SELECTION OF S_1 and S_2 LONGE 4 MAIZE LINES FOR TOLERANCE TO LOW SOIL NITROGEN IN UGANDA

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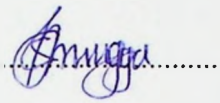
DECLARATION

This thesis is my original work and has never been submitted for a degree in any other university



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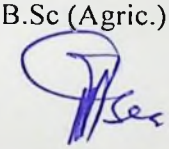

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DEDICATION

To my dear mother

To my brothers and sisters

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ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
CIMMYT	International Maize and Wheat Improvement Centre
DTP	Drought Tolerant Population
GxE	Genotype x Environment Interaction
KYU	Kyambogo University
MAK	Makerere University
N	Nitrogen
NaCRRI	National Crop Resources Research Institute
NUE	Nitrogen Use Efficiency
OPV	Open Pollinated Variety
QTL	Quantitative Trait Loci
S_1	Selfed progeny 1
S_2	Selfed progeny 2
S_2R	Selfed progeny 2 randomly selected families
UBOS	Uganda Bureau of Statistics

ABSTRACT

Low nitrogen is a major environmental stress leading to low yields in maize. Identification of varieties able to perform well under low soil N is of great significance to the resource poor farmers in the country especially when input costs are high and fertilizer use is very low (3%). This study was conducted with the following objectives; (1) to compare the yield performance of Longe 4 families in S_1 and S_2 generations under low and optimal N conditions, (2) to estimate the heritability of yield in S_1 & S_2 generations and (3) predict expected gain under low N conditions for different selection approaches and to determine the association of yield with key secondary traits for possible use in indirect selection to improve yield. For this purpose S_1, S_2 and S_2R (S_2 random) families derived from Longe 4; a popular early maturing OPV, were evaluated during the 2 growing seasons of 2009A and 2009B at National Crops Resources Research Institute (NaCRRI) under low and optimal nitrogen conditions. S_1-derived S_2 families were randomly selected (S_2R) to produce an unselected control population using a random number generation procedure. Yield, yield components and other agronomic traits were assessed among families to compare mean performance of generations, to estimate heritability and the amount of genetic variance in the three populations, and to determine the magnitude of correlation between traits and between environments. Results obtained indicated that S_1 families in both testing environments yielded higher than S_2 families and gave larger predicted gains from selection. Genetic variances were higher for S_1 families in both environments. Therefore, more rapid gains for yield in Longe 4 are expected from S_1 compared to S_2 selection. The overall yield reduction in the low-N test was 35% and 34% in 2009A and 2009B, respectively. However, some S1 families

performed well in both low N and optimum N experiments. Average broad sense heritability estimates under low N were 21% for grain yield, 17% for ASI (S_2), 18% for ears per plant, 18% for leaf senescence and 21% for yellowing. Narrow-sense heritability was 0.24 and 0.52 for grain yield and ASI respectively. Genotype means under low N showed correlations between grain yield and: ASI, -0.23; plant height, 0.52; leaf senescence, 0.15 and leaf nitrogen concentration, 0.12. In conclusion, sufficient amount of genotypic variation was found for low N tolerance among Longe 4 S_1 and S_2 families which can be improved by selection. Selection should be done at S_1 to save time and avoid an extra season of selfing and the associated resources required. Predicted gains were higher for S_1 families and when ranks of grain yield were averaged across N-levels, therefore both environments should be used in selection of genotypes for low nitrogen performance if feasible.

CHAPTER ONE

INTRODUCTION

1.1. Background

In Uganda maize is a strategic food and feed crop providing a significant amount of protein and energy for both humans and livestock (FAO, 1992). Maize ranks first among cereals in this country with an estimated 2,000, 000 MT produced annually (UBOS, 2010). Of this, half is exported, mainly to neighboring Kenya (Ackello *et al.*, 1997) and the other half is locally consumed. Other important purchases of maize are made by the World Food Programme and NGOs for use in food aid programmes in regional countries. These purchases are sometimes made directly from large Ugandan grain merchants, but more often large scale farmers. Plans are also underway to buy grain directly from small scale farmers. Large domestic purchases are made by government-controlled Tender Boards, which supply the army, police, schools and hospitals, and by local millers and processors (CMIS/Foodnet, 1999).

About 43% of maize produced in the country is consumed as roasted or steamed green cobs or as porridge by families on their own farm. Maize stover and bran also constitute major ingredients in livestock feeds (Ntege *et al.* 1997). However, the rest of the maize grain is used by most farmers as a cash crop. Maize has the advantage of being relatively nonperishable if properly dried and is fairly easy to store and transport in bags. Almost all the maize in Uganda is grown in small-scale units, intercropped with other crops. There are emerging large scale maize farms in Uganda, with acreage of more than150 acres.

Although maize is grown throughout the country, the main producing areas are Kasese, Masindi, Sironko, Kapchorwa and Iganga.

1.2. Global Production trends of Maize

Globally, maize ranks second in cereal production after wheat, with an annual production of about 600 million tonnes (Pingali, 2001). It is estimated that by the year 2020, demand for maize in developing countries will surpass the combined demand for both wheat and rice. Global and sub-Saharan Africa consumption is projected to increase by 50% and 93%, from 1995 to 2020 respectively (Pingali, 2001). In 2000, of the 140 million ha of maize grown globally, approximately 96 million hectares were in the developing world (Pingali, 2001). The average maize yield in the industrialized countries is more than 8 t/ha, while in the developing world it is slightly less than 3 t/ha. In many of the developing countries, such as Guatemala, Mexico, Kenya, Zambia and Zimbabwe, maize is the basic staple food, with a per capita consumption average of more than 100 kilograms per year, supplying approximately 40% of the total calorie needs (Morris, 1998). The average yield of maize in the tropics is 1.8 t/ha, against the global average of 4.2 t/ha (CIMMYT, 1994).

In Uganda, maize production is carried out by two groups of farmers, the predominant small scale and the emerging medium/large scale commercial farmers. Mostly small scale and medium scale farmers have holdings of between 0.2-0.8ha and 0.8-2.0ha under maize respectively. Maize production in Uganda is generally characterized by low yields regardless of farm size that result in high unit costs and lead to low returns. Yield levels for maize range between 1.0 and 1.8 mt/ha (Private sector foundation report (PSF),

2003). This is partly because of low soil fertility and input use on smallholder farms. Specifically, nitrogen (N) deficiency has become one of the most important abiotic factors limiting maize yields (Kikafunda *et al.*, 2001). Therefore, for improved yields, organic or inorganic fertilizers are needed to meet the nitrogen requirement of the crop. Options for N inputs are organic materials such as compost, farm yard manure, and legumes that can fix atmospheric nitrogen. Unfortunately, the nitrogen content of organic materials (e.g. leaves of trees and shrubs, farm yard manure) are generally low and do not provide enough N for annual crops (Kikafunda *et al.*, 2001). Inorganic mineral N fertilizers are a good option (Kikafunda *et al.*, 2001), but soluble N fertilizers remain prohibitively expensive for most smallholder farmers. For instance, in Uganda, N costs over US$500 per tonne (USD 40/bag currently) compared to $120-150 in the global markets. In fact, fertilizer consumption on crop land averages 25kg/ha (or less, according to local estimates) and seems to have decreased over the past 10 years (FAOSTAT, 2003) as farmers have faced increasing input costs and decreasing market prices. Although maize prices are somewhat high now, prices fluctuate widely and are still very low at harvest time; when most producers must sell their maize since they have poor storage facilities.

1.3. Justification of the study

Low N maize efficient varieties can be very useful to alleviate effects of low nitrogen due to declining soil fertility. Unfortunately, most high yielding commercial maize varieties were selected under optimum soil fertility and therefore are unstable when grown in low N soils such as those that most resource poor farmers use. For this reason, attention has

been focused on genetic improvement of maize for tolerance to low nitrogen (Banziger *et al.*, 2000, Kikafunda *et al.*, 2001).

The Cereal Program of the National Crop Resources Research Institute (NaCRRI) began improving maize varieties for nitrogen tolerance. Initially, the program targeted popular farmer-preferred open-pollinated varieties (OPV) such as Longe 4 for improvement through recurrent selection. OPVs are targeted because are mostly grown by small scale farmers who do not regularly buy seed. Longe 4 a popular OPV that is early maturing (105-110 days) was released in 2000 and in 2006 in Kenya and Tanzania and grown mainly in drought prone areas. As an OPV, it also provides ready seed from season to season unlike an equivalent hybrid variety that would require continuous purchase of seed. Improving this variety for low N tolerance should increase production, and therefore the availability of maize for both home consumption and sale, resulting in greater food security and improved livelihoods.

Although various selection methods are available, recurrent selection is the best approach to maximize genetic improvement in an OPV. However, it is not clear which recurrent selection method will be the most efficient, and which test environment will result in higher selection gains. Therefore, heritability estimates of traits and their correlation with grain yield, and estimates of potential gains from selection in Longe 4 are needed. These estimates will be important in predicting breeding progress for low N environment. Therefore, this study compared the performance of S_1 and S_2 selected progenies for heritability, potential gains and the possibility of indirect selection based on key

secondary traits related to yield. Selection and evaluation were carried out in two contrasting, carefully managed N levels (low and optimal).

Additionally, genotype by environment interactions (G x E) are very common in maize, especially when low soil nitrogen and drought are involved. The aim of this study was to establish the level of genotype and environment interaction (GxE) for grain yield between low and optimal nitrogen levels and therefore help predict gain in low N from selecting under high N.

1.4. Objectives

1.4.1. Overall objective

- To improve the performance of Longe 4 by recurrent selection method under low N conditions in Uganda

1.4.2. Specific objectives

- To compare the yield performance of Longe 4 families in S_1 and S_2 generations under low and optimal N conditions
- To estimate the heritability of yield in S_1 and S_2 generations and predict expected gain under low N conditions for different selection approaches
- To determine the association of yield with key secondary traits for possible use in indirect selection to improve yield

1.5. Hypotheses tested

1) There is no mean difference in performance of Longe 4 S_1 and S_2 families in low-N and optimal N test sites.

2) Heritability and predicted gains for yield are equal in low-N and optimal N environments, and in S_1 and S_2 generations.

3) There is a strong relationship between yield and secondary traits of longe 4 S_1 and S_2 families in low-N and optimal N test sites.

4) An adequate amount of genetic variation exists among Longe 4 families for low N tolerance that can be improved by selection

CHAPTER TWO
LITERATURE REVIEW

2.1. Origin, production and uses of maize

Maize (*Zea mays* L.) is a member of the grass family *Poaceae* (*Gramineae*), tribe *Maydeae* and is the only cultivated species in this genus. The crop originated from two primary centers in the highlands of Mexico: Oaxaca and the Valley of Mexico, from where it expanded throughout Americas (Eagles and Lothrop, 1994). Within a few years after the arrival of Europeans in New World in 1492, maize had spread through Northern France, Southern Europe, and Northern Africa. Portuguese and other Europeans travelers took it to Africa and Asia, where it rapidly spread over the two continents (Dowswell *et al.*, 1996). By the 1600s, maize was a major crop in sub-Guinean zone and the Congo basin. In 1800s, it expanded to Eastern and Southern Africa from the sea coast especially from Zanzibar and Mombasa, where it was introduced at the beginning of 16th century.

Maize is the most productive food plant with a multiplication ratio of 1: 600 or more per plant basis under optimum conditions (Aldrich *et al.*, 1975). It is used in more ways than any other cereal (Morris, 1998). It is used as human food, as feed for livestock, and for several industrial purposes (Morris, 1998). Every part of the plant has economic value. The grain; leaves, stalk, tassel, and even the cob are used to produce hundreds of food and non-food products (Morris, 1998). In developed countries, maize is mainly used as animal feed and in the manufacture of food and non-food products whereas in the developing world, it is used as both food and feed. However, in the Sub-Sahara region,

and much more in East-Africa, maize is essentially used as human food. In East Africa, only Uganda uses a significant proportion of maize for purposes other than direct human food (Ngaboyisonga, 2008).

2.2. Constraints in production of Maize in Uganda

Maize production in Uganda is constrained by several factors which include poor adoption of improved varieties among farmers (Ntege *et al.*, 1997). Many farmers usually plant seed retained from the previous crop; and they mainly prefer open pollinated varieties due to their resistance to known diseases mainly maize streak virus and perceived high cost of seed (Balirwa, 1992). The key pests are mainly stalk borers (*Chilopertellus*) in mid altitude and (*Busseola fusca*) mainly in highland ecology; and storage weevils. Significant yield loss in the lowland tropical and highland agroecologies of Uganda is attributed to Maize streak virus (MSV), *Turcicum* leaf blight (*Exserohilum turcicum*) (Opio et al., 2010).

Among other production-related constraints there is lack of effective technology transfer mechanisms that can expose farmers to improved technologies and practices, extension services have greatly deteriorated in the country so farmers receive little or no guidance regarding improved farming methods and new technologies. This has resulted in consistently low yields. Besides extension services, provisions such as training, research and related infrastructures are also quite limited (PSF, 2003). Lack of reliable and knowledgeable input supply stockists who can sell genuine inputs and advise farmers accordingly.

Low producer prices, which tends to deter production, inadequate financial resources and the prevailing high interest rates in commercial banks. This has curtailed the extent to which farmers can either adopt improved technologies/practices or expand on their maize enterprise. Prices are highly volatile and respond to much localized supply and demand situations. High cost of production as exhibited by expensive farm inputs such as implements, seeds, fertilizers and pesticides, and high cost of farm labour. Poor post-harvest handling methods and lack of proper post-harvest handling, and poor storage facilities. This results in high post-harvest losses and poor quality of product. Inadequate market information flow between middlemen and producers has resulted in unfair marketing practices. Inadequate quality standards, weights and measure, have undermined crop quality improvements. As a result, premium prices for quality products are not widespread in the country. Unpredictable weather patterns make it difficult for farmers to plan their operations (PSF, 2003). Poor roads and the lack of an effective communications network mean that local markets are isolated and very small.

The peak period labor constraints in the farming system face intense competition from other farm activities for limited labour. Farmers entirely rely on family labour and this influences adoption of new techniques and enterprises that require more labour than there is in the family. Farmers also lack the required capital to hire labour and or to adopt other improved techniques. Weed infestation is another constraint that limits maize production in Uganda; by making seed bed preparation and weeding very labour intensive operations especially under small holder conditions. An important weed in Uganda is striga, mainly

in the eastern, northern and to some extent in the western parts of the country. It's a parasitic and can cause 100% yield loss (Mukiibi, 2001).

2.3. Effects of low soil nitrogen on maize yields

A very serious constraint emerging is the general decline in soil fertility and low nitrogen stress that reduces grain yield by 37 to 78% (Banziger *et al.*, 1997). The importance of adequate soil N to crop yield has been well documented, (Liu *et al.*, 2008). Many studies have demonstrated that low N not only resulted in reduced grain yield, but also affected nutrient levels in kernels, particularly of protein content (Bertin and Gallais, 2001). Unlike drought, the pattern of N stress through the season is usually very similar from location to location. At the beginning of the season, especially with fertilizer application, N supply usually exceeds crop demand. As the season progresses, however, soil N is depleted by plant absorption and by leaching. Mineralization of soil N is usually less than 1 kg N/ha/day, whereas a healthy maize crop can take up and assimilate 4 to 5 kg N/ha/day. As the season progresses, this requirement leads to depletion of N in the soil and N stress occurs in the plant.

The morphological and physiological responses of maize to a continuous N deficiency include smaller plants, reduced radiation-use efficiency, accelerated leaf senescence, increased mobilization of vegetative N to the grain, and a lower plant N concentration. According to Presterl *et al.*, (2002), N-deficiency delays shoot elongation and leaf growth, but increases root growth. Under high N supply, uptake depends mainly on the growth-related demand for N, whereas under low N, uptake depends on morphological and physiological characteristics. Plants adjust to some extent to N stress by

remobilizing N from older tissue; a mechanism that does not affect yield in the case of tissue that contributes little to photosynthesis (Banziger *et al.*, 2000). Depending on the timing of N stress in different plant parts, different yield-determining factors are affected. Nitrogen stress before flowering reduces leaf area development, photosynthesis rate, and the number of ears and spikelets (potential grains). Nitrogen stress during the flowering stage results in kernel and ear abortion, whereas stress during grain-filling accelerates leaf senescence and reduces crop photosynthesis and kernel weight (Banziger *et al.*, 2000).

An average reduction in grain yield of 65% was noted for OPVs under low N conditions (Monneveux *et al.*, 2005). Plant and ear heights were significantly reduced under low N conditions and no correlations were found between these traits and grain yield, while significant correlations were observed between yield and ears-per-plant under stress conditions. In some genotypes the days-to-silking are lengthened under low N conditions than under the optimum soil fertility conditions, implying that days-to-silking was influenced by soil fertility conditions. The top yielding genotypes under both fertility environments had a relatively reduced anthesis-silking interval (ASI). Variation for ears per plant, selection index, leaf senescence and disease reaction was also observed (Worku *et al.*, 2001).

2.4. The need for selection under stress environments

Breeding for tolerance to low N may be related to tolerance to other stress factors, and vice versa. Tropical maize selected for drought tolerance has been shown to be tolerant to low N (Banziger *et al.*, 2002). These authors have proposed the presence of constitutive

stress tolerance mechanisms that increase yield and yield stability. The most elegant breeding scheme is to select genotypes in early generations that give high grain yield under both high and low N. At CIMMYT - Zimbabwe, selection was made in maize for S1 lines that yielded well either with or without N topdressing. These were compared with S1 lines that yielded well under the higher N regime but poorly in the low N environment. S2 lines were derived from the S1 lines and the ability to perform well in both N regimes was inherited (Short, 1994).

Breeding methodologies in the tropics are strongly influenced by maize breeding in temperate areas (Banziger *et al.*, 2000). In temperate environments maize is grown under relatively stress-free conditions, and farm yields are comparable to yields obtained from experiment stations. On the contrary, in tropical environments, maize is frequently stressed and on-farm yields fall far below those obtained on breeding stations. This means that selection under high yielding conditions may not be the best way to increase yields in farmers' fields. In developing countries, farmers in high yielding, high input areas are attractive marketing targets for the private sector rather than the average, often resource-poor farmers. As a result, commercial breeders often ignore abiotic stress tolerance. The public sector is influenced by the same view although their responsibility and target environments include areas not served by the private sector (Banziger *et al.*, 2000).

The physiology of maize shows that certain plant characteristics that are less relevant under non-stressed conditions become important for yield under drought and N stress.

For example, under drought conditions maize genotypes have been observed to produce grain-bearing ears at flowering. These findings have led to experimental methodologies. In the case of drought, experiments are conducted partly or entirely during dry seasons and the stress managed through irrigation. In the case of low N, experiments are typically conducted in fields that are depleted of nitrogen. The objective of such experiments is to measure the genotypic drought tolerance and or the genotypic tolerance to low N levels (Banziger *et al.*, 2000).

During the early 1990's, CIMMYT developed lowland tropical maize genotypes with improved grain yield under low N, while maintaining yield under high N (Lafitte and Edmeades, 1994 a, b). Improvements in grain yield under low N were reported to be 75 - 100kg/ha^{-1} per cycle of recurrent selection, and somewhat higher at high rates of applied N (Lafitte and Banziger, 1997). Additionally, improvement in the drought tolerance of maize (through better anthesis- silking synchrony under water deficit) was found to be closely associated with improved N use (Banziger *et al.*, 1999).

Eberhart-Russell stability analysis of the improved tropical maize populations estimated a 40% yield advantage at the one tonne level, which decreased to 2.5% at yields of 10 tonnes (Banziger *et al.*, 2004). Those results suggested that simultaneous selection for tolerance and resistance to abiotic and biotic stress while monitoring performance under high potential conditions could result in significant progress for target environments where a combination of stresses occur at lower yield levels. In a study to determine the effects of drought screening on the genetic variance and covariance in Pool 16 DT maize

populations, Badu-Apraku *et al.*,(2004) found narrow-sense heritability estimates of 73% for grain yield. Although the induced stress appeared to be too severe to properly elicit the true differences among families, they found sufficient genetic variance to warrant continued selection for drought tolerance among the white early-maturing populations.

A similar study examined the performance of hybrid progenies of drought–tolerant populations (DTP- white and DTP- yellow of CIMMYT) in stressed (drought and low-N) and unstressed environments (Zaidi *et al.*, 2004). The compared a set of high-yielding normal single-cross hybrids, developed using inbred lines selected primarily on yield per se under optimal conditions, with DTPc9S3 top-crosses, across environments. They found that the performance of normal hybrids was slightly higher than that of DTP top-crosses under optimal conditions. However, normal hybrids performed poorly, with an average yield of 3-5% under drought and 35-36% under low N conditions. Hybrid progenies from DTP yielded up to 32-42% under drought and 49-64% under low-N compared to yields without stress. When DTPs were selected under mid-season drought, the estimated selection gains for yield were 90% and 39% higher, respectively, for performance in drought and low N conditions compared to when selection was made under optimal conditions. They attributed the improved performance of DTP hybrids across environments to improvement in secondary traits, such as reduced anthesis-silking interval (ASI), increased number of ears per plant, delayed senescence, and relatively high levels of leaf chlorophyll during late grain filling.

Similarly, in Ghana, nine early-maturing maize composites, including drought-tolerant selections were assessed under stressed and non-stressed conditions (Sallah *et al.*, 2002). In these studies, the effects due to Genotype x Environment (GxE) were found to be highly significant ($P<0.01$) for grain yield, ASI, plant height, lodging, ears per plant and ear acceptability ratings for both drought-stressed and unstressed conditions. The average yield in stressed environments ranged from 2.21 to 3.1 t/ha while in favorable environments it was 4.2 to 6.0 t/ha. Two drought-tolerant selections out-yielded the improved check and the local landrace in stressed environments. In non-stressed environments, the grain yield was similar (5.9 t/ha) for the two selections and the improved check.

2.5. Nitrogen Use Efficiency

Because of the economic and environmental costs of excessive use of N for maize production, more emphasis has shifted to the selection of varieties with greater N use efficiency (NUE) (Lucia *et al.*, 2001). In fact it has been shown that NUE at low soil N levels may not be comparable to crop responsiveness to high soil N levels (Lucia *et al.*, 2001). Others have proposed the selection of maize for yield and NUE should be more appropriately done under low soil N conditions (Lafitte and Edmeades, 1994a). During the early 1990's, CIMMYT developed lowland tropical maize genotypes with improved grain yield under low N, while maintaining yield under high N (Lafitte and Edmeades, 1994a and b). These improvements resulted in grain yield under low N of between 75 and 100kg ha^{-1} per cycle of recurrent selection. At high rates of applied N higher yields of maize were achieved (Lafitte and Banziger, 1997). Additionally, improvement in the

drought tolerance of maize through better anthesis-silking synchrony under water deficit was found to be closely associated with improved N use (Banziger *et al.*, 1999).

The relationships between the use of N fertilizers, plant growth and grain yield in maize have been studied (McCullough., *et al.* 1994). Different maize hybrids respond differently to N availability (Perez and Long, 1994). Various genotypes demonstrate variability in their ability to absorb natural or fertilized N from the soil, as well as in their efficiency in using acquired N to increase biomass and improve yield (Costa *et al.*, 2002). The quantitative genetic parameters of NUE in European maize have previously been estimated (Presterl *et al.*, 2003), and quantitative trait loci (QTL) related to secondary traits of NUE have been investigated using a set of F2.3 populations derived from two inbred lines, B73 and G79 (Agrama *et al.*, 1999). The QTLs for NUE were found to be linked to key agronomic and physiological traits (Hirel *et al.*, 2001). A divergent recurrent selection adapted to low- and high-N fertilization conditions have been reported, based on a recombinant inbred line (RIL) population that were used in mapping QTLs for NUE (Bertin and Gallais, 2001).

Heritability and genetic variance for grain yield usually decreases under abiotic stress as yield levels fall. The difference between entries is often non-significant, and the expected selection gain is less than under conditions where yields are high. Because of the high Genotype x Environment interactions involved, stress experiments often produce rankings that differ significantly from one experiment to another, making it difficult to identify the best germplasm (Banziger *et al.*, 2000). In a series of studies where yield reductions due to stress were more than 43%, broad sense heritability of grain yield under

low N averaged 29% smaller than under high N, and the differences in heritability levels between low and high N were significant at $P<0.01$(Banziger *et al.*, 1997). Average genotypic variance for grain yield under low N was about one third of that under high N (significant at $P<0.001$), while average error variance was similar at both N levels (Banziger *et al.*, 1997). They found that genetic correlations were generally positive with a magnitude of 0.38 between yields under low and high N conditions. Relative yield reduction under low N explained 27% of the variation in genetic correlations between experiments, and 61% of the variation in the ratio of genotype and N-level variance to the total of genotypic plus genotype x N-level variances. As the relative yield reduction under low N increased, genetic correlation between grain yields under low and high N decreased, and the proportion of genotype x N-level variance to the total genotypic plus genotype x N-level variance became larger (Banziger *et al.*, 1997).

2.6. Relation between key agronomic traits under stress

In Eberhart & Russel's study (1966), grain yield in stressed environments was positively correlated with yield in non-stressed environments ($r=0.71$, $P<0.01$). Estimates of the coefficient of regression across environments were $b=1.04$ for improved varieties and $b=0.65$ for the local varieties, indicating that improved varieties were more responsive to higher yielding environments. Deviations of 0.13 and 0.22, respectively, for improved and landrace varieties indicated that improved maize varieties had yields closer to their regression line than the local landrace varieties. The positive association of grain yield in stressed environments to that in high yielding environments indicated that generally, varieties that were outstanding in the stressed environment were also high yielding in the optimal environment, and hence the methodology for improving populations and varieties

for stressed environments enhanced productivity across both low and high yielding environments (Eberhart and Russell, 1966).

Significant positive correlations were found between plant and ear height and days-to-anthesis in OPVs and hybrids. Silking was significantly delayed when stressed by low N (Monneveux *et al.*, 2005). Chlorophyll concentration was strongly affected by stress conditions. The special products analysis division (SPAD) values were lower under low N and yield was positively correlated with chlorophyll concentration measured 4 weeks after male flowering. An association was also found between yield and the number of green leaves above the ear either 6 or 8 weeks after male flowering (Monneveux *et al.*, 2005).

2.7. Possibilities for Indirect selection

The consideration of secondary traits could improve selection efficiency as indirect selection for a single secondary trait results in greater progress for grain yield than direct selection for grain yield and may prove more useful in stress environments where heritabilities for grain yield are low (Banziger and Lafitte, 1997). In a study on efficiency secondary traits for improving maize for low- nitrogen target environments (Banziger and Lafitte, 1997); indirect selection for single secondary traits was predicted to be superior to direct selection for grain yield for improving grain yield under low N in 7 of the 19 experiments. Ears per plant was the most effective secondary selection criterion, followed by leaf senescence. In none of the experiments was selection for ASI or leaf chlorophyll concentration predicted to be more effective at improving grain yield than direct selection for grain yield. The study made comparisons between predicted responses in individual

experiments and standard errors of estimation were not considered and thus predicted responses of grain yield to selection for any single trait were compared across experiments using pairwise t-tests. When responses to selection for single secondary traits were compared, response of grain yield to selection for either ears per plant or leaf senescence was predicted to be significantly larger than response of grain yield to selection for ASI or leaf chlorophyll concentration. The observed genetic correlations of grain yield with ASI, ears per plant, leaf chlorophyll concentration and leaf senescence confirmed that these traits are generally linked with N- stress tolerance and therefore very useful in selecting genotypes for low N performance.

On the other hand, anthesis-silking interval (ASI) was found to be an effective predictor of grain yield under low N conditions in a study on population density and low nitrogen on yield associated traits in tropical maize Monneveux *et al.*, (2005). Miti *et al.*, (2010) considered indirect selection under optimal environments to select genotypes that could yield well under low N conditions and the importance of indirect selection for grain yield under optimal conditions for the low N environment, depended on the genetic correlation of grain yield under optimal to that under low N conditions.

CHAPTER THREE

MATERIALS AND METHODS

3.1. Experimental site

This study was conducted at National Crops Resources Research Institute (NaCRRI), at Namulonge, Uganda. The research station is 1,150 meters above sea level (masl), with a bimodal rainfall of 1,270 mm/year and it lies 00 32" N of the equator and 32^0 37" E. The station is located 27Km North of Kampala with mean temperatures of 28.4^o C maximum, 15.9^o C minimum, and 22.2^o C annually. The soils at NaCRRI are ferralitic (red sandy clay loams), with a pH of 4.9-5.0, with tall grassland vegetation.

3.2. Experimental environments

The study was conducted for two seasons during 2009: March to June (2009A) and October to January, (2009B) under both optimal and low N conditions in fields adjacent to each other. The trials were rainfed, except in 2009A when sprinkle irrigation was used twice to supplement the insufficient rainfall whenever the soil moisture was very low. Initial soil analysis at the site was conducted before planting at the National Agriculture Research Laboratories (NARL) to establish the levels of nitrogen, potassium, phosphorus and other nutrients. (Table 1).

Table 1: Results of soil analysis at trial sites for low and optimal N sites

Trial	Texture	PH	Organic matter (%)	N (%)	P (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)
Optimal N	Sandy- clay	4.62	3.85	0.21	5.42	353	1222	302.29
Low N	Clay	5.35	3.74	0.2	3.64	760	2176	389.42
Critical values		5.2	3.00	0.20	5.00	150	350	100
Sufficient values		5.2-7.0	5.0 -6.0	0.3	20.0	500	2000	

3.2.1. Environment 1: Optimal conditions

Nitrogenous fertilizers were applied at rate of 120kg/ha at planting and 80 kg/ha side dressed thirty days after planting. Triple-super phosphate was applied at a rate of 60 kg P/ha at planting time, and muriate of potash at 30 kg K/ha was added 30 days after emergence.

3.2.2. Environment 2: Low N conditions

The trials were conducted for two seasons; 2009A and 2009B at NaCRRI. The trial depended on rainfall, except in the season of 2009A where sprinkle irrigation was used to supplement the insufficient rainfall. The site had been maintained without application of N fertilizers either as inorganic or organic form for a long period (over 20 years) (G.Asea, personal communication). In addition continuous cultivation of maize and sorghum at a high density was maintained producing high biomass to deplete the soil of nitrogen. All crop residues were immediately removed after harvest. Nitrogen was not applied to the trial but single super phosphate used to supply phosphorus and muriate of potash for potassium at a rate of 60 Kg P/ha at planting and 30Kg K/ha 30 days after crop emergence respectively.

3.3. Description of the source population, Longe 4

Longe 4 is a popular OPV in Uganda. It is earlier maturing (105-110 days from planting to physiological maturity) than other OPVs and hybrids, and is very popular in drought prone areas where the rains tail off early. It was developed by crossing Longe 1–an earlier developed OPV (semi flint) and the Pool 16 population (early maturing white flint), and was released in Uganda in 2002 (Cereals annual report, 2007).

3.4. Generation of S_1 and S_2 families (selfed first and second generations)

Longe 4 seed was obtained from a Longe 4 bulk population and planted in a nursery at NaCRRI in 2008A under optimal N conditions. The plot size was 5m long, with 0.75m between rows and 0.35m between plants, with two seeds planted per hill and later thinned to one plant per hill two weeks after germination. At least 10 plants per plot were selfed. All standard agronomic practices were followed. Planting, self pollination and harvesting were done by hand. Selfed ears were harvested and shelled, with the resulting S_1 seed kept separate by family.

From this harvested seed, 198 S_1 families were grown in a nursery under optimal N conditions at NaCRRI during the 2008B season and standard agronomic practices were followed. S_2 seed was produced by selfing 3-5 desirable plants within each S_1 row. At harvest, 60 S_1 families were selected for replanting as S_2 families based on general plant performance, plant architecture and ear aspect (texture- dentiness and flintiness; ear rot and cob size). After harvest, 50 of the 60 families were selected for further evaluation based on available seed. The seed from each selfed progeny was shelled and stored ear to

packet. At planting, approximately equal number of kernels from each ear were bulked to form an S_1-derived S_2 family.

3.5. Evaluation of selected families

Based on S_1 visual selection, 50 S_1 families (S_1) were replanted from remnant seed, along with their corresponding 50 S_2 families. Concomitantly, an equal number (50) of S_1-derived S_2 families were randomly selected to produce an unselected control population using a random number generation procedure. Eleven of the randomly selected families overlapped with those previously, thirty nine random selections did not overlap. This process resulted in a total of 150 families (S_1, S_2 and random families) that were grown together. The Longe 4 population was also used as a check in the second season of evaluation.

3.5.1 Experimental design and field management

Each trial was established as a 10 x 15 alpha lattice design with two replications in each environment. Planting was done on 15th April in 2009A and on 21st September in 2009B. The plot size measured 5m long, 0.75m between rows with one plant per hill spaced at 0.35m. Data was recorded on agronomic traits and other traits associated with low N. Two border rows were included in each trial to minimize boarder effect and protect the trials but excluded at harvesting. The trials were maintained free of weeds by hand weeding. Planting and harvesting were also done by hand. Fertilizers were applied as previously described.

3.5.2 Data collection

The number of plants was counted in each plot after emergence and gap filling was done where necessary. Anthesis date (AD) and silking date (SD) were recorded as the number of days after planting that 50% of the plants shed pollen or their silks emerged, respectively. The anthesis-silking interval (ASI) was determined as days between silking date and anthesis date. Data was collected on the plant height (measured in cm as the distance from the base of the plant to the height of the first tassel branch), ear height (base to the node bearing the upper ear), leaf senescence and yellowing. Leaf senescence was scored on 2-3 occasions, 7-10 days apart during the latter part of grain filling, using a scale of 0-10, where 0 = no senescence and 10 =100% of leaves dead (Banziger *et al.*, 2000). Yellowing was scored from 1-5, where 1 = all plants in a row completely green and 5 = all plants completely yellow (G. Asea, Personal communication).

Two key diseases were also recorded following natural infestation as; *Turcicum* leaf bright (TLB) was scored on a scale of 1-5, where 1 = no symptoms and 5 = all plants in a row severely infected while maize streak virus disease (MSV) was scored as the number of plants affected in each plot (Pernet *et al.*, 1999). At tasselling, ear leaves from five plants in a row were bulked from each site, dried and crushed. Using Kjeldahl's method, the percentage nitrogen was determined and expressed as leaf nitrogen concentration (LNC). At harvest the number of plants per plot and the number of ears per plot were counted, and field weight and grain moisture were recorded. In 2009A, shelling percent was assumed to be 80% for all entries. In 2009B, 3-4 representative ears from each plot were weighed and shelled for calculation of shelling percentage. Grain yield was

calculated from field weight and shelling percentage, and standardized to 12.5% moisture. In 2009A, shelling percent was assumed to be 80% for all entries.

3.6 Data analysis

Data within each trial were subjected to REML and ordinary analysis of variance using Genstat 12th edition. Generation and family means were computed and were compared by Fisher's protected LSD at 5% ($P<0.05$) when significant differences were present. Skeleton ANOVA for expected results are presented in Table 2 and 3.

Table 2: Skeleton ANOVA for within season analysis

Source	Model term	df	Expected mean squares[1]	F-test denominator
N-level	Fixed	1	σ^2_e+15 N-level*Rep*Block +150Rep*N-level 300N-level	N-level*Rep
N-level*Rep	Random	2	σ^2_e+15 N-level*Rep*Block +150Rep*N-level	N-level*Rep*Block
N-level*Rep*Block	Random	36	σ^2_e+ 15 N-level*Rep*Block	Error
Generations	Fixed	2	σ^2_e+ 200G	Error
Generation/Families	Fixed	147	σ^2_e+4GF	Error
N-level*Generation	Fixed	2	σ^2_e+100GN-level	Error
N-level*Generation/ Families	Fixed	147	σ^2_e+2GFN-level	Error
Error			σ^2_e	
Mean				
LSD (0.05)				
CV (%)				

Rep=Replication, B=Block, G=generation, and F=families

Table 3: Skeleton ANOVA for combined season analysis

Source	Model term	df	Expected mean squares[1]	F-test denominator
Season	Random	1	σ^2_e + 600S	Error
N-level	Fixed	1	σ^2_e + 300SN-level + 600 N-level	Season*N-level
Season* N-Level		2	σ^2_e + 300SN-level	Error
Generations	Fixed	2	σ^2_e + 200GS + 400G	Season*Generations
Season*Generations		2	σ^2_e + 200GS	Error
N-level*Gen	Fixed	2	σ^2_e . 100GSN-level + 200GN-level	Season*N-level* Generations
Season*N-level* Generations		2	σ^2_e + 100GSN-level	Error
Families within generations	Fixed	147	σ^2_e + 4GFS-level+8GF	Season*Generations*Family
Season*Families within generations		147	σ^2_e +4GFS	Error
N-level*Families within Generations	Fixed	147	σ^2_e + 2GFSN-level +4 GF N-level	Seasons*N-level* Generations/Families
Season*N-level* Families within Generations		147	σ^2_e + 2GF SN- level	Error
Pooled Error		524	σ^2_e	
Mean				
LSD (0.05)				
CV (%)				

[1] S=season, G=generation, and F=families

Broad sense heritability estimates were calculated as $H^2 = V_G/V_F$ where V_G = genotypic variance and V_F = phenotypic variance among families. The parent-offspring regression coefficient "b" was used as an estimate of narrow sense heritability (Vogel *et al.*, 1980, Falconer and Mackay, 1996). Predicted gains based on parent-offspring regression were calculated from $G = K^* b\sqrt{V_F}$ where **G** is the predicted gain, **K** is the standard selection differential, **b** is the narrow sense heritability estimate and $\mathbf{V_F}$ is the phenotypic family variance (Allard, 1960). Correlations of entry means were determined between grain yield and secondary traits, and between N-levels and generations.

CHAPTER FOUR

RESULTS

4.1 General performance of S_1 and S_2 families

4.1.1 Comparison of seasons and N-levels

During 2009A, there was an incidence of drought for about one month and supplementary irrigation was applied but the trial was still affected. Leaf senescence set in early and the effect of turcicum leaf blight (TLB) was more pronounced. Significant differences were observed for grain yield and plant height within generations ($P \leq 0.001$). Families showed significant differences for grain yield, maize streak virus (MSV), TLB and plant height. However no variation was observed at nitrogen levels for all the traits except for anthesis silking interval and maize streak virus (Table 4).

Table 4: ANOVA for grain yield and other agronomic traits of selfed progenies evaluated under low and optimum nitrogen conditions at NACRRI during 2009A

		MS						
Source	Df	GY	ASI	EPP	MSV	TLB	PH	LNC
N-level	1	35.15	191.16'	2.061	252.72*	1.27	42444	6.12
N-level*Rep	2	20.79***	12.02	0.792***	9.92*	0.93	6451***	6.72***
N-level*Rep*Block	36	1.08***	7.52	0.052	2.62**	0.58***	494***	0.45***
Generations	2	8.15***	3.29	0.000	0.05	0.00	35***	0.00
Generation/Families	147	0.85*	8.37	0.052	3.79***	0.32*	640***	0.18
N-level*Generation	2	8.65***	9.04	0.000	0.03	0.03	5*	-0.00
N-level*Generation/ Families	147	0.89**	9.04*	0.049	2.65***	0.31*	618***	0.21
Error	262	0.44	6.67	0.052	1.43	0.21	210	0.127
Total	599							

*, **, *** Significant at P≤0.05, 0.01, and 0.001, respectively. The appropriate denominator MS used in the F-tests are shown in Table 2. GY, Grain yield, ASI, anthesis silking interval, EPP, Ears per plant, PH, plant height, MSV, Maize streak virus and TLB, turcicum leaf blight.

In 2009B growth conditions were optimum and a good stand count was recorded but a number of plants were damaged by termites and stem borers. Significant differences were observed for grain yield and turcicum leaf blight in the testing environments. Variations within generations and families within generations were observed for all the traits except ears per plant suggesting that ear size other than number of ears caused the difference in generations (Table 5).

Table 5: ANOVA for grain yield and other agronomic traits of selfed progenies evaluated under low and optimum nitrogen conditions at NACRRI during 2009B

		MS					
Source	**Df**	**GY**	**ASI**	**EPP**	**MSV**	**TLB**	**PH**
N-level	1	96.55*	84.36	0.0005	0.00	13.14**	18667.00
N-level*Rep	2	2.30*	13.58*	0.046	8.37*	0.12	13526***
N-level*Rep*Block	36	0.70*	2.91	0.090	2.25	0.15***	421.00
Generations	2	44.82***	99.91***	0.142	51.61***	4.84***	42507***
Generation/Families	147	2.09***	7.95***	0.090	3.92***	0.28***	1222.00**
N-level*Generation	2	0.78	21.58**	0.103	25.66*	0.17*	415.00
N-level*Generation/ Families	147	0.52*	3.08	0.088	1.93	0.08***	407.00*
Error	262	0.40	3.05	0.084	1.93	0.049	309.00
Total	599						

*,**,***Significant at P≤0.05, 0.01, and 0.001, respectively. The appropriate denominator MS used in the F-tests are shown in Table 2

GY, Grain yield, ASI, anthesis silking interval, EPP, Ears per plant, PH, plant height, MSV, Maize streak virus and TLB, Turcicum leaf blight.

The results revealed that the two planting seasons were significantly different ($P \leq 0.001$) for grain yield and all other agronomic traits except yellowing (Table 6). Significant differences were observed on all traits for N-levels and across seasons. In each season and across seasons, as expected mean grain yields were higher for optimal nitrogen conditions than for low nitrogen. In both seasons, replication and blocking effects were significant implying that they were beneficial in the lattice design (Tables 4 and 5). Overall means for some important traits were; 2.31 t/ha for grain yield, 2.5 d for ASI and 0.96 ears per plant. For low N- stress indicators, the results showed no significant effects for yellowing in either season or N- level (Table 7). The effect of N-levels was significant on leaf nitrogen concentration ($P \leq 0.001$) but no significant differences were observed among families within generations. Seasonal effects on leaf senescence were significant ($P \leq 0.01$) across the two seasons but significant differences were observed among families within generations.

Table 6: Combined ANOVA for grain yield and agronomic traits of S_1 & S_2 selfed progenies evaluated under low and optimal N conditions during 2009A and 2009B

		MS						
Source	**Df**	**GY(T/ha)**	**ASI(d)**	**EPP(no.)**	**MSV (no. plants)**	**TLB score**	**PH(cm)**	**F-test denominator**
Season	1	40.86***	88.94***	0.672***	13.59***	95.34***	179003***	Error
N-level	1	130.17*	83.63	0.478	255.74***	5.64	1199	Season*N-level
Season* N-Level	2	2.50***	22.04***	0.554***	0.02	1.56***	29393***	Error
Generations	2	8.48*	2.62	0.007	2.64	0.47	5431	Season*Generations
Season*Generations	2	0.13	4.24	0.006	3.73*	0.14	441*	Error
N-level*Gen	2	2.35	0.26	0.007	0.69	0.49	275	Season*N-level*Generations
Season* N-Level* Generations	2	0.87*	4.59	0.015	3.28*	0.63***	228	Error
Families within generations	147	0.82***	5.59***	0.034***	2.80***	0.21***	525***	Season*Generations*Family
Season*Families within generations	147	0.46***	2.31	0.012	0.89	0.09**	224***	Error
N-level*Families within Generations	147	0.26	3.43*	0.026**	1.35**	0.09	197	Seasons*N-level*Generations/Families
Season*N-level*Families within Generations	147	0.26**	2.43	0.017	0.83	0.10***	216***	Error
Pooled Error	524	0.19	2.58	0.029	0.84	0.07	130	
Mean		2.31	2.45	0.96	1.58	2.07	120	
LSD (0.05)		0.08	0.28	0.02	0.09	0:05	2.31	
CV (%)		18.90	65.60	17.70	58.00	12.80	9.50	

*,**,***Significant at P≤0.05, 0.01, and 0.001, respectively. The appropriate denominator MS used in the F-tests are shown in Table 3. GY, Grain yield, ASI, anthesis silking interval, EPP, Ears per plant, PH, plant height

MSV, Maize streak virus and TLB, Turcicum leaf blight.

Table 7: Combined ANOVA for leaf senescence, yellowing and leaf nitrogen concentration under low N- conditions

		MS			
Source	**Df**	**LS**	**YW**	**LNC**	**F-test Den**
Season/N-level	1	2.42**	0.29	3.06***	Error
Generation	2	1.15	0.11	0.04	Season/N-level*Gen
Season/N-level*Generation	2	0.55	0.17	0.00	Error
Families/generation	147	0.23	0.06	0.07	Fam/Gen*Season/N-level
Family/generation*Season*N-level	147	0.19	0.08	0.08	Error
Error	262	0.24	0.08	0.13	
Mean		2.02	1.55	2.02	
LSD(0.05)		0.06	0.06	0.06	
CV (%)		35.7	18.40	17.90	

*, **,***Significant at P≤0.05, 0.01, and 0.001, respectively.

LS=Leaf senescence, YW= Yellowing, and LNC= Leaf nitrogen concentration

4.1.2. Comparison of generations

The mean grain yield for the trial was 2.31tha^{-1}. S_1 families in each season and across seasons significantly out-yielded S_2 families and random selections (S_2R) while means for selected (S_2) and random (S_2R) families did not differ significantly in both seasons. S_1 and S_2 means differed significantly in each N-level averaged over the two seasons even though there was no significant difference between S_1 and S_2 families under low nitrogen in 2009A (Table 8). For all traits, significant differences were observed between seasons and N-levels (appendix Tables 1-8). Overall, generational differences were significant only for grain yield and plant height in 2009A (Table 4). In 2009B, significant differences were observed for all traits shown except for EPP (Table 5). There was an interaction of N-levels with seasons for all traits except MSV (Table 6). Seasonal effects on generational performance were only significant for MSV and plant height ($P \leq 0.05$) while the effect of N-levels were consistent across generations in all traits, as shown by the lack of any significant N-level x generation interactions when compared to the season x N-level x generation interaction (Table 6).

Table 8: Mean performance of S_1 and S_2 families for grain yield across seasons and environments

		SEASON		
		2009A	2009B	Comb
N-level	GEN			
LN	S_1	1.94a	1.93a	**1.94a**
	S_2	1.89a	1.63b	**1.76b**
	S_2R	1.94a	1.61b	**1.78b**
Opt N	S_1	3.39a	2.86a	**3.13a**
	S_2	2.84b	2.44b	**2.64b**
	S_2R	2.72b	2.48b	**2.60b**
Comb	S_1	2.67a	2.39a	**2.53a**
	S_2	2.37b	2.04b	**2.21b**
	S_2R	2.33b	2.05b	**2.19b**
	Mean	**2.46**	**2.16**	**2.31**
LN		1.92b	1.72b	**1.82b**
Opt N		2.97a	2.59a	**2.79a**
Overall yield reduction (1-Low N/ Optimal N)		35%	34%	
Comb		**2.45**	**2.16**	**2.31**

[1] Comb, combined seasons, GEN, generation, Opt N, optimal nitrogen, LN, low nitrogen conditions, S_1, progenies selfed once, S_2, progenies selfed twice and S_2R, randomly selected S_2s.

[2] LSD: Season=0.08, N-level=0.08, Season*N-level=0.12, GEN=0.10, Season*GEN= 0.14, N-level *GEN=0.14, Season* N-level*.GEN=0.20 (the appropriate denominator MS used as error terms for calculating LSD's are shown in Table 2)

[3] Means within a column followed by a common lower case letter are not significant by F- protected LSD (α=0.05)

4.1.3. Comparison of families within generations

S_1 and S_2 families showed significant differences for grain yield, plant height and reaction to TLB and MSV in both seasons while ASI was significant only in 2009B (Tables 4 and 5). Significant interactions of family within generations with N-levels were observed for grain yield, ASI, plant height, TLB, and MSV in 2009A, whereas a significant interaction was observed for grain yield, plant height, and reaction to TLB in 2009B (Tables 4 & 5). Across seasons, families showed significant differences for all the variables (Table 6). Families showed no significant differences for leaf indicators of stress (senescence, yellowing and leaf nitrogen concentration) in either season or across seasons (Table 7). Significant interactions of families within generations across seasons were observed for grain yield, TLB and plant height. Interactions of families with N-levels were significant for ASI, ears per plant and MSV and significant three-way interactions were observed for families with N-levels by Seasons for grain yield, TLB and plant height (Table 6).The effect of N-levels was significant on leaf nitrogen concentration ($P \leq 0.001$) but not significant among families within generations for this trait (Table 7). Seasonal effects on leaf senescence were significant ($P \leq 0.01$), but no significant differences were observed among families within generations for the same trait (Table 7).

4.1.4. Performance of selected families between the two N-levels

The overall average yield reduction of low-N compared to optimal nitrogen was 35% and 34% during the seasons of 2009A and 2009B, respectively (Table 8). When averaged over the two fertility environments, ranks showed seven and eight families appearing in both generations during 2009A and 2009B, respectively. Eight S_1 families ranked well in both seasons while only two S_2 families showed up in both seasons (Tables 9 and 10). Average ranks were more consistent in both seasons and a high selection differential in measured units was noted in 2009B. Ranking by low N showed five and nine families in both generations during 2009A and 2009B, respectively. When ranks were averaged across N-levels, six S_1 families performed well in both seasons, and only two S_2 families performed well in both seasons. In all cases, ranks suggested moderate consistency in performance and that selection of these families was not by chance (Table 11 and 12).

Table 9: Performance of S_1 and S_2 families under low and optimal nitrogen conditions, ranked by average over N-levels

Rank			Grain yield (t/ha)		Average yield(t/ha)	Rank
Family	Low N	Opt N	Low N	Opt N		
2009A S_1 families						
105	50	1	1.38	5.58	3.48	1
123	44	2	1.60	5.20	3.40	2
12	15	4	2.05	4.74	3.39	3
41	42	3	1.69	4.88	3.29	4
15	14	6	2.07	4.46	3.27	5
185	24	5	1.98	4.54	3.26	6
170	4	8	2.31	4.17	3.24	7
8	8	9	2.18	4.03	3.11	8
68	13	11	2.07	3.97	3.02	9
01	5	13	2.29	3.75	3.02	10
138	25	10	1.97	4.02	2.99	11
191	6	14	2.27	3.63	2.95	12
78	1	24	2.45	3.30	2.87	13
181	49	7	1.41	4.30	2.86	14
54	16	15	2.03	3.60	2.82	15
Mean of selected families			**1.98**	**4.28**	**3.13**	
Mean of all 50 Families			**1.94**	**3.39**	**2.27**	
LSD (family)			**0.19**	**0.27**	**0.23**	
2009A S_2 families						
9	2	1	2.46	4.57	3.52	1
63	1	4	2.63	3.81	3.22	2
41	23	2	1.90	4.46	3.18	3
75	16	3	1.99	4.07	3.03	4
181	11	7	2.05	3.71	2.88	5
121	3	11	2.45	3.26	2.86	6
12	12	8	2.04	3.62	2.83	7
158	30	5	1.81	3.77	2.79	8
170	4	12	2.29	3.23	2.76	9
105	35	6	1.73	3.73	2.73	10
78	39	9	1.69	3.62	2.65	11
45	44	10	1.65	3.56	2.61	12
107	14	13	2.02	3.03	2.52	13
185	7	21	2.15	2.85	2.50	14
111	15	15	2.01	2.97	2.49	15
Mean of selected families			**2.06**	**3.62**	**2.84**	
Mean of all 50 Families			**1.89**	**2.84**	**2.37**	
LSD (family)			**0.19**	**0.27**	**0.23**	

Table 10: Performance of S_1 & S_2 families under low and optimal nitrogen conditions, ranked by average over N-levels

	Rank		Grain yield (t/ha)		Average yield(t/ha)	Rank
Family	**Low N**	**Opt N**	**Low N**	**Opt N**		
2009B S_1 families						
181	1	1	4.09	5.81	4.95	1
123	6	2	3.57	5.27	4.42	2
105	2	3	3.93	4.80	4.36	3
41	4	4	3.63	4.23	3.93	4
12	3	16	3.76	3.18	3.47	5
93	8	6	2.91	4.03	3.47	6
15	5	19	3.59	3.12	3.36	7
140	9	14	2.90	3.36	3.13	8
68	11	8	2.53	3.67	3.10	9
47	24	5	1.85	4.20	3.02	10
9	13	9	2.37	3.48	2.92	11
159	12	11	2.40	3.41	2.90	12
121	22	7	1.90	3.80	2.85	13
170	14	18	2.32	3.15	2.73	14
1	15	22	2.31	2.97	2.64	15
Mean of selected families			**2.94**	**3.90**	**3.42**	
Mean of all 50 Families			**1.93**	**2.86**	**2.39**	
LSD (family)			**0.19**	**0.25**	**0.22**	
2009B S_2 families						
123	3	1	2.49	4.05	3.27	1
52	10	3	2.18	3.42	2.80	2
47	12	5	2.12	3.34	2.73	3
8	15	4	2.03	3.37	2.70	4
105	7	10	2.44	2.93	2.68	5
170	13	7	2.09	3.23	2.66	6
185	25	2	1.60	3.60	2.60	7
45	11	9	2.15	2.94	2.55	8
1	4	17	2.49	2.60	2.54	9
12	1	20	2.54	2.53	2.53	10
93	9	12	2.19	2.72	2.45	11
140	24	6	1.62	3.27	2.44	12
138	5	24	2.45	2.33	2.39	13
191	6	27	2.44	2.28	2.36	14
15	14	14	2.05	2.64	2.35	15
Mean of selected families			**2.19**	**3.02**	**2.61**	
Mean of all 50 Families			**1.63**	**2.44**	**4.07**	
LSD (family)			**0.19**	**0.25**	**0.22**	

Table 11: Performance under low and optimal nitrogen of the 15 families in each generation that yielded best in low-N conditions

	2009A S_1 families				
	Yield Rank		Grain yield (t/ha		
Family	Low N	Opt N	Low N	Opt N	Relative yield reduction
78	1	24	2.45	3.30	0.26
29	2	26	2.34	3.27	0.28
121	3	25	2.32	3.29	0.29
170	4	8	2.31	4.17	0.45
101	5	13	2.29	3.75	0.39
191	6	14	2.27	3.63	0.38
158	7	29	2.25	3.24	0.31
8	8	9	2.18	4.03	0.46
74	9	44	2.17	2.49	0.13
45	10	27	2.15	3.26	0.34
9	11	39	2.13	2.81	0.24
63	12	21	2.11	3.37	0.37
68	13	11	2.07	3.97	0.48
15	14	6	2.07	4.46	0.54
12	15	4	2.05	4.74	0.57
Mean of selected families			**2.21**	**3.59**	
Mean of all 50 Families			**1.94**	**3.39**	
LSD(family)			**0.19**	**0.27**	
	2009A S_2 families				
63	1	4	2.63	3.81	0.31
9	2	1	2.46	4.57	0.46
121	3	11	2.45	3.26	0.25
170	4	12	2.29	3.23	0.29
1	5	38	2.26	2.36	0.04
173	6	41	2.26	2.30	0.02
185	7	21	2.15	2.85	0.25
149	8	44	2.13	2.15	0.01
2	9	33	2.06	2.59	0.20
178	10	29	2.06	2.70	0.24
181	11	7	2.05	3.71	0.45
12	12	8	2.04	3.62	0.44
123	13	36	2.03	2.53	0.20
107	14	13	2.02	3.03	0.33
111	15	15	2.01	2.97	0.32
Mean of selected families			**2.19**	**3.05**	
Mean of all 50 Families			**1.90**	**2.86**	
LSD(family)			**0.19**	**0.27**	

Table 12: Performance under low and optimal nitrogen conditions of the 15 families in each generation that yielded best in low -N conditions

	Rank		Grain yield (t/ha)		
Family	Low N	Opt N	Low N	Opt N	Relative yield reduction
2009B S_1 families					
181	1	1	4.09	5.81	0.30
105	2	3	3.93	4.80	0.18
12	3	16	3.76	3.18	-0.18
41	4	4	3.63	4.23	0.14
15	5	19	3.59	3.12	-0.15
123	6	2	3.57	5.27	0.32
191	7	37	2.98	2.29	-0.30
93	8	6	2.91	4.03	0.28
140	9	14	2.90	3.36	0.14
185	10	28	2.53	2.52	0.00
68	11	8	2.53	3.67	0.31
159	12	11	2.40	3.41	0.29
9	13	9	2.37	3.48	0.32
170	14	18	2.32	3.15	0.26
1	15	22	2.31	2.97	0.22
Mean of selected families			**3.05**	**3.68**	
Mean of all 50 Families			**1.93**	**2.86**	
LSD(family)			**0.19**	**0.25**	
2009B S_2 families					
12	1	20	2.54	2.53	-0.00
68	2	35	2.53	2.16	-0.17
123	3	1	2.49	4.05	0.38
1	4	17	2.49	2.60	0.04
138	5	24	2.45	2.33	-0.05
191	6	27	2.44	2.28	-0.07
105	7	10	2.44	2.93	0.17
76	8	25	2.20	2.31	0.05
93	9	12	2.19	2.72	0.19
52	10	3	2.18	3.42	0.36
45	11	9	2.15	2.94	0.27
47	12	5	2.12	3.34	0.36
170	13	7	2.09	3.23	0.35
15	14	14	2.05	2.64	0.22
8	15	4	2.03	3.37	0.39
Mean of selected families			**2.29**	**2.86**	
Mean of all 50 Families			**1.63**	**2.44**	
LSD(family)			**0.19**	**0.25**	

The results further showed that the selection differential was higher for the best yielding families in each season under low N conditions compared to optimal conditions (Table 14). S_1 selection gave a larger selection differential for grain yield than did S_2 in 2009B, with only a minor difference between generations in 2009A. ASI selection differentials were larger in S_2 families compared to S_1, but ASI responded to yield selection in different directions in the two seasons, and showed a larger effect of yield selection by seasonal mean in low N than in opt N. For the number of ears per plant, the selection differential was small and inconsistent between seasons and among generations suggesting that the observed differences in yield could be due to ear size rather than number of ears.

The selection differential is greater under low nitrogen conditions as expected when average ranks are used, but the gain in optimal nitrogen conditions was low. The combined selection gain was higher when yield across N-levels was ranked by average of grain yield (Table 13).

Table 13: Means in low-N and opt-N of the 15 highest yielding families (as ranked by average over N-levels)

		2009A			2009B		
GY(t/ha)		**Low N**	**Opt N**	**LN+OptN**	**Low N**	**OptN**	**LN+OptN**
S_1	Selected families	1.98	4.28	3.13	2.94	3.90	3.42
	All (50)	1.94	3.39	2.67	1.93	2.86	2.39
	Sel. Diff.	**0.04**	**0.89**	**0.47**	**1.01**	**1.04**	**1.03**
S_2	Selected families	2.06	3.62	2.84	2.19	3.02	2.61
	All (50)	1.89	2.84	2.37	1.63	2.44	2.04
	Sel. Diff	**0.17**	**0.78**	**0.48**	**0.56**	**0.58**	**0.57**

Sel. Diff., selection differential in measured units

Table 14: Means in low-N and opt-N of various characteristics for the 15 highest yielding families (as ranked in low N)

		2009A			2009B		
GY(t/ha)		**Low N**	**Opt N**	**LN+OptN**	**Low N**	**Opt N**	**LN+OptN**
	Selected						
S_1	families	2.21	3.59	2.90	3.05	3.68	3.37
	All (50)	1.94	3.39	2.67	1.93	2.86	2.39
	Sel. Diff.	**0.27**	**0.20**	**0.24**	**1.12**	**0.82**	**0.97**
	Selected						
S_2	families	2.19	3.05	2.62	2.29	2.86	2.58
	All (50)	1.90	2.86	2.38	1.63	2.44	2.04
	Sel. Diff	**0.29**	**0.19**	**0.24**	**0.66**	**0.42**	**0.54**
	Selected						
ASI(d)	families	3.83	2.62	3.23	1.94	1.58	1.76
S_1	All (50)	3.72	2.47	3.09	2.36	2.02	2.19
	Sel. Diff	**0.11**	**0.15**	**0.13**	**-0.42**	**-0.44**	**0.43**
	Selected						
S_2	families	4.13	3.01	3.57	2.23	2.05	2.14
	All (50)	3.47	2.77	3.12	2.96	2.35	2.66
	Sel. Diff.	**0.66**	**0.24**	**0.45**	**-0.73**	**-0.30**	**-0.52**
	Selected						
EPP(no.)	families	1.05	0.95	1.00	0.91	0.87	0.89
S_1	All (50)	1.08	0.93	1.01	0.92	0.93	0.93
	Sel. Diff	**-0.03**	**0.02**	**-0.01**	**-0.01**	**-0.06**	**-0.04**
	Selected						
S_2	families	1.01	1.00	1.01	0.90	0.90	0.90
	All (50)	1.04	0.93	0.99	0.93	0.92	0.93
	Sel. Diff	**-0.03**	**0.07**	**0.02**	**-0.03**	**-0.02**	**-0.03**

Sel. Diff., selection differential in measured units

4.2. Variance components and heritability

4.2.1 Variance-component heritability estimates

Broad–sense heritabilities were estimated within each generation as V_G/V_F, where V_G is the genetic variance and V_F is the family variance. Large among-family variance compared to error variance was reflected in high heritability estimates. Generally, S_1 families had higher heritability estimates for grain yield in both environments in 2009A while S_2 families (selected or random) had inconsistent heritability estimates for all traits in both seasons and testing environments (Table 15). For S_1 families in 2009A, grain yield heritability estimates were 0.49 and 0.42 under optimal nitrogen conditions and low N respectively. For ASI, heritability estimates were greater for both S2 selected and random families than for S1 families, suggesting that homozygosity increased the frequency of alleles favouring flowering in the longe 4 populations. Genetic variances varied greatly between N-levels and among different traits: no consistency was observed (Tables15-18).

Table 15: Variance components and broad sense heritability estimates of grain yield and ASI for different generations and different N-levels during 2009A and 2009B

Season			2009A				2009B			
Variable	N-level	GEN	MS Family(V_F)	MS error	Genetic variance(V_G)	H^2	MS Family	MS error	Genetic variance	H^2
GY	Opt N	S_1	0.58	0.30	0.28	**0.49**	0.57	0.52	0.05	**0.09**
		S_2	0.42	0.30	0.12	**0.29**	0.19	0.52	-0.33	0.00
		S_2R	0.48	0.30	0.18	**0.38**	0.35	0.52	-0.479	0.00
	LN	S_1	0.25	0.15	0.11	**0.42**	0.19	0.25	-0.05	0.00
		S_2	0.18	0.15	0.03	**0.16**	0.09	0.25	-0.16	0.00
		S_2R	0.10	0.15	-0.05	**0.00**	0.18	0.25	-0.07	0.00
ASI	Opt N	S_1	2.68	2.47	0.21	**0.08**	1.75	2.73	-0.99	**0.00**
		S_2	3.71	2.47	1.24	**0.33**	2.24	2.73	-0.49	**0.00**
		S_2R	3.93	2.47	1.46	**0.37**	2.87	2.73	0.14	**0.05**
	LN	S_1	4.38	4.13	0.25	**0.06**	3.23	3.39	-0.16	**0.00**
		S_2	5.29	4.13	1.15	**0.22**	3.81	3.39	0.43	**0.11**
		S_2R	6.02	4.13	1.89	**0.31**	2.45	3.39	0.93	**0.38**

Table 16: Variance components and broad sense heritability estimates of ears per plant and plant height for different generations and different N-levels during 2009A and 2009B

Season			2009A				2009B			
Variable	N-level	GEN	MS Family(V_F)	MS error	Genetic variance(V_G)	H^2	MS Family	MS error	Genetic variance	H^2
EPP	Opt N	S_1	0.02	0.03	-0.02	**0.00**	0.19	0.14	0.05	**0.27**
		S_2	0.05	0.03	0.02	**0.35**	0.01	0.14	-0.14	**0.00**
		S_2R	0.01	0.03	-0.02	**0.00**	0.01	0.14	-0.13	**0.00**
	LN	S_1	0.03	0.02	0.01	**0.35**	0.02	0.03	-0.00	**0.00**
		S_2	0.01	0.02	-0.01	**0.00**	0.01	0.03	-0.01	**0.00**
		S_2R	0.01	0.02	-0.01	**0.48**	0.02	0.03	-0.00	**0.00**
PH	Opt N	S_1	360	94	266	**0.74**	249	231	19	**0.08**
		S_2	340	94	246	**0.72**	215	231	-16	**0.00**
		S_2R	372	94	277	**0.75**	323	231	93	**0.29**
	LN	S_1	101	111	-15	**0.00**	403.85	389	15	**0.04**
			241	111	125	**0.52**	261.43	389	-127	-
		S_2								**0.49**
		S_2R	161	111	45	**0.28**	455.52	389	67	**0.15**

Table 17: Variance components and broad sense heritability estimates of leaf nitrogen concentration, leaf senescence and yellowing for different generations and different N-levels during 2009A and 2009B

Season			2009A				2009B			
Variable	N-level	GEN	MS Family(V_F)	MS error	Genetic variance(V_G)	H^2	MS Family	MS error	Genetic variance	H^2
LNC	Opt N	S_1	0.10	0.14	-0.04	**0.00**				
		S_2	0.07	0.14	-0.07	**0.00**				
		S_2R	0.06	0.14	-0.08	**0.00**				
	LN	S_1	0.06	0.14	-0.08	**0.00**				
		S_2	0.06	0.14	-0.07	**0.00**				
		S_2R	0.08	0.14	-0.06					
LS	LN	S_1	0.17	0.12	0.05	**0.31**	0.27	0.25	0.01	**0.05**
		S_2	0.17	0.12	0.05	**0.31**	0.21	0.25	-0.04	**0.00**
		S_2R	0.09	0.12	-0.02	**0.00**	0.34	0.25	0.09	**0.25**
YG	LN	S_1	0.046	0.043	0.003	**0.07**	0.10	0.07	0.03	**0.34**
		S_2	0.044	0.043	0.001	**0.02**	0.08	0.07	0.01	**0.15**
		S_2R	0.039	0.043	-0.004	**0.00**	0.12	0.07	0.05	**0.42**

Table 18: Variance components and broad sense heritability estimates of MSV and TLB for different generations and different N-levels during 2009A and 2009B

Season			2009A				2009B			
Variable	N-level	GEN	MS Family(V_F)	MS error	Genetic variance(V_G)	H^2	MS Family	MS error	Genetic variance	H^2
MSV	**Opt N**	S_1	2.69	1.02	1.68	**0.62**	1.48	2.66	-1.18	**0.00**
		S_2	3.09	1.02	2.08	**0.67**	1.81	2.66	-0.85	**0.00**
		S_2R	2.13	1.02	1.11	**0.52**	2.66	2.66	0.00	**0.00**
	LN	S_1	0.45	0.41	0.04	**0.09**	0.85	1.22	-0.37	**0.00**
		S_2	0.36	0.41	-0.05	**0.00**	1.11	1.22	-0.11	**0.00**
		S_2R	0.39	0.41	-0.02	**0.00**	0.67	1.22	-0.54	**0.00**
TLB	**Opt N**	S_1	0.10	0.08	0.03	**0.27**	0.04	0.04	0.00	**0.11**
		S_2	0.17	0.08	0.10	**0.57**	0.07	0.04	0.03	**0.44**
		S_2R	0.18	0.08	0.11	**0.59**	0.09	0.04	0.05	**0.57**
	LN	S_1	0.18	0.14	0.05	**0.25**	0.10	0.06	0.04	**0.42**
		S_2	0.14	0.14	0.00	**0.02**	0.08	0.06	0.02	**0.24**
		S_2R	0.14	0.14	0.00	**0.03**	0.16	0.06	0.09	**0.61**

[1]Heritability was indicated as 0 when the estimate for genetic variance was negative.

[2]Where GEN, generation, H^2= Broad sense heritability estimate, GY=Grain yield, ASI=Anthesis silking interval, EPP=Ears per plant, MSV=Maize streak Virus, TLB=Turcicum leaf blight, PH= Plant height, LNC= Leaf nitrogen concentration LS=Leaf senescence and YW= Yellowing.

4.2.2 Parent-offspring regression

Heritability estimates ("b" values) from parent- offspring regression analysis for grain yield and ASI were only significant in 2009B for both N-levels (Table 19).

Low heritability estimates for grain yield in 2009A show that a large proportion of the estimated genetic variance was non-additive or errors of estimation were more than heritable variation. This could be due to the poor plant stand and irregularities in water supply during irrigation. However, the significant heritability estimates in 2009B suggest moderately high heritable variation and additive genetic effects.

Table 19: Parent-offspring regression for grain yield and ASI of S_2 on S_1 families during 2009A and 2009B

		Mean Squares							
		GY				ASI			
		2009A		2009B		2009A		2009B	
Source	**Df**	**Low N**	**Opt N**	**Low N**	**Opt N**	**Low N**	**Opt N**	**Low N**	**Opt N**
Regression	1	0.002	1.27	0.56*	2.33***	3.72	3.73	41.99***	22.26**
Residual	48	0.179	0.40	0.08	0.15	5.32	3.71	3.02	1.82
Total	49	0.176	0.42	0.09	0.19	5.27	3.71	3.81	2.24
"b"		0.01	0.21	0.24*	0.29***	0.13	0.17	0.52***	0.51**
S.E (b)		0.120	0.12	0.009	0.073	0.16	0.17	0.14	0.15

Opt N, Optimal nitrogen, LN, Low nitrogen, S_1 and S_2 generations.

*, **, ***, ns Significant at P≤0.05, P≤0.01, P≤0.001, and non significant respectively.

4.2.3 Predicted gains in the selected families

Predicted gains for grain yield were higher during 2009B under optimal conditions for both generations and very low under low nitrogen conditions during 2009A (Table 20). In both seasons and environments predicted gains for ASI were higher for S_2 than for S_1, resulting from a greater spread in ASI among S_2 families. Gains predicted from parent-offspring heritability and from the selection differential in measured units were lower compared to those calculated using the standardized selection differential (Table 21).

Table 20: Predicted gains from selection for yield and for ASI of S_1 and S_2 under low and optimal nitrogen conditions using parent-offspring heritability and standardized selection differential

	2009A		2009B		2009A		2009B	
	GY(t/ha)				ASI (d)			
	Low N	OptN	Low N	OptN	Low N	Opt N	Low N	OptN
S_1	0.01	0.19	0.12	0.25	0.32	0.32	1.084	0.783
S_2	0.01	0.16	0.07	0.15	0.35	0.38	1.177	0.885

Selection intensity = 30% (15/50 families), K=1.16 (standardized selection differential), among family variances taken from Table 15.

Table 21: Predicted gains from selection for yield of S_1 and S_2 under low and optimal nitrogen conditions using parent offspring heritability and selection differential in measured units

	2009A		2009B	
	GY(t/ha)		GY(t/ha)	
	Low N	Opt N	Low N	Opt N
S_1	0.0032	0.042	0.27	0.24
S_2	0.0035	0.040	0.16	0.12

Selection differential used is in measured units (t/ha); Table 13

4.3. Correlations

4.3.1. Correlation of secondary traits with yield

There was a weak, but statistically significant, negative correlation of yield with ASI, and weak positive correlations with plant height and LNC (Table 22). The positive correlation with TLB is unexplained, since one would expect that any correlation of yield with TLB would be negative. ASI correlated negatively with plant height and positively with ears per plant and TLB. Weak positive but significant correlations (r= 0.13-0.26) were observed between plant height and leaf senescence; yellowing and leaf senescence; TLB and yellowing. A significant negative correlation was observed between TLB and plant height.

Table 22: Correlation of grain yield and other agronomic traits, based on all 150 family means averaged across seasons and N-levels.

VARIABLE	GY	ASI	EPP	PH	LNC	LS	YW	MSV
GY								
ASI	-0.15**							
EPP	-0.07	0.12*						
PH	0.26***	-0.19***	-0.06					
LNC	0.12*	-0.03	0.05	-0.18**				
LS	-0.03	0.04	0.07	0.16**	-0.03			
YW	-0.03	0.08	-0.00	-0.05	0.07	0.27**		
MSV	0.01	0.01	-0.07	-0.08	-0.04	0.10	0.03	
TLB	0.11*	0.14*	0.05	-0.47**	0.26**	-0.05	0.13*	-0.04

*, **, ***Significant at P≤0.05, 0.01, and 0.001, respectively

GY=Grain yield, ASI=Anthesis silking interval, EPP=Ears per plant, MSV=Maize streak Virus, TLB=Turcicum leaf blight, PH= Plant height, LNC= Leaf nitrogen concentration LS=Leaf senescence and YW= Yellowing

4.3.2. S_1 and S_2 correlation

The results showed fairly strong correlations of family means between N-levels and generations in 2009B while only a small number of comparisons showed significance in 2009A (Table 23). Few significant correlations in 2009A could be attributed to the poor plant stand due to uneven germination and perhaps the non-uniformity of water supply to the two N-levels during irrigation. Correlations of S_1 family means in different N-levels in 2009B were 0.74 and 0.53 for grain yield and ASI, respectively, and 0.47 and 0.42, respectively, for S_2s (Table 23). The number of ears per plant was only significantly correlated between optimal and low nitrogen conditions for $S_{2\ in}$ 2009B. Family means for MSV rating correlated across both generations and N-levels during 2009B and only significant for S_1 families in low and optimal conditions in 2009A. For leaf senescence and yellowing, significant correlations ($P \leq 0.01$) were only observed during 2009B. No significant correlation was observed between generations and N-levels for leaf nitrogen percentage during 2009A.

Table 23: Correlation of family means in different generations in low and optimal conditions during the seasons of 2009A and 2009B

VARIABLE	GY	ASI	EPP	PH	LNC	LS	YG	MSV	TLB
N-level, Generation			**2009A**						
$OptNS_1$&LNS_1	-0.03	0.22	0.09	0.23	-0.03			0.41**	0.01
$OptNS_2$&LNS_2	0.02	-0.05	0.02	0.00	0.02			0.27	0.08
$OptNS_1$&$OptNS_2$	0.25	0.14	0.14	0.45***	0.25			0.49***	0.37**
LNS_1&LNS_2	-0.01	0.12	-0.04	-0.17	-0.01	-0.17	-0.01	-0.09	0.23
$OptNS_1$&LNS_2	0.05	0.20	0.03	-0.06	0.05			-0.06	0.11
$OptNS_2$&LNS_1	-0.01	0.07	0.12	0.25	-0.01			0.29*	-0.04
			2009B						
$OptNS_1$&LNS_1	0.74***	0.53***	-0.07	0.48***				0.36**	0.33*
$OptNS_2$&LNS_2	0.47***	0.42**	0.33*	0.39**				0.36*	0.53***
$OptNS_1$&$OptNS_2$	0.25	0.45**	0.17	0.34*				0.36**	0.50***
LNS_1&LNS_2	0.26	0.47***	0.17	0.40**		0.37**	0.37**	0.19	0.34*
$OptNS_1$&LNS_2	0.27	0.49***	-0.03	0.49***				0.34*	0.37**
$OptNS_2$&LNS_1	0.19	0.34*	0.08	-0.07				0.28	0.47***

*, **, ***Significant at P≤0.05, 0.01, and 0.001, respectively

GY=Grain yield, ASI=Anthesis silking interval, EPP=Ears per plant, MSV=Maize streak Virus, TLB=Turcicum leaf blight, PH= Plant height, LNC= Leaf nitrogen concentration LS=Leaf senescence and YW= Yellowing.

CHAPTER FIVE

DISCUSSION

5.1 Soil analysis of low and optimal nitrogen experimental fields

The low and optimal N treatments were grown in fields adjacent to each other and were identically managed apart from N fertilization. The level of N stress was sufficient to allow evaluation of the effect of selecting genotypes under the two N levels. Worku *et al.*,(2001) reported that yield was greatly reduced different under low N for all seasons and generations. It is assumed that nitrogen was the major factor leading to yield differences observed between low and optimal N environments, though other factors like soil texture, soil PH and depth might have influenced the results (Table 1). The soil analysis results indicated that the level of nitrogen was low for a nitrogen study; percentage nitrogen in the soil was 0.2%. Kikafunda *et al.* (2001) found the same percentage when evaluating maize varieties for N-use efficiency in Uganda using the same trial site. Organic matter content (3.74%) was in the lower end of the normal range of 3.0-6.0%, implying that the soil had little mineralization capacity for nitrogen.

5.2 Performance of S1 and S2 families

The low mean performance of S_2 compared to S_1 in both N-levels in 2009B and in optimum N in 2009A would seem to be due to inbreeding depression that was especially evident in some families. Indeed, Longe 4 is susceptible to inbreeding depression (G. Asea, Personal communication). The genotypes had longer days to silking under the low N conditions than under optimal soil fertility conditions, indicating the sensitivity of

silking date to N stress. The increase of average ASI from 2.4 days in optimal to >3.1 days under low N conditions reflects the delay in silking, and is in agreement with the results of Monneveux *et al.* (2005).

Ranks of average yield showed more consistency compared to ranking by low N. More S_1 families appeared in the selected group of S_1 in both seasons than did S_2s, showing greater consistency of S_1 performance and/or evaluation. Average ranks were more consistent in 2009B and a high selection differential in measured units was observed. The following S_1s appeared in the best selected fifteen families in both seasons; 105, 123, 12, 41, 15,170, 68 and 181) while the S_2 families appearing in both seasons were 105 and 170 (Tables 9 and 10). Inbreeding depression, due to reduced heterozygosity may have made the S_2 performance more variable across seasons. The good performance of selected S_1s under low N conditions shows that the inherent ability of these families to tolerate the abiotic stress, making them potentially useful in crop improvement targeting low N environments. Miti *et al,.* (2010) found some tolerant S_1 families in S_1 selection of local maize landraces for low soil nitrogen tolerance in Zambia. This indicates that the S1 tolerant lines found in this present study can be similarly used to improve Longe 4 for low nitrogen tolerance in Uganda.

5.3 Heritability estimates of grain yield and secondary traits

Broad sense heritability (H^2) for grain yield was 0.42 for S_1 families and 0.16 for S_2 families under low N conditions, and was higher than that of ASI, ears per plant and leaf senescence. The low to intermediate heritabilities meant that much of the grain yield was

not determined by genotypic effects suggesting that selection based on grain yield alone under low N conditions would produce limited gains. Under optimal conditions H^2 for grain yield was 0.49 and 0.29 for S_1 and S_2 families respectively and was also higher than that of other secondary traits except plant height. Average broad sense heritability estimates were 0.29 and 0.39 under low and optimal nitrogen conditions respectively, and not surprisingly, these were higher than the parent-offspring heritability estimates (0.13 under low N and 0.25 under optimal conditions). This is because parent offspring heritability estimates narrow sense heritability. Low N generally resulted in lower broad-sense heritabilities than optimal N, due to decreased genotypic variances in low N rather than increased error variances. Lower genotypic variances and lower heritabilities for grain yield under stressed conditions were reported by Banzinger *et al.*, (1997). In the same study error variances did not differ significantly between low and optimal nitrogen conditions, but they tended to decrease with increasing relative yield reduction of low N experiments. In the present study error variances for grain yield decreased modestly under low N compared to the optimal conditions (Table 15).

5.4 Prediction of gains

Predicted gains from selection were higher for S_1s under both environments due to greater genotypic variation among the S_1 families compared to S_2s. Predicted gains for grain yield were higher for optimal N than for low-N in both seasons (Table 17 &18). There was moderately good consistency between the two N-levels in respect to the families selected. Therefore, if only one of the fertility environments is used for selection, selections may yield poorly in the opposite selection environment (Worku *et al.*, 2001). Predicted gains were higher for ASI from S_2 selection, suggesting that selfing increased genetic variation for silking delay.

5.5 Correlation of yield with secondary traits and implications for indirect selection

The magnitude of the correlations between traits, based on overall family means, was shown in Table 19. Significant but weak correlations of grain yield with leaf nitrogen concentration (r= 0.12), with plant height (r=0.26), and with *Turcicum* leaf blight (r= 0.11) were found implying that the traits explained only a small portion of the variation in grain yield. A negative correlation of grain yield with ASI (r = -0.15) was found under low N conditions, but its small magnitude implies that this trait had little potential for indirect selection of yield in the conditions of this trial. Comparatively, Banziger and Lafitte (1997) found correlations of grain yield with ears per plant (r= 0.78), with ASI (r=-0.47) with leaf senescence (r=0.42), and with leaf chlorophyll concentration (r= 0.24). Similar findings were reported by Lafitte and Edmeades (1994a). A significant positive correlation between grain yield and LNC under low N conditions w

as noted in the present study, similar to results reported by Monneveux *et al.*, (2005) and to other studies of OPVs and hybrids under low N (Bazinger *et al.*, 1997). The correlations between grain yield and secondary traits were not strong enough to be used in selection for high yielding genotypes. This is contrary to the results of nitrogen studies Miti *et al.*, (2010) and Banziger *et al.*, 2000, 1997).

5.6 Correlation of yield across N-levels and across generations

Grain yield correlations between low and optimal nitrogen environments were found to be 0.74 for S_1 and 0.47 for S_2. These moderately strong correlations meant that genotypes selected for grain yield in one environment are likely to express their superiority at least partly under the other environment. Banziger *et al.*,(1997 also found positive genetic correlations of grain yield between low and optimal conditions which decreased with when N stress was more severe, indicating the importance of specific adaptability of genotypes, especially for severe stress.

A moderately strong positive correlation existed between low and optimal nitrogen conditions for both generations, indicating that the two fertility environments were sufficiently related to anticipate that selection in one environment may provide good results in the other. Ranks of average yield (low and optimal N) showed more consistency than ranks in low N, suggesting that using both environments gives a better estimate of the genetic performance of families. S_1 families consistently yielded higher and overall showed higher genetic variance and higher heritabilities than S_2 families. Hence, S_1 selection should be used rather than S_2 selection.

CHAPTER SIX

CONCLUSIONS

This study determined the yield performance of Longe 4 families in S_1 and S_2 generations under low and optimal N conditions; estimated heritability of yield in S_1 & S_2 generations and predicted expected gain under low N conditions for different selection approaches. The study also determined the association of yield with key secondary traits for possible use in indirect selection to improve yield.

Sufficient genetic variation was present among the selfed progenies to anticipate reasonable yield improvement in Longe 4 for low N conditions.

The correlation of family-mean grain yield between low N and optimal N-levels was moderately strong for S_1 families (0.74) and weak for S_2 families (0.48), meaning that indirect selection for low N under optimal conditions could be effective, but simultaneous selection in both N-levels may be better. Variance-component, broad-sense heritability for grain yield was moderate for S_1 families (0.42) and low for S_2 families (0.16) in 2009A, and was estimated to be 0 for S_2R (a random set of S_2 families for comparison) families in 2009A, and for all three sets of families in 2009B.

Parent-offspring (PO) regression estimates of narrow sense heritability in the two seasons were 0.21 and 0.29 in optimal N, and 0.01 and 0.24 in low N. It is not clear why the PO regression values were higher than the variance-component heritabilities in three of the

four environments. Nevertheless, the PO regression estimates appear to be more reliable than the variance-component heritability estimate in this experiment.

Based on the results of this study, S_1 families were more consistent than S_2 families in their performance in both fertility conditions, providing more potential for improving the population for low nitrogen tolerance. When yields were averaged across N-levels, the ranks gave better correspondence to each N-level than the ranks of each N-level compared to the other. The level of correlation between grain yield and key secondary traits was weak, explaining only a small part of the variation in grain yield. Therefore, indirect selection did not appear promising based on the present study. However, several nitrogen studies have revealed the potential of secondary traits to improve selection for grain yield and hence they should be evaluated further for their potential to improve the efficiency of selection for the target environment (Banziger and Lafitte, 1997).

RECOMMENDATIONS

Selection should be done at S_1 to save time by avoiding an extra season of selfing and the associated resources required. The lower predicted gain for S_2 indicates that the extra season and extra resources required for selfing to obtain S_2 seed would not be justified. Both testing environments should be used for selection if feasible.

S_1 recurrent selection is recommended; involving low-N and opt-N test environments, if feasible, the selected families should be planted and recombined in a recurrent selection cycle to determine actual selection gains.

Future low-N research would benefit from both the low N and optimal N fields being made more uniform, and the optimal N site being limed to at least partly neutralize the Acidic pH.

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APPENDIX

Table 1: Mean performance of S1 and S2 families across seasons and N-levels for ASI

		SEASON		
		2009A	2009B	Comb
N-level	GEN			
Opt N	S1	2.47a	2.02b	**2.25a**
	S2	2.73a	2.35a	**2.54a**
	S2R	2.5a	2.18a	**2.34a**
LN	S1	3.72a	2.36b	**3.04a**
	S2	3.45a	2.96a	**3.21a**
	S2R	3.93a	2.31b	**3.12a**
Comb	S1	3.09a	2.19b	**2.64a**
	S2	3.09a	2.66b	**2.88a**
	S2R	3.22a	2.25b	**2.74a**
	Mean			
OptN		2.57a	2.18a	**2.38a**
LN		3.69a	2.54b	**3.12b**
Comb		**3.13**	**2.36**	**2.75**

LSD: Season=0.28, N-level=0.28, Season*N-level=0.39 GEN=0.34, Season*GEN= 0.48, N-level *GEN=0.48, Season* N-level*.GEN=0.67

Table 2: **Mean performance of S1 and S2 families across seasons and N-levels for ears per plant**

		SEASON		
		2009A	**2009B**	**Comb**
N-level	**GEN**			
Opt N	**S1**	0.931a	0.933a	**0.932a**
	S2	0.931a	0.924a	**0.928a**
	S2R	0.938a	0.925a	**0.932a**
LN	**S1**	1.079a	0.919a	**0.999a**
	S2	1.041b	0.931a	**0.999a**
	S2R	1.032b	0.919a	**0.976a**
Comb	**S1**	1.005a	0.926a	**0.966a**
	S2	0.986a	0.928a	**0.964a**
	S2R	0.985a	0.922a	**0.954a**
	Mean	**0.992**	**0.925a**	**0.961**
OptN		0.933a	0.927a	**0.93a**
LN		1.051a	0.923b	**0.987b**
Comb		**0.992**	**0.925**	**0.959**

LSD: Season=0.02, N-level=0.02, Season*N-level=0.03, GEN=0.03, Season*GEN= 0.04, N-level *GEN=0.04, Season* N-level*.GEN=0.06

Table 3: Mean performance of S1 and S2 families across N-levels for plant height

		SEASON		
		2009A	2009B	Comb
N-level	GEN			
LN	S_1	96.07a	150.44a	**123.26a**
	S_2	95.55a	140.38b	**117.97b**
	S_2R	92.1b	138.54b	**115.32b**
Opt N	S_1	117.9a	139.44a	**128.67a**
	S_2	110.13b	130.26b	**120.19b**
	S_2R	106.17c	126.15c	**116.16c**
Comb	S_1	106.99a	144.94a	**125.97a**
	S_2	102.84b	135.32b	**119.08b**
	S_2R	99.14b	132.35b	**115.95c**
	Mean	**102.99a**	**137.54b**	**120.33c**
LN		94.58a	143.12b	**118.85a**
Opt N		111.4a	131.95b	**121.68b**
Comb		**102.99**	**137.54**	**120.27**

LSD: Season=2.31, N-level=2.31, Season*N-level=3.27, GEN=2.83, Season*GEN= 4.00, N-level *GEN=4.00, Season* N-level*.GEN=5.66

Table 4: Mean performance of S1 and S2 families across N-levels for Leaf Nitrogen Concentration

N-level	GEN	2009A
LN	S_1	1.937a
	S_2	1.894a
	S_2R	1.937a
Opt N	S_1	2.31a
	S_2	2.108b
	S_2R	2.136b
Comb	S_1	2.124a
	S_2	2.001b
	S_2R	2.037b
	Mean	
LN		1.923a
Opt N		2.125b
Comb		**2.024**

LSD: N-level=0.06, GEN=0.08, N-level *GEN=0.11

Table 5: Mean performance of S1 and S2 families across seasons and N-levels for leaf senescence

		SEASON		
		2009A	2009B	COMB
N-level	GEN			
LN	S_1	1.327a	1.348a	1.338a
	S_2	1.335a	1.652b	1.494b
	S_2R	1.188a	1.389b	1.289a
		1.283	1.46	1.372

LSD: Season=0.06, GEN=0.08, Season*GEN= 0.11

Table 6:Mean performance of S1 and S2 families across seasons for Yellowing

		SEASON		
		2009A	2009B	COMB
N-level	GEN			
LN	S_1	1.583a	1.451a	1.517a
	S_2	1.568a	1.598b	1.583a
	S_2R	1.592a	1.506b	1.549a
		1.581a	**1.518b**	**1.549**

LSD: Season=0.06, GEN=0.08, Season*GEN= 0.11

Table 7: Mean performance of S1 and S2 families across seasons and N-levels for *Maize Streak Virus disease*

		SEASON		
		2009A	2009B	Comb
N-level	GEN			
LN	S_1	0.727a	0.941a	**0.834a**
	S_2	0.784a	1.295a	**1.039a**
	S_2R	0.826a	0.98a	**0.903a**
Opt N	S_1	2.181a	1.941a	**2.061a**
	S_2	2.063a	2.514b	**2.289a**
	S_2R	1.986a	2.702b	**2.344a**
Comb	S_1	1.454a	1.441a	**1.448a**
	S_2	1.424a	1.905a	**1.665a**
	S_2R	1.406a	1.841a	**1.624a**
	Mean	**0.4281**	**1.729**	**1.579**
LN		0.779a	1.072b	**0.926a**
Opt N		2.077a	2.386b	**2.233b**
Comb		**1.428a**	**1.729b**	**1.579**

LSD: Season=0.17, N-level=0.17, Season*N-level=0.24, GEN=0.21, Season*GEN= 0.0.29, N-level *GEN=0.29, Season* N-level*.GEN=0.41

Table 8: Mean performance of S1 and S2 families across seasons and N-levels for TLB

		SEASON		
		2009A	2009B	Comb
N-level	GEN			
LN	S_1	2.58a	1.73a	**2.16a**
	S_2	2.59a	1.87b	**2.23a**
	S_2R	2.38b	1.86b	**2.12a**
Opt N	S_1	2.31a	1.46a	**1.89a**
	S_2	2.44b	1.56a	**2b**
	S_2R	2.52b	1.56a	**2.04b**
Comb	S_1	2.45a	1.59a	**2.03a**
	S_2	2.52a	1.72b	**2.12a**
	S_2R	2.45a	1.71b	**2.08a**
	Mean	**2.47**	**1.67**	**2.08**
LN		2.52a	1.82b	**2.17a**
Opt N		2.42a	1.52b	**1.97b**
Comb		**2.47a**	**1.67b**	**2.07**

LSD: Season=0.05, N-level=0.05, Season*N-level=0.07, GEN=0.06, Season*GEN= 0.0.09, N-level *GEN=0.09, Season* N-level*.GEN=0.12