.

Under optimum conditions it was plant height and Chlorophyll content 45% and 26%, respectively that had significant influence on GY of inbred lines. This was so because genotypes with greater plant height are often larger in overall plant size, intercept more light and use water faster by transpiration leading to increased production of assimilates (Edmeades et al., 1999). Cattivelli et al., (2008) noted that the capacity of mobilizing assimilates depended on plant height and stem diameter, thus the findingin the current study relating yield to plant height was in line with their assertions. Plant height of N stressed plants had been shown to be a good indicator of biomas at anthesis and delayed senescence measured as Chlorophyll Content, contributes to larger biomass gains under severe N stress (Lafitte and Edmeades, 1988; Zeiger and Taiz, 2006).

Increased chlorophyll content or delayed senescence was related to increased grain yield. This was so because as Araus et al., (2008) reported that delayed senescence led to sustained leaf photosynthesis during grain filling, which has been associated with increases in dry matter accumulation and increase in kernel number and weight due to higher assimilate partitioning to the kernels during the sensitive period of kernel number determination. Edmeades et al., (1999) also supports this finding arguing that increased chlorophyll content increased production and transport of energy from photosynthesis. In addition, Cattivelli et al., (2008) reported that delayed leaf senescence diminish evaporation while increasing water use and water use efficiency which is critical at flowering and grain filling stages for increased successful ears and kernel formation that are components of yield in maize. However, Bullock and Anderson, 1998reported that there was no association between Maize grain yield and Chlorophyll Content.

For hybrids, it was plant height and ears per plant that had significant influence on GY, 11.4% and 37%, respectively, under low N conditions. In addition plant height had important and significant influence (35%) on GY under optimum conditions. With regards to plant height,the same explanation by Edmeades et al., (1999) holds, that genotypes with greater plant height are often larger in overall plant size, intercept more light and use water faster by transpiration, leading to lower plant water status and higher grain yield under optimum N.Ears per plant influenced grain yield becauseprolific and semi prolific cultivars start the process of ear formation earlier which increased chances of ear survival under stress (Monneveux et al., 2005).

Generally, the results showed that PH, EPP and CC had been related to GY especially under low N stress conditions. Bänziger et al., (2000),Bänziger et al., (1997) and, Bolaños andEdmeades(1996) showed that relationships of PH, EPP and ASI to GY were more pronounced under severe low N stress conditions than under optimum N.

These results meant that PH, EPP and CC can be used to aid in selection for superior genotypes for use in maize improvement programmes under low N stress conditions

5.3 Relationship of hybrid performance to inbred lines secondary traits

A step wisemultple regression analysis of GY of hybrids on inbred lines traits under low N showed that PH of inbred lines had a significant influence on hybrid grain yield explaining 28% of the variation observed;Chlorophyll content also showed a significant influence explaining 7 % of the total variation in Hybrid grain yield.

The current study has shown that secondary traits of inbred lines explained the variation observed in the GY of the hybrids under low Nconditions but such a relationship could not be established under optimum conditions. This implied that it is more efficient to use PH and CC as secondary traits when selecting superior inbred lines under low N conditions than under optimum N conditions. This was in line with Bolaños et al. (1993),Edmeades et al.,(1993) and Lafitte and Edmeades,(1994) who concluded from their studies that efficient breeding for low N stress should be done under low stress conditions.

Furthermore, this meant thattall inbred lines and those with higherChlorophyll contentwould give hybrids that would yield more under low N stress conditions. This finding was in line with works byBänziger et al., (2000) and Edmeades et al.,(1999) who foundthat plant height was heritable and related to grain yield under low N. This was in such a way that taller plants gave higher yields, but these plants had negative consequences of increased lodging. Results showed that under low N, PH and CC were related to grain yield in such a way that genotypes with higher values of PH and CCgave higher yielding hybrids.

CHAPTER 6: CONCLUSIONS

Low N depressed performance, in terms of yield, of both inbred lines and hybrids. The reduction in yield was associated with a reduction in EPP, PH and CC and an increase in ASI.

Plant height influenced grain yieldof the inbred linesunder low N stress conditions. Under optimum conditions it was plant height and CCthat positively influenced grain yield. For hybrids under low N conditions, plant height and ears per plant positively influenced grain yield of the hybrids, while under optimum conditions, only plant height positively influenced grain yield. Thus, plant height was directly related to grain yield under low and optimum N conditions in both inbred lines and hybrids and was found to be an important secondary trait in selecting for performance for low N.

Under low N conditions, plant height and CCof inbred linespositively influenced hybrid grain yield. Nonetheless, under optimum conditions secondary traits of inbred lines did notinfluence yield of hybrid in any way.

On the basis of this study conclusions were that if inbred lines with increased chlorophyll content measured as CC in this study, and plant height under low N were selected, they would give Hybrids with higher yields under small holder farmer conditions which are generally low N conditions in Zambia.

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7. List of Appendices

Appendix 1: Analysis of Variance and Means Tables for Inbred lines under Low N level

Variate: GYG

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
REP stratum	2		18.315	9.158	4.76	
GENOTYPE	39		88.200	2.262	1.18	0.270ns
Residual	76	(2)	146.203	1.924		
Total	117	(2)	247.290			

Variate: EPP

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
REP stratum	2		0.05884	0.02942	0.69	
GENOTYPE	39		2.79599	0.07169	1.69	0.025*
Residual	76	(2)	3.21966	0.04236		
Total	117	(2)	5.99102			

Variate: ASI

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	4.850	2.425	0.49	
GENOTYPE	39	278.000	7.128	1.43	0.091
Residual	78	389.150	4.989		
Total	119	672.000			

Variate: PH

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
REP stratum	2		518.8	259.4	0.29	
GENOTYPE	39		33364.1	855.5	0.95	0.559
Residual	77	(1)	69235.7	899.2		
Total	118	(1)	102755.2			

Variate: CHLOROPHYL CONTENT

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	789.45	394.72	5.43	
GENOTYPE	39	3215.75	82.46	1.13	0.314
Residual	78	5673.48	72.74		
Total	119	9678.69			

Appendix 2: Analysis variance and Means for Inbred Lines under Optimum N Level

Variate: GY

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	7.3328	3.6664	3.95	
GENOTYPE	39	1033.4843	26.4996	28.54	<.001
Residual	78	72.4334	0.9286		
Total	119	1113.2505			

Variate: EPP

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	0.08650	0.04325	1.00	
GENOTYPE	39	4.12352	0.10573	2.44	<.001
Residual	78	3.37735	0.04330		
Total	119	7.58736			

Variate: ASI

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	22.017	11.008	3.81	
GENOTYPE	39	306.533	7.860	2.72	<.001
Residual	78	225.317	2.889		
Total	119	553.867			

Variate: PH

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	249.24	124.62	1.76	
GENOTYPE	39	82110.88	2105.41	29.79	<.001
Residual	78	5513.30	70.68		
Total	119	87873.42			

Variate: CHLOROPHYL CONTENT1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	90.2	45.1	0.34	
GENOTYPE	39	14254.6	365.5	2.75	<.001
Residual	78	10356.8	132.8		
Total	119	24701.7			

Appendix 3: Analysis of variance and mean tables for Hybrids under Low N Level

Variate: GYG

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
REP stratum	2		0.279	0.139	0.09	
GENOTYPE	39		65.383	1.676	1.12	0.327
Residual	77	(1)	114.965	1.493		
Total	118	(1)	180.622			

Variate: ASI

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
REP stratum	2		40.507	20.253	5.01	
GENOTYPE	39		298.139	7.645	1.89	0.009
Residual	77	(1)	311.003	4.039		
Total	118	(1)	631.580			

Variate: EPP

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	0.00277	0.00139	0.11	
GENOTYPE	39	1.14195	0.02928	2.22	0.001
Residual	78	1.02689	0.01317		
Total	119	2.17162			

Variate: PH

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
REP stratum	2		1269.2	634.6	3.04	
GENOTYPE	39		25273.8	648.0	3.10	<.001
Residual	77	(1)	16071.2	208.7		
Total	118	(1)	41178.9			

Variate: CHLOROPHYL CONTENT

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	50.31	25.15	0.30	
GENOTYPE	39	2505.55	64.24	0.76	0.822
Residual	78	6567.50	84.20		
Total	119	9123.36			

Appendix 4: Analysis of variance and Mean Tables for Hybrids under Optimum N Level

Variate: GY

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	0.314	0.157	0.10	
GENOTYPE	39	147.476	3.781	2.44	<.001
Residual	78	121.068	1.552		
Total	119	268.859			

Variate: ASI

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	0.817	0.408	0.32	
GENOTYPE	39	98.592	2.528	1.99	0.005
Residual	78	99.183	1.272		
Total	119	198.592			

Variate: EPP

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	0.00236	0.00118	0.10	
GENOTYPE	39	0.58717	0.01506	1.29	0.167
Residual	78	0.90849	0.01165		
Total	119	1.49802			

Variate: PH

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	1785.54	892.77	9.94	
GENOTYPE	39	14116.11	361.95	4.03	<.001
Residual	78	7002.13	89.77		
Total	119	22903.78			

Variate: SEN

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	0.8375	0.4188	2.13	
GENOTYPE	39	10.1583	0.2605	1.33	0.145
Residual	78	15.3292	0.1965		
Total	119	26.3250			

Variate: CHLOROPHYL CONTENT

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	611.8	305.9	0.89	
GENOTYPE	39	10783.1	276.5	0.80	0.775
Residual	78	26921.3	345.1		
Total	119	38316.1			

Appendix 5: Stepwise Regression Analysis for Grain yield of inbred lines on secondary traits under low N

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics					Durbin-Watson
					R Square Change	F Change	df1	df2	Sig. F Change	
EPP	.170[a]	.029	.003	.86655	.029	1.136	1	38	.293	
ASI	.170[b]	.029	-.023	.87818	.000	.000	1	37	.985	
PH	.432[c]	.187	.119	.81476	.158	6.984	1	36	.012	
CC	.501[d]	.251	.165	.79309	.064	2.995	1	35	.092	1.212

Appendix 6: Stepwise Regression Analysis for Grain yield of Hybrids on secondary traits under low N

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics					Durbin-Watson
					R Square Change	F Change	df1	df2	Sig. F Change	
EPP	.608[a]	.370	.353	.60144	.370	22.303	1	38	.000	
ASI	.609[b]	.371	.337	.60894	.001	.071	1	37	.792	
PH	.697[c]	.485	.443	.55841	.114	7.998	1	36	.008	
CC	.730[d]	.533	.479	.53974	.047	3.534	1	35	.068	2.254

Appendix 7: Stepwise Regression Analysis for Grain yield of inbred lines on secondary traits under Optimum N

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics					Durbin-Watson
					R Square Change	F Change	df1	df2	Sig. F Change	
EPP	.036[a]	.001	-.025	3.004	.001	.050	1	38	.824	
ASI	.138[b]	.019	-.034	3.017	.018	.669	1	37	.419	
PH	.670[c]	.449	.403	2.292	.430	28.083	1	36	.000	
CC	.841[d]	.708	.675	1.692	.259	31.077	1	35	.000	1.424

Appendix 8: Stepwise Regression Analysis for Grain yield of Hybrids on secondary traits under Optimum N

Variable	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics					Durbin-Watson
					R Square Change	F Change	df1	df2	Sig. F Change	
EPP	.093[a]	.009	-.017	1.132	.009	.331	1	38	.569	
ASI	.094[b]	.009	-.045	1.148	.000	.005	1	37	.946	
PH	.594[c]	.353	.299	.940	.344	19.133	1	36	.000	
CC	.597[d]	.357	.283	.951	.004	.205	1	35	.653	2.036

Appendix 9: Stepwise Regression Analysis for Grain yield of Hybrids on secondary traits of inbred lines under low N

Variable	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics					Durbin-Watson
					R Square Change	F Change	df1	df2	Sig. F Change	
GY	.256[a]	.065	.041	.73244	.065	2.662	1	38	.111	
EPP	.261[b]	.068	.018	.74121	.003	.106	1	37	.746	
ASI	.275[c]	.075	-.002	.74846	.007	.286	1	36	.596	
PH	.596[d]	.355	.281	.63415	.279	15.148	1	35	.000	
CC	.652[e]	.425	.340	.60745	.070	4.144	1	34	.050	2.510

Appendix 10: Stepwise Regression Analysis for Grain yield of Hybrids on secondary traits of inbred lines under optimum N

Variable	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics					Durbin-Watson
					R Square Change	F Change	df1	df2	Sig. F Change	
GY	.209[a]	.044	.019	1.112	.044	1.742	1	38	.195	
EPP	.213[b]	.045	-.006	1.126	.001	.057	1	37	.812	
ASI	.304[c]	.093	.017	1.113	.047	1.878	1	36	.179	
PH	.381[d]	.145	.047	1.096	.052	2.144	1	35	.152	
CC	.423[e]	.179	.058	1.089	.034	1.408	1	34	.244	2.116

Appendix 11:Effects of Nitrogen level on inbred lines

Appendix 12: Effects of Nitrogen level on inbred lines

	Inbred lines Performance under low N					Change in performance = (Optm N - Low N)/Optn N					Inbred lines Performance under optimum N				
Inbred lines	GY	EPP	ASI	PH	SPAD	ΔGY	Δ EPP	Δ ASI	Δ PH	Δ SPAD	Gy	EPP	ASI	Ph	SPAD
CML491	1.55	0.92	0.33	119.70	14.33	0.69	0.31	0.84	0.30	0.65	4.98	1.33	2.00	171.43	40.90
CLQRCWQ26	1.76	0.73	3.00	177.30	24.73	0.58	0.37	0.25	0.03	0.32	4.18	1.16	4.00	183.23	36.30
CLWN273	2.69	0.80	4.67	146.00	31.03	0.05	0.33	-0.75	0.15	0.11	2.82	1.20	2.67	171.70	34.80
CML495	3.05	1.01	2.00	154.50	34.33	0.22	-0.03	0.00	0.09	0.13	3.91	0.98	2.00	169.47	39.50
CLRCW96	1.07	0.89	2.00	104.10	23.77	0.75	0.18	0.40	0.47	0.27	4.25	1.09	3.33	194.93	32.70
CLRCW98	2.12	0.81	2.33	142.10	24.27	0.62	0.24	0.56	0.30	0.34	5.58	1.07	5.33	203.40	36.70
CLRCY030	2.57	1.01	4.67	149.80	30.13	0.51	0.04	-1.34	0.11	0.34	5.25	1.06	2.00	168.27	45.80
CLRCW106	1.92	0.91	3.67	135.30	21.27	0.39	-0.28	0.15	0.38	0.41	3.15	0.71	4.33	216.50	35.80
CLRCW105	1.21	0.83	5.67	134.90	16.90	0.72	0.23	-1.12	0.35	0.43	4.39	1.08	2.67	209.10	29.40
CLWN234	0.85	0.80	3.67	102.80	19.07	0.76	0.07	-0.10	0.47	0.36	3.55	0.86	3.33	192.37	29.80
CLYN251	0.92	0.47	7.67	118.20	16.77	0.77	0.68	-6.67	0.12	0.70	4.02	1.47	1.00	134.47	56.80
La Posta Seq C7-F64-2-6-2-2-B-B-B	1.81	1.30	3.00	112.00	18.63	0.36	-0.04	5.48	0.26	0.16	2.85	1.26	-0.67	151.87	22.20
CLYN255	1.35	0.69	3.67	126.50	18.53	0.61	0.42	0.00	0.21	0.53	3.46	1.19	3.67	159.23	39.10
CLYN256	1.77	0.85	6.00	129.80	25.83	0.49	0.09	-0.28	0.20	-0.05	3.45	0.93	4.67	162.57	24.50
CLYN259	1.36	0.88	6.33	106.70	21.33	0.67	0.22	-1.37	0.37	0.49	4.08	1.13	2.67	168.57	42.20
CKL5003	1.67	0.75	3.33	145.60	35.47	0.71	0.25	0.33	0.20	0.16	5.79	1.01	5.00	181.57	42.30
CLWQ212	1.63	0.85	3.33	137.20	32.13	0.00	0.23	0.50	0.28	0.20	1.64	1.11	6.67	189.73	40.20
CLWN318	1.27	0.72	5.00	138.10	15.77	0.76	0.42	-1.99	0.19	0.58	5.28	1.25	1.67	170.57	37.20
CLWN243	2.73	0.74	2.67	116.50	25.97	0.49	0.32	-2.99	0.31	0.33	5.40	1.10	0.67	169.00	39.00
CLQRCWQ123	1.56	1.09	2.33	136.70	26.40	0.72	0.22	0.50	0.36	0.44	5.53	1.41	4.67	214.53	47.50
CLYQ216	2.23	0.98	3.33	130.20	29.73	0.24	-0.20	-0.43	0.35	0.16	2.95	0.82	2.33	201.23	35.60
CLYQ231	1.20	0.78	2.00	110.70	18.93	0.71	0.28	-0.20	0.31	0.57	4.17	1.08	1.67	160.37	44.50
CLYQ296	1.47	0.81	1.67	128.20	23.50	0.65	0.38	0.44	0.12	0.24	4.24	1.29	3.00	145.03	30.80
CLQRCWQ133	1.52	0.79	2.00	156.90	21.47	0.62	0.29	0.62	0.21	0.43	4.00	1.12	5.33	198.60	37.60
CLQRCWQ48	1.58	0.93	1.33	133.60	24.50	0.41	0.07	0.43	0.36	-0.03	2.68	1.01	2.33	209.57	23.70
P30 C9 -119-1-1-2-1-1-B	1.25	0.74	4.00	124.60	22.30	0.56	0.41	-0.72	0.25	0.42	2.82	1.26	2.33	165.90	38.70
P30 HSRS C10-160-1-2-1-B	3.22	0.72	4.00	159.10	26.03	0.01	0.52	-2.01	-0.01	-0.01	3.27	1.51	1.33	157.13	25.90
P30 HSRS C10-191-1-1-1-B	1.27	0.85	3.67	137.10	24.70	0.63	0.31	-0.37	0.13	0.02	3.45	1.23	2.67	157.87	25.10
P30 HSRS C10-58-2-1-1-B	1.13	1.15	0.67	97.10	20.10	0.56	0.24	0.86	0.26	0.17	2.54	1.51	4.67	131.87	24.20
CLRCY039	1.49	0.89	3.33	132.70	25.43	0.69	0.23	0.29	0.22	0.44	4.79	1.16	4.67	170.30	45.80
CLQRCWQ117	1.65	1.09	3.33	150.30	22.43	0.69	0.09	-0.43	0.19	0.46	5.27	1.20	2.33	185.53	41.20
CLRCY040	1.61	0.78	3.00	120.30	31.67	0.63	0.11	0.31	0.20	0.04	4.35	0.87	4.33	150.77	32.90
CLRCY044	1.41	0.64	5.67	138.70	25.57	0.57	0.39	-7.46	0.08	0.13	3.27	1.05	0.67	150.33	29.40
CLWN228	1.66	0.85	3.00	137.90	26.53	0.72	0.46	-0.50	0.26	0.22	5.91	1.56	2.00	186.57	33.80
CLWN240	1.17	0.67	3.67	118.30	30.40	0.78	0.44	0.21	0.43	0.15	5.26	1.20	4.67	206.30	35.90
CLWN237	0.12	0.57	3.33	115.20	21.47	0.97	0.43	-9.09	0.33	0.30	3.87	1.01	0.33	171.47	30.50
CLWN236	1.15	0.93	5.67	143.30	17.13	0.71	0.17	-3.26	0.20	0.27	4.02	1.12	1.33	179.43	23.50
Mean	1.62	0.84	3.49	131.57	24.01	0.60	0.26	-0.20	0.25	0.32	4.07	1.14	2.91	175.97	35.45

	Inbred lines Performance under low N					Change in performance = (Optm N - Low N)/Optn N					Inbred lines Performance under optimum N				
Inbred lines	GY	EPP	ASI	PH	SPAD	ΔGY	Δ EPP	Δ ASI	Δ PH	Δ SPAD	Gy	EPP	ASI	PH	SPAD
CML491	3.38	0.98	2.00	201.10	26.63	0.69	0.26	-1.01	0.18	0.47	10.79	1.33	1.00	246.00	49.90
CLQRCWQ26	4.36	0.90	3.00	218.80	31.43	0.57	0.32	-1.89	0.13	0.42	10.03	1.33	1.04	251.00	54.30
CLWN273	3.16	0.89	2.33	223.20	21.43	0.68	0.33	-1.08	0.13	0.63	9.93	1.33	1.12	257.37	58.70
CML495	4.91	0.94	4.00	200.00	35.07	0.51	0.53	-3.06	0.19	0.25	10.07	2.00	0.99	246.10	47.00
CLRCW96	2.78	0.69	7.00	209.80	21.97	0.74	0.74	-5.74	0.17	0.50	10.76	2.67	1.04	251.53	44.30
CLRCW98	3.17	0.90	3.00	209.00	26.17	0.66	0.61	-2.30	0.21	0.43	9.19	2.33	0.91	265.30	46.20
CLRCY030	4.52	0.96	3.33	204.70	21.00	0.60	-1.89	-2.44	0.18	0.60	11.20	0.33	0.97	250.00	53.10
CLRCW106	2.44	0.77	2.33	200.10	22.93	0.76	0.67	-1.45	0.20	0.58	10.28	2.33	0.95	249.13	54.30
CLRCW105	4.15	0.88	1.33	231.90	28.80	0.58	0.34	-0.33	0.10	0.41	9.94	1.33	1.00	258.77	49.10
CLWN234	2.25	0.85	0.09	175.20	22.37	0.77	-0.26	0.91	0.26	0.54	9.62	0.67	1.01	235.57	48.30
CLYN251	3.29	0.63	1.33	180.70	27.03	0.68	0.07	-0.20	0.21	0.51	10.19	0.67	1.11	228.20	55.30
La Posta Seq C7-F64-2-6-2-2-B-B-B	3.94	0.90	5.00	206.70	26.23	0.60	0.61	-4.00	0.19	0.38	9.81	2.33	1.00	253.63	42.40
CLYN255	2.97	0.98	3.33	187.70	26.60	0.64	0.51	-2.56	0.20	0.49	8.33	2.00	0.94	233.63	51.90
CLYN256	3.06	0.84	2.00	177.40	24.67	0.69	0.72	-0.89	0.28	0.42	9.95	3.00	1.06	246.60	42.90
CLYN259	2.67	0.89	3.00	200.70	31.83	0.66	0.62	-2.06	0.17	0.33	7.86	2.33	0.98	241.73	47.50
CKL5003	2.51	0.74	3.67	189.60	28.77	0.73	0.78	-2.44	0.20	0.58	9.32	3.33	1.07	236.60	69.00
CLWQ212	4.26	0.91	3.00	217.30	30.27	0.57	0.77	-1.60	0.14	0.12	9.94	4.00	1.15	252.30	34.30
CLWN318	3.60	0.85	2.67	193.00	34.50	0.59	0.15	-1.81	0.15	0.00	8.74	1.00	0.95	225.90	34.60
CLWN243	3.01	0.82	3.00	197.00	35.80	0.67	0.51	-2.17	0.17	0.08	9.10	1.67	0.95	237.97	39.10
CLQRCWQ123	3.24	0.85	3.67	210.60	28.93	0.72	0.64	-2.22	0.16	0.41	11.53	2.33	1.14	250.27	48.80
CLYQ216	3.06	0.86	4.00	202.60	31.43	0.65	0.63	-2.84	0.18	0.40	8.79	2.33	1.04	247.63	52.60
CLYQ231	3.27	0.79	3.67	191.00	24.10	0.61	0.53	-2.63	0.13	0.51	8.38	1.67	1.01	220.00	49.40
CLYQ296	4.30	1.05	5.00	189.60	18.83	0.54	0.48	-3.40	0.19	0.63	9.31	2.00	1.14	232.73	51.10
CLQRCWQ133	3.90	1.03	4.33	212.50	23.17	0.65	0.66	-2.91	0.15	0.48	11.25	3.00	1.11	250.57	44.90
CLQRCWQ48	4.13	0.94	2.67	210.40	28.40	0.51	0.60	-1.86	0.17	0.31	8.38	2.33	0.93	254.50	41.40
P30 C9 -119-1-1-2-1-1-B	2.65	0.84	4.33	189.90	21.73	0.65	0.77	-2.93	0.21	0.55	7.50	3.67	1.10	239.97	47.90
P30 HSRS C10-160-1-2-1-B	4.49	0.98	3.67	181.90	26.60	0.39	0.42	-2.30	0.19	0.35	7.35	1.67	1.11	225.43	40.90
P30 HSRS C10-191-1-1-1-B	3.39	0.86	5.00	184.00	22.83	0.62	0.57	-3.12	0.18	0.60	9.03	2.00	1.21	223.43	56.40
P30 HSRS C10-58-2-1-1-B	2.46	0.76	5.67	164.10	29.53	0.68	0.71	-4.25	0.25	0.29	7.67	2.67	1.08	220.20	41.60
CLRCY039	3.64	0.89	3.00	205.40	34.03	0.67	0.67	-1.76	0.15	-0.09	10.98	2.67	1.09	242.10	31.20
CLQRCWQ117	3.23	0.78	2.67	204.20	25.73	0.67	0.53	-1.72	0.16	0.56	9.91	1.67	0.98	243.33	57.90
CLRCY040	1.87	0.65	7.33	173.90	24.47	0.79	0.67	-5.61	0.28	0.44	8.99	2.00	1.11	239.90	43.50
CLRCY044	3.71	0.95	1.00	186.70	25.47	0.53	0.05	0.11	0.20	-0.04	7.87	1.00	1.13	233.53	24.50
CLWN228	3.66	0.98	4.33	201.60	20.43	0.65	0.26	-3.11	0.20	0.54	10.35	1.33	1.05	252.10	44.90
CLWN240	2.46	0.78	5.67	192.70	22.23	0.73	0.80	-4.41	0.21	0.65	8.97	4.00	1.05	244.70	63.30
CLWN237	3.48	0.88	1.67	195.50	29.97	0.58	0.56	-0.54	0.16	0.43	8.35	2.00	1.09	231.60	52.20
CLWN236	4.28	0.88	2.33	195.50	34.13	0.62	0.56	-1.25	0.20	0.31	11.31	2.00	1.04	245.73	49.40
MRI 694	2.67	0.67	4.33	199.20	21.63	0.72	0.84	-3.35	0.19	0.62	9.54	4.33	1.00	245.43	56.40
MRI 514	4.15	0.79	3.67	218.00	33.90	0.62	0.66	-2.67	0.12	0.28	10.95	2.33	1.00	249.03	47.10
ZMS 606	4.62	0.86	6.67	218.30	23.67	0.50	0.68	-5.52	0.14	0.69	9.16	2.67	1.02	252.67	76.60
Mean	3.43	0.86	3.50	198.79	26.87	0.64	0.47	-2.36	0.18	0.42	9.52	2.14	1.04	242.80	48.61