Genetic studies of secondary traits and yield heterosis in maize under high and low nitrogen conditions, incorporating farmer perceptions and preferences for varieties

By

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Thesis abstract

In Tanzania, 85% of the population depend on maize for food security; it provides 60% of their dietary calories and about 50% of their protein. It is not only a source of food security, but is also a cash crop on which they depend as a source of income. Small-scale farmers contribute over 80% of Tanzania's total maize production. Of this, 50% is produced in the Southern Highlands agro-ecological zone, where the crop is grown under low-fertiliser input conditions — especially nitrogen (N), which compromises grain yield. Other constraints include the use of unimproved varieties and lack of access to other technologies. Any threat to maize production in this zone may impact negatively on food security and therefore the livelihoods of all Tanzanians.

The major objectives of the present study were to document farmers' perceptions of their preferred elite varieties and their production constraints and to determine the genetic effects and heterosis of maize hybrids among local (Tanzanian) and exotic inbred lines under low and high N conditions. Relationships among secondary traits in these hybrids were also studied under both N conditions. This knowledge is very important for developing strategies for breeding maize cultivars that are high-yielding, early-maturing and adapted to the intermediate altitudes of Tanzania.

Prior to undertaking the genetic studies, formal questionnaires and informal surveys, such as focused group discussions were conducted in eight maize farming communities in the Mbeya region, involving 214 randomly selected farmers.

In order to determine the genetic effects, six generations that included parents P_1 and P_2, F_1 and $F_{2,}$ and backcross progenies BCP_1 and BCP_2, were generated from two of the crosses with contrasting inbred parents for the traits under the study. These crosses included divergent parents for leaf chlorophyll concentration and one cross of each selected for plant and ear heights, number of leaves above the ear and number of kernels per ear. The six generations of each cross were evaluated in a randomized complete block design — with two replications under high and low N conditions (120 and 60 kg N ha^{-1}, respectively) — in separate trials that were conducted side by side at one location for two seasons. To determine the genetic effects governing the traits, generation mean analyses were performed with SAS computer software on the data collected under both N conditions.

Forty-eight F_1 hybrids were developed from local and exotic inbred lines from CIMMYT (Southern Africa) and IITA (West Africa) to form six hybrid sets. The experimental hybrids, plus six check varieties, were evaluated in a 9 x 6 alpha lattice design, with two replications under both N conditions. Heterosis for yield was measured under both conditions. Relative effects of low N on yield and other traits were calculated. Multiple trait linear regression, correlations, and path coefficient analyses were performed to determine the associations among secondary traits and grain yield.

The survey revealed a marked variation among the farmers in background training and experience, access to new technology and preferences for maize cultivars. They required maize cultivars that:

- matured in two months (extra early);
- had fast kernel dry-down rates;
- stayed green longer upon maturity;
- were drought tolerant;
- had large cobs; and
- had high single plant yield that was used to select seed for the next planting.

In addition to outside influences helping to develop elite varieties that incorporate these traits, any improvement made to the farmers' socio-economic situation through poverty reduction might also impact positively on yield.

The study established that different genes govern leaf chlorophyll concentration character after mid-silking through to physiological maturity. The ratio of fixable (additive and additive x additive) to non-fixable (dominance, additive x dominance and dominance x dominance) genetic effects was 74% to 26% under HN and 35% to 65% under LN in the cross T20 x C58. In sharp contrast, the non-additive genetic effects were predominant in the cross T20 x N_G8, whose ratio of fixable to non-fixable effects was 37% to 63% under HN and 20% to 80% under LN. It is suggested that the fixable genetic effects with N regimes is genotype specific.

Additive genetic effects prevailed during early grain filling stages, whereas non-additive gene effects were preponderant during the later grain filling stages for leaf chlorophyll concentration.

It was found that the nitrogen conditions in which the trait and crosses were evaluated influenced a number of factors, including genetic effects on the plant height, ear height, number of leaves above the ear and the number of kernels per ear. Non-fixable genetic effects predominated for plant height in the cross T20 x N_G8, with negligible epistasis under both N conditions. For ear height in the same cross, the dominance effects had 90% of the non-fixable effects under HN, but both fixable and non-fixable effects were almost equal under LN. Fixable genetic effects controlled the number of leaves above the ear in the cross N_G2 x C3 under both N conditions, although the frequency distribution curves were discontinuous and discrete, which suggested the predominance of a few major genes operating in an additive manner. The number of kernels per ear was controlled by non-fixable effects under HN, while the fixable gene effects conditioned this trait under LN. About 20% additive x additive effects and 30% dominance x dominance effects prevailed for the same trait under high and low N, respectively. Recurrent selection is suggested to improve the cross where it was found that fixable genetic effects prevailed, whereas reciprocal recurrent selection may be effective where both fixable and non-fixable genetic effects were about equal. For the hybrid-oriented programmes, it was found that selection, accompanied by inbreeding, could be used, firstly to exploit the additive genetic effects, and secondly, to do the same to non-additive effects such as dominance and positive epistasis, through the creation of hybrids between the inbred progenies.

Results from the heterosis study indicated that grain yield could be improved by 30%, if recommended fertiliser rates were used. Maize farmers lose about 20% to 30% of grain yield by applying less N. Generally, hybrids that involved one or both local inbred parents exhibited a higher tolerance towards low N for yield than exotic x exotic inbred combinations. The study revealed three types of hybrids with general adaptation and specific adaptation to different N conditions. Four hybrids displayed high yield across N conditions and two exhibited high yield under low N. The other two hybrids were outstanding under high N. Application of low N fertiliser rate did not affect final calendar physiological maturity and kernel moisture content at the harvest of hybrids. However, low N reduced flowering dates by four days, an average of 18% to 25% for leaf chlorophyll concentration, quickened kernel dry-down by a proportion of about one to nine, and increased duration of grain filling by two to four days. Hybrid combinations involving West Africa inbred lines possessed genes for early maturity. Early- and high-yielding hybrids to be grown under low N conditions may therefore be developed if inbred lines with high grain yield potential are crossed with inbreds from West Africa. Generally, hybrid sets displayed higher standard heterosis under LN, compared with HN conditions, suggesting that the use of hybrids in low N input environments may improve maize grain yields and therefore have a positive impact on household food and cash security.

The results demonstrated that yield can be improved under LN by increasing the number of ears per plant, whereas under HN, the yield could be raised by increasing the number of kernels per ear. Three factors — plant and ear heights, plant stand at harvest and the number of kernels per ear — showed a consistent, significant positive correlation with yield across N regimes and seasons. The grain fill duration and mean leaf chlorophyll concentration were strongly positively correlated but in most cases they correlated negatively with other traits. Strongly positively correlated traits suggested indirect selection and a separate selection programme to that of negatively associated traits. Direct effects to grain yield were higher under LN compared with HN for kernels per ear and plant height. The indirect effects to grain yield were higher under HN than LN. Under HN, grain yield was significantly reduced by plant stand at harvest via ears per plant (-0.418), followed by mean chlorophyll concentration through 50% anthesis date (-0.376), whereas yield could be raised by plant height via 50% anthesis date (0.294), then by kernels per ear via plant stand at harvest (0.229). Furthermore, it would be simple to raise dry matter accumulation, as the vegetative phase could be reduced and grain filling duration increased due to the fact that leaf chlorophyll concentration and duration of grain fill correlated negatively with flowering dates.

It was established that breeding, both for high grain yield potential and earlier-maturing maize cultivars for production under low soil N in tropical maize, was possible — and that varieties that performed above average, under both N conditions, could be developed. The

identified significant relative yield loss of about 30% that was observed when maize hybrids were grown under low soil N may justify the need for development of maize hybrids that are adapted to low soil N conditions. For the new hybrids to meet farmers' requirements in the Southern Highland Zone of Tanzania, the ideotype (preferred variety) should have a combination of the following traits:

- functional leaf chlorophyll concentration until around physiological maturity;
- high grain filling duration;
- many viable kernels per ear maintained until physiological maturity;
- many ears per plant (i.e. prolific); and
- non-dwarf plant stature.

Declaration

I, Arnold Mushongi, declare that:

1. The research report in this thesis, except where otherwise indicated, is my original research.
2. The thesis has not been submitted for any degree or examination at any other university.
3. This thesis does not contain other person's data, pictures, graphs or other information, unless specifically acknowledged as being sourced from those persons.
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Signed:

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Arnold Mushongi (PhD Candidate)

As research supervisors we agree to submission of this dissertation for examination:

Signed: ……………………………………………..Date……………………………..

Prof. John Derera (Supervisor)

Signed: ……………………………………………..Date………………………………

Prof. Pangirayi Tongoona (Co-supervisor)

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Dedication

To The Great Family of Mushongi owa Kasheshe, Karagwe, Tanzania.

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List of Acronyms

Abbreviation	Definition
R_1	mid-silking stage
R_2	blister stage
R_3	milk stage
R_4	dough stage
R_5	dent stage
R_6	physiological maturity stage
a	additive genetic effects
aa	additive by additive interaction genetic effects
ad	additive by dominance interaction genetic effects
ASI	calendar anthesis-to-silking interval
BCP_1	parent one crossed back to F_1
BCP_2	parent two crossed back to F_1
C_3 plants	photosynthesis in which atmospheric carbon is first fixed in 3-carbon chain; indicated anatomically by irregular chlorenchyma with four or more cells between adjacent cells Characteristic of temperate and shade-loving grasses
C_4 plants	photosynthesis in which atmospheric carbon is first fixed in 4-carbon chain; indicated anatomically by only two to four chlorechymatous cells between adjacent bundle-sheaths (Kranz anatomy). Characteristic of tropical grasses
CAN	calcium ammonium nitrate
Check	Commercial hybrid check
CIMMYT	Maize and Wheat International Research Centre
CoB	client oriented breeding
d	dominance genetic effects
DAP	di-ammonium phosphate
dd	dominance by dominance interaction genetic effects
DM	dry matter
DMA	dry matter accumulation
DS	dry season
EFPD	effective grain filling period duration
EPP	number of ears per plant
F_1	first filial generation
F_2	second filial generation
FAO	United Nations Food and Agriculture Organisation
FFI	floret fertility index
Fixable genetic effects	additive and additive by additive genetic effects
GCA	general combining ability
GExI	genotype by environment interaction

Abbreviation	Definition
GMA	generation mean analysis
GY	grain yield (t ha^{-1})
H^2	broad sense heritability
h^2	narrow sense heritability
HI	harvest index
HN	high nitrogen fertiliser application rate (120 kg N per hectare)
HNDS	high nitrogen fertiliser application rate (120 kg N per hectare) for dry season
HNWS	high nitrogen fertiliser application rate (120 kg N per hectare) for wet season
Inferior parent	parent without or with low level of genes of interest
KDD	index of kernel dry-down
KPE	number of kernels per ear
KPP	number of kernels per plant
LCC	leaf chlorophyll concentration in SPAD units
LN	low nitrogen fertiliser application rate (60 kg N per hectare)
LNDS	low nitrogen fertiliser application rate (60 kg N per hectare) for dry season
LNWS	low nitrogen fertiliser application rate (60 kg N per hectare) for wet season
$LSD_{(0.05)}$	least significant differences at 5% level of probability
MPH	mid-parent heterosis
Mean LCC	leaf chlorophyll concentration for average of grain filling stages (trait 5 of chapter 3)
N	nitrogen
$NO3^-$	nitrate ion
Non-fixable genetic effects	dominance, additive by dominance, and dominance by dominance genetic effects
NUE	nitrogen use efficiency
P_1	parent one
P_2	parent two
P_{ij}	path coefficients for measuring direct influence between variables to grain yield
PRA	participatory rural appraisal
PROC CORR	SAS procedure for computing Pearson's correlation coefficients
PROC GLM	SAS procedure dealing with generalised linear models
PROC REG	SAS procedure for computing regression
QTL	quantitative trait loci
r	simple Pearson's correlation coefficient
R stage	Reproductive stage
R_a^2	adjusted coefficient of determination
r_g	Pearson's genotypic correlation coefficient
r_{ij}	simple Pearson's correlation coefficients for measuring the mutual association of two variables

Abbreviation	**Definition**
$r_{ij}P_{ij}$	indirect effects of variables upon another through the other variable
r_p	Pearson's phenotypic correlation coefficient
RRS	reciprocal recurrent selection
RS	recurrent selection
R^2	coefficient of determination
SAS	Statistical Analysis System
SCA	specific combining ability
SG	leaf chlorophyll concentration at physiological maturity (i.e. stay-green trait)
LCC_1	SPAD values at 50% silking stage (wave lengths) = trait one of chapter 3
LCC_2	SPAD values at milk stage (wave lengths) = trait two of chapter 3
LCC_3	SPAD values at dent stage (wave lengths) = trait three of chapter 3
LCC_4	SPAD values at physiological maturity stage (wave lengths) = trait four of chapter 3
SHZ	Southern Highlands Agro-ecological Zone of Tanzania
SNP	single nucleotide polymorphism
SPAD-502	Chlorophyll Meter (Model 502 Konica Minolta, Japan)
SPS	Sucrose phosphate synthase
SPSS	Statistical Package for Social Sciences
SSA	sub Sahara Africa
Superior parent	parent with relatively high level of genes of interest
V_A	additive genetic variance
WS	wet season
X_{int}	plant growth rate at which kernels begin forming a second ear
Y_{int}	number of kernels on the primary ear if kernels begin forming a second ear

Introduction to thesis

Importance of maize in Tanzania

In Tanzania, not only does maize provide 60% of dietary calories and 50% of protein, but it is also a cash crop for 85% of the population (RATES, 2003). Maize is synonymous with food security in the country (RATES, 2003) and Tanzania ranks 23rd in maize production in the world (FAOSTAT, 2007), with small-scale farmers contributing over 80% of the total production (RATES, 2003). The Southern Highlands Agro-ecological Zone (SHZ), covering about 250,000 km^2 (i.e. 28% of the country), produces about 50% of all maize in Tanzania (Lyimo, 2006). This constitutes 90% of the National Strategic Grain Reserve, indicating that the SHZ contributes significantly to the grain basket of the country. Any threat to maize production in the SHZ will, therefore, compromise not only national food security and the livelihood of countless Tanzanians but also result in a secondary effect in neighbouring countries that import maize grain from this zone.

Constraints to maize production in the Southern Highlands of Tanzania

Resource-poor farmers in the intermediate altitudes of the SHZ produce maize under multiple stresses such as diseases, drought, low soil fertility (nitrogen, phosphorus, potassium, and micronutrients such as iron and zinc), and insect pests (Lyimo, 2006). Despite impoverished soils in the SHZ, farmers in Tanzania apply less inorganic fertiliser to farms compared to many countries in the world (Table 1.1). The low production level in the SHZ is in part associated with the low application of chemical fertilisers. The intermediate altitudes experience a wide gap between farmers' yields (<1.5 t ha^{-1}) and potential yields for most of the hybrids that are available of 8 t ha^{-1} (Lyimo, 2006). Such farmers' yield figures are slightly above the range of 0.9-1.2 t ha^{-1} in sub-Saharan Africa (SSA), which is very low yield level in relation to the average world maize production by resource-poor farmers in the intermediate altitudes (Elings et al., 1997). Therefore, breeding efforts should concentrate on stability of grain yields by identifying and using traits that increase tolerance and resistance to many stresses at farmers' production conditions.

Table 1.1: Consumption of fertilisers and estimates of maize grain production in Tanzania compared with selected regions and countries

Region/Country	Grain production† (million MT)	Amount of inorganic fertilisers applied (kg ha^{-1})
Industrialised countries*	352.15	206
South East Asia	30.46	135
South Asia	NA	100
Latin America	84.61	73
South Africa	7.13	50
Malawi	3.23	27
Tanzania	3.66	9

Source: http://www.tzonline.org, adopted from Abuja Africa Fertiliser Summit, June 2006)

* = Western Europe and United States of America, MT = metric tons, NA = data not available; †FAOSTAT (2007)

Problem of low soil nitrogen in sub Sahara Africa

The Abuja Fertiliser Summit (2006) reported that the low productivity of the agricultural sector in Africa could be precipitated by low use of fertiliser, since only 8 kg ha^{-1} is applied, which is 10% of global fertiliser consumption. Nitrogen (N) is a key nutrient in maize productivity, but tropical maize has been produced under low N either by farmers applying little N or to soils inherently impoverished of N. At the regional level in SSA, Worku et al. (2007) reported that farmers apply as little as 20 kg N ha^{-1} to maize fields. In response to this problem, farmers opted for the establishment of new farms for maize. However, opening up new farms is not feasible in areas with high population density and it would increase soil degradation in areas with low population density. Since farmers use less N (i.e. than recommended) at varying rates on unimproved varieties and on soils where N mining is high, maize cultivars that use N efficiently (measured as yield per plant per unit amount of applied N) are relevant with regards to tropical maize.

Application of low fertiliser dosage

Farmers' use of low N may be determined by factors such as affordability, availability, soil conditions, and the likelihood of decent rainfall to minimise the risk of N investments. While the first two factors could be corrected by socioeconomic interventions, the other factors may require the breeding of cultivars adapted to such production environments. The application of fertilisers in maize by farmers in the SHZ is highly variable and may range from nothing at all to anything below the recommended rates of 120 kg N ha^{-1} and 20-30 kg P_2O_5 ha^{-1}. Continuous application of suboptimal rates of N in maize in this zone would further reduce maize yields, which are already low and erratic thus precipitating a high variability in yields for farmers.

Problems of low nitrogen are exacerbated by other factors

The bulk of maize production in Tanzania is typically rain-fed under a unimodal pattern of rainfall. Dry spells that are erratic in such a rainfall regime would precipitate unstable and low-average yields. It is known that the effects of drought and low soil N are compounded in the farmers' production environment. Moisture stress renders soil nutrients unavailable to maize, even if the soils are fertile. The FAO (1968) reported that maize is prone to drought but, under favourable water conditions, improved maize varieties use water efficiently and respond to N fertiliser far better than other crops in most soils. High plant density and weed infestation are also associated with the effects of low N. The identification and genetic analysis of and association between adaptive traits under low soil N would improve yields in tropical maize that is produced under such conditions.

Association of secondary traits with and adaptability of maize cultivars to low nitrogen

Leaf chlorophyll concentration and kernel dry-down

One of the pertinent breeding strategies in areas where soils are impoverished of N and the growing seasons are decreasing is to study the profiles of the post mid-silking leaf chlorophyll concentration (LCC) to maintain the photosynthetic capacity of maize cultivars until physiological maturity i.e. stay-green (SG). The profiles of LCC in the post-mid-silking stages may relate to the rate of kernel dry-down (KDD) and these traits may be associated with, or determine the time to physiological maturity, of maize cultivars since the SG cultivars are characterised with high leaf water and N at physiological maturity (Hawkins et al., 2007). The SG genotypes do likely have high root density, as they may promote photosynthetic capacity, thus regulating the ratio of source to sink. In addition, such cultivars correlate with nitrogen-use efficiency (Bertin and Gallais, 2000) and limit stresses from defoliation (i.e. loss of chlorophyll) at the grain filling stages thus have the dual purpose of providing grain and high-quality feed to ruminant livestock. Studying how LCC diminishes at the grain filling stages may determine the rate and duration of dry matter accumulation in maize (Hageman and Lambert, 1996). However, overwhelming evidence of how the LCC character leads to the SG trait and how these traits relate with final grain yield in tropical maize under N regimes is not clearly established in literature.

Varieties with rapid KDD may reduce costs to farmers (and the seed industry) who dry their maize, and limit the proliferation of post-harvest pests. Such varieties also germinate early and so escape early season stress (Dhillon et al., 2002). Despite the importance of the LCC and KDD traits, they are less studied and have been treated differently in the literature — particularly low soil N under which tropical maize is produced.

Leaf chlorophyll concentration vs. grain filling stages

Precise factors determining final maize grain yield are not clear, although targeting the appropriate crop growth stage where sink strength is maximised may improve the grain yield. Plant N relates with LCC and it has been reported to decrease with growth stage and this is relevant from post mid-silking until the attainment of physiological maturity (Hageman and Lambert, 1996). The rate and duration of decline of the leaf N embedded in the chlorophyll pigment may have consequences to final grain yield. The slow rate of loss of LCC may imply that the genotypes could accumulate, over a longer time, high dry matter into the kernels, and so maintain the kernels to physiological maturity. On the other hand, genotypes that lose LCC earlier may be low yielders. However, the challenge has been to increase and maintain sink (kernels per ear) until physiological maturity, particularly under low soil N. Furthermore, the genetic control of post mid-silking LCC and the influence of N treatments on the inheritance of LCC profiles during the grain filing period in tropical maize are not well documented either.

Kernel productivity vs. maturity character

The improvement of traits related to increased kernel productivity per plant and per unit area would lead to the development of maize cultivars that remain stable under a range of production conditions (Mock and Pearce, 1975). Early maturity is an important requirement in the cultivars that farmers choose. The challenge would be to increase grain yield in early cultivars but enhancing yield in them under low N conditions could prove difficult. Most secondary traits related to maturity are negatively associated with grain yield (GY) components, therefore the value of secondary traits would be to ensure high kernel set, and maintenance of the set kernels to physiological maturity, particularly under low N.

Under low N most of the size and contribution of secondary morpho-physiological traits to final yield in maize are reduced (Bertin and Gallais, 2000) such that there could be genotype x N interaction effects that might complicate selection. This interaction may be associated with the low GY at farmers' level in tropical maize. Costa et al. (2002), Shaver (1983) and Chase and Nanda (1967) have reported that the number of leaves improve maturity and GY, whereas plant height and ear height may also relate directly and indirectly with final GY. However, the genetics and correlations of secondary traits such as plant and ear heights and the number of leaves above the ear in relation to kernel productivity under low N conditions in tropical maize have been barely mentioned in the literature. Since maize in SSA is typically produced under low N conditions, it would be worthwhile to identify and study secondary traits under these conditions before maize ideotypes are developed for SSA.

Relationships between traits

If multiple trait models and interrelationships among traits could be established and applied over time, at least in a modified form, they would improve kernel productivity (Lizaso et al. 2003), especially under low N. Making available cultivars adapted to low soil N to small-scale farmers would be a pertinent and affordable way of improving the livelihood of small-scale farmers who are encountering unaffordably high N fertiliser prices and rainfall regimes that are decreasing in an unpredictable manner. However, Edmeades et al. (1997) reported lack of quantitative estimates of the incidence and intensity of important stresses such as drought and low N, and their associated grain yield loss. This suggests the need for studies not only to establish relative yield losses under low and/or application of low N in tropical maize but also to help improve yields in SSA.

Exploitation of exotic germplasm lines in breeding varieties with local adaptation

Although the need for early-maturing tropical maize cultivars that do not compromise yields has been increasing, it is unfortunate that only limited information exists on both the concept and definition of early maturity under stress conditions (Pswarayi and Vivek, 2007) in SSA. The best way to fix the segregating behaviour of the hybrids for early maturity would be to introduce and adapt an exotic germplasm into the local environment by conducting investigations on heterosis for grain yield that refer to a measure of local adaptation (Kang, 1994). The importation and use of foreign germplasm to breed for early maturing and high-yielding genotypes has been reported four decades ago by Giesbrecht (1960). With such a breeding objective, local inbred lines that are high yielding but lack genes for earliness could be crossed to early exotic donor inbreds. However, such a breeding objective in tropical maize under low N has not been systematically documented in SSA, Tanzania in particular.

Heterosis under low soil nitrogen

The stay-green trait, tolerance to high population density stress and heterosis for grain yield have been reported to increase maize yields, especially in developed countries. Nonetheless, studies and practical application of heterosis for yield with regards to such traits have not extensively boosted yields in SSA, particularly where maize is produced under low soil N. Heterosis under low N, if it exists, would therefore improve maize yields for SSA region. And from plant breeder’s viewpoint, standard heterosis is the most practical and relevant aspect (Virmoni, et al., 1982), since heterosis is a direct measure of value for cultivation. However, this has not been the case in developing countries — particularly in SSA, where farmers continue to grow landraces under low N, so partly explaining low productivity levels for the crop. Breeding maize cultivars tolerant to low N would create a positive impact on yield to this region. Whereas heterosis studies have been widely conducted under optimum production conditions such as high N fertiliser levels, heterosis for final yield as a measure of adaptation under low N in tropical maize does not feature widely in the literature. However, in rice, Kush (2004), and in transgenic (Bt) hybrid cotton, Kuruviganti (2009) reported heterosis to increase yield gain of 20-30%. Low N has been reported to have significant effects on the patterns and trends of heterosis for grain yield (Meseka et al., 2006). This may thus suggest that selection under N stress is necessary while generating maize genotypes tolerant to low N.

Breeding maize varieties for tolerance to low soil nitrogen

Little has been reported about varieties that perform well under both high and low and specific soil N conditions in SSA, Tanzania in particular. It would be better to test and breed genotypes at N rates that are closer to the affordable amounts used by farmers. Breeding varieties that are tolerant to low N are required, since soils in Tanzania are impoverished of N and the use of fertilisers is probably among the lowest in the world, as indicated in Table 1.1. Such varieties, if bred, would improve maize productivity.

Farmers' requirements concerning elite varieties in the Southern Highlands

Farmers producing maize in the multiple stressed production environments situated in the intermediate altitudes of Tanzania require maize varieties that are early maturing and locally adapted (Gibson et al., 2005; Lyimo, 2006). Edmeades et al. (1997) asserted the common situation in tropical maize that the early maturing landraces are found in dry lands and less fertile soils. This may justify breeding strategies targeting low N tolerant and earlier maturing cultivars. Participatory maize breeding would therefore be crucial in the development of elite maize cultivars that are not only early maturing and locally adapted, but also have end user preferred traits.

Summary of research focus

To recap, studies are needed that target farmers' perceptions and important constraints or challenges to maize production. Knowledge is also required on the inheritance and adaptability of traits related to productivity of intermediate maturing maize cultivars, particularly under low N, since tropical maize is produced under such conditions. The thrust is put on the intermediate maturing cultivars because the bulk of maize in Tanzania comes from the intermediate zones. Additionally, breeders require knowledge of (i) the levels of heterosis for yield, (ii) appropriate use of exotic germplasm lines to enhance yield, and (iii) how to effectively exploit associations among secondary traits while breeding new varieties for production under low N conditions. Specifically, the challenge at hand in Tanzania remains breeding for high yielding and early maturing maize cultivars under N impoverished soils, which the present study was geared to attempt.

Research objectives

The major objective of the present study was to breed maize cultivars that are high yielding, early maturing, and adapted to the intermediate altitudes of Tanzania. The specific objectives were to:

1) determine farmers' perceptions, preferences and production constraints on maize cultivars,
2) determine the genetic effects controlling leaf chlorophyll concentration from mid-silking to physiological maturity under low- and high-nitrogen conditions,
3) investigate the genetic effects that govern plant and ear heights, the number of leaves above the ear and the number of kernels per ear under low and high nitrogen conditions,
4) determine heterosis of hybrids for grain yield and calendar physiological maturity under low and high nitrogen, and
5) establish the relationships between secondary traits and grain yield under low and high nitrogen regimes.

Research hypotheses

The study was undertaken to test the following hypotheses:

1) Perceptions, preferences and selection criteria of small-scale farmers for maize cultivars do not match those of empirical maize breeders' in the design of cultivars that are early maturing, locally adapted and high yielding.
2) There are high levels of exploitable genetic variability for leaf chlorophyll concentration, from mid-silking to physiological maturity, and their genetic effects are independent of nitrogen conditions.
3) The genetic effects and gene action for plant and ear heights, the number of leaves above the ear, and the number of kernels per ear are independent of nitrogen conditions.
4) Heterosis under low-nitrogen conditions can be enhanced by the exploitation of exotic germplasm lines, in combination with local inbred lines under low soil nitrogen.
5) Low soil nitrogen levels do not affect relationships among secondary traits and the relationship of these traits with final grain yield and calendar physiological maturity.

Organisation of the thesis

The thesis has seven chapters as follows:

1) Review of the literature.
2) Farmers' perceptions of production constraints and preference for maize varieties and other supporting technologies in the intermediate altitude zone in Tanzania.
3) Generation mean analysis of leaf chlorophyll concentration from mid-silking to physiological maturity in three tropical maize inbred lines under low and high nitrogen conditions.
4) Generation mean analysis of four secondary traits in crosses involving five tropical maize inbred lines under low and high nitrogen conditions.
5) Heterosis of hybrids among local and exotic inbred maize lines under low and high N conditions in Tanzania.
6) Relationships between secondary adaptive traits and grain yield in tropical maize hybrids among exotic and local inbred lines under low and high nitrogen conditions in Tanzania.
7) Overview of research findings.

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Chapter 1:
Literature review

1.1 Introduction

This review of the literature discusses the following: i) the role of nitrogen in maize breeding, ii) generation mean analysis, iii) heterosis and its role in maize under low-nitrogen conditions, iv) response of genotypes to low-nitrogen stress, v) secondary traits and stability of grain yield and maturity, vi) integrating molecular markers and conventional approaches, vii) relationships between grain yield and secondary traits, and viii) the overall summary.

1.2 Role of soil nitrogen in maize breeding

1.2.1 Improving maize varieties for low-nitrogen tolerance

Nitrogen (N) is the most limiting nutrient for maize growth (FAO, 1968; Lafitte, 1994). Bertin and Gallais (2000) reported high N in kernels for non-senescent cultivars of maize. Such cultivars should be judged according to their final value as efficient users of unit amount of applied N, and the status of plant and soil N consequently depend on the management of N fertilisers (Thomas and Smart, 1993). However, low soil N and drought are associated (Bänziger et al., 1999; 2000), because water is a solvent through which the plants take up and assimilate N. Varieties that tolerate drought absorb fertiliser nutrients efficiently as they have well-developed root systems (Olson et al., 1964; Foth and Turk, 1972; Hatlitligil et al., 1984; Borrell et al., 2001). It could thus be hypothesised that such cultivars take up N throughout the growing season and lose it slowly. Cultivars that are efficient in the uptake of N reportedly experience enhanced uptake of other nutrients, even those that are less mobile (Hatlitligil et al., 1984). The cross B73 x Mo17 has been cited to have high N uptake efficiency (Hatlitligil et al., 1984; Rajcan and Tollenaar, 1999), which may also indicate that it would be possible to breed varieties that use N efficiently. It may be more feasible and economical to breed for efficient use of N, compared with efficient uptake of N since, by definition, efficient use considers final yield whereas efficient uptake is about N content in the plant. Therefore, final yield may be seen as both a measure of performance for cultivars that use N efficiently and low N-stress index.

Varieties performing well under both high and low N conditions have been reported to use N efficiently (Lafitte and Edmeades, 1995; Kikafunda et al., 2001; Basra and Goyal, 2002).

However, Bruetsch and Estes (1976) could not find a consistent relationship between physiological maturity and genotypes' nitrogen use efficiency (NUE). Bolton (1971) conducted a study in Tanzania and found significant association between optimum plant density and N x P (phosphorus) with yield components such as cob size and the number of ears per plant (EPP), however, there was no appreciable significance between the treatments, with 50% anthesis thus partly agreeing with Bruetsch and Estes (1976). The author added that the efficiency of N x P treatments were high >900 metres above sea level (m.a.s.l.) with intermediate maturing cultivars performing better than early genotypes. The response was much smaller or did not exist in <900 m.a.s.l. On the other hand, Willman et al. (1987) differed by reporting that soil fertility regimes directly affect maturity of genotypes and a trend is known at least for N, compared to other nutrients. Efficiency for N use in maize has been considered to be high under low N conditions (Bruetsch and Estes, 1976; Bertin and Gallais, 2000) and also in genetically weak plants (Bertin and Gallais, 2000).

However, a penalty to maize yields, due to reduced plant morphological size and small architecture in maize grown under low N conditions have not been reported extensively in literature (Hageman and Lambert 1996). A few studies indicate that small morphological structures such as dwarf stature and small cobs point to low yield. Sinclair and Horie (1989) further asserted that C_4 plants like maize have higher dry matter accumulation in reduced N conditions than C_3 plants and vice versa. The upper canopy has also been reported to have high leaf N and radiation use efficiency and leaf N correlated well to canopy under no stress (Hammer and Wright, 1994). In such cases the actual yield would approximate potential yield (Tollenaar and Lee, 2002). However, the number of kernels per ear has been considered as a yield component that differentiates well between genotypes under low N (Lafitte and Edmeades, 1995; Bertin and Gallais, 2000; Varga et al., 2004). This may justify the need of P, which, with other numerous including other nutrients, is responsible for root development. Strong root mass would support the additional harvest index from NUE cultivars. However, early cultivars have been found to accumulate high P in kernels, unlike later maturing cultivars, but the efficiency for yield is better in later maturing genotypes (Bruetsch and Estes, 1976). Although N regulates 12 other nutrients important to the development of maize, the balance between nutrients is crucial to the optimum production of maize. Researchers will be interested to know how much farmers lose by applying sub-optimal rates of N; one aspect is the gene action governing the inheritance of agronomically important traits under low and high N, among others.

1.2.2 Genetic control of maize traits under low nitrogen conditions

The genotype x N interaction has been reported under low N conditions (Bertin and Gallais, 2000; Worku et al., 2007). This interaction would suggest variable performance of maize genotypes under farmers' production environments since these farmers apply differential marginal rates of N. This could be contributing to low average grain productivity of maize (per plant and per unit area) observable in SSA. The wide variations for genotypes across low regimes of N may suggest that selection of genotypes for differential responses to regimes of N would be possible (Worku et al., 2007). Such variation under low N could suggest the prevalence of additive genetic effects for secondary traits (Betran et al., 2003a, b). Despite the association between low soil N and drought suggested by Bänziger et al. (2000), which would permit use of a correlated response to breeding approach, Falconer (1989) reported on the difficulty of indirect selection in maize. Bänziger et al. (2000) and Betran et al. (2003) reported the preponderance of additive variance (V_A) for secondary traits (i.e. EPP, ASI, and leaf chlorophyll concentration) in maize under stress. However, Robinson et al. (1949) had asserted much earlier that both V_A and the response to selection diminish with cycles of selection. To the contrary, Meseka et al. (2006) and Medici et al. (2004) reported the preponderance of non-additive genetic effects for most of the traits under low N in maize. Furthermore, other researchers have reported on the preponderance of epistasis under suboptimal regimes of production environments (Gorsline, 1961; Wolf and Hallauer, 1977; Ceballos et al., 1998). In crops such as maize, where heterosis is crucial, Kearsey and Pooni (1996) reported that even a trace of non-additive gene effects would cause immense and unexpected heterosis. Therefore, this suggests the need for detailed detection, estimation and interpretation of the genetic parameters to improve maize, particularly under sub-optimal environments, such as experienced with low N. Nevertheless, most studies in tropical maize have not only disregarded the existence and importance of epistasis under optimum production and evaluation environments but they have done so too under sub-optimum conditions.

However, a few studies had been conducted either on the inheritance of secondary traits and their relationships with yield or interrelationships among secondary adaptive traits, specifically under low N in tropical maize in SSA. This may therefore indicate that either previous efforts to improve maize in SSA had not been that successful or little had been documented. It would be worthwhile to breed for tolerance to low N and report research findings to improve maize productivity under low N conditions.

1.2.3 Correlation between traits under N conditions

Nitrogen is a mobile element, so it diminishes with growth stage and its effects would be more visible at the grain filling stage (Friedrich and Shrader, 1979; Hageman and Lambert, 1996). A negative relationship was reported between yield of dry matter and N at silking stage (r= -0.92), although this association fades with age of grain filling (Bertin and Gallais, 2000). Since N conditions photosynthesis, the genetic variability for photosynthetic activity may be higher at the grain fill stage compared with the vegetative stage (Ahmadzadeh et al., 2004). Hageman and Lambert (1996) who studied the field-grown single crosses that were bred for the period 1930-1970 reported significant differences in genotypic responses of such hybrids to environmental differences with time. Under optimum N conditions, Ahmadzadeh et al. (2004) and Beauchamp et al. (1976) asserted that remobilisation of N to developing kernels is slow during the first two weeks after silking and increases thereafter. The situation would be reversed under LN conditions. Although the maize plant requires more N starting at the mid-vegetative stage, with maximum requirements at silking stage, the maximum yield will depend on initial soil N status, including genotype differences for acquiring and using N (Binder et al., 2000). The lesser the initial soil N, the earlier the N has to be applied (Azeez et al., 2006). The authors studied four maize genotypes at 0, 30, 60, and 90 kg N ha^{-1} and added that N uptake increased with these rates and with 90 kg N ha^{-1} about 45.3 and 8.8 g N kg^{-1} were found in shoots and grain, respectively. Later N application, especially at R_3 stage may not recover yield because it promotes photosynthates to source strength rather than strengthening the sink, thus there is a net yield loss (Binder et al., 2000). The challenge has been to ensure kernel set and its maintenance to physiological maturity in efforts to improve yield under low N. However, the literature — whether it pertains to genetic control and/or physiology of photosynthetic potential or machinery against specific grain filling stages onto which to concentrate breeding efforts under low N regime — is silent on this subject. This knowledge, however, would help to reduce breeding cycles by not waiting until the final harvest of the crop.

Tsai et al. (1984) described two types of maize hybrids in relation to NUE and concentration of plant N at R stages, based on 201 and 447 kg N ha^{-1} application rates. A high N fertility hybrid contained about 65% of its final N at the mid-silking stage, regardless of N levels. The remaining portion had to be absorbed subsequently from the soil after the mid-silking stage. A low N fertility hybrid contained high percentages of its final N by the mid-silking stage at different N fertiliser levels i.e. 93 and 81% at 201 and 447 kg N ha^{-1} respectively. This would imply that the benefits of the leaf chlorophyll concentration character are relevant to later-maturing genotypes compared with earlier cultivars (Capristo et al., 2007). The low yield of a short-season genotype would therefore be due to limited sink strength during the grain filling stage, compared to its long-season counterpart. A yield penalty would be serious at low N, compared with high N conditions.

Dry matter (DM) in maize plant is comprised of approximately 1.5% N and 43.6% carbon (C) (Hageman and Below 1990). The high concentration of C demonstrates the predominant role of photosynthesis in achieving maximum yield (Swank et al., 1982; Hageman and Below 1990). Besides its low proportion, N has a vital regulatory role in DM production, in that N and C are closely connected and interrelated (Stulen, 1990). This could be demonstrated by the fact that maize needs for N are lower early in the season, which increases as dry matter production increases later in the season. Carbon provides the skeleton onto which DM is mounted and the role of N would be to regulate DMA since N is the major component of chlorophyll pigment eventually promoting grain yield. In this case, the efficiency of C_4 plants, e.g. maize, as DM producers at low water, high temperatures, and high light intensity would be justified (Godwin and Mercer, 1988). However, the relationship between high yield and mineral and protein contents in the grain under N conditions is not clear (Feil et al., 2005).

The capacity of the leaves to produce photosynthates through the first half of the grain filling period has been reported to exceed the needs of ear and/or the capacity of the transport system (Swank et al., 1982). Thus such period may be where N is needed the most in maize. However, much earlier Hanway (1962) reported that low N reduces dry matter accumulation

(DMA) but it does not alter partitioning of DM. Furthermore, under low N, the photosynthetic capacity through grain filling period decreases compared with high N conditions, resulting in low yield under low soil N (Swank et al., 1982). This would not only affect the rate and proportion of DMA in kernels but could also influence the kernel moisture content at harvest and the rate of kernel dry-down (KDD). Tsai et al. (1984) asserted that the inefficiency of N uptake after the mid-silking stage and shorter duration in grain filling may, however, have a secondary effect in reducing yield potential of low soil N fertility genotype (Pioneer 3732), as compared with high soil N fertility hybrid (B73 x Mo17). Contrarily, Azeez et al. (2006) found that low-N pool C2 genotype had the highest grain N concentration and a shoot uptake significantly higher than TZB-SR, which is a high N genotype. However, factors that condition grain filling processes are not clear: is it the availability of DM, their translocation or the sink capacity such as EPP or KPE? (Duncan, 1975; Loiva, 1993). Whereas the sink capacity may be observed and quantified under N regimes, the first two factors are not easily measured under field conditions. The relationships among leaf photosynthetic capacity and KDD under low and high N conditions in tropical maize have not been established either.

Prevention of pollination resulted into accumulation of soluble solids in stalk (Hume and Campbell 1972). Ear removal caused same effect (Christiansen et al., 1981). Moreover, the barren plants senesced earlier than one with ears (Christiansen et al., 1981). However, the correlation between leaf chlorophyll concentration status across grain filling stages and EPP under low N in tropical maize is not known.

To recap, the characteristics associated with high yield in maize can therefore be considered as component characters of N uptake, assimilation, translocation, and sink strength (Swank et al., 1982; Tsai et al., 1984; Hageman and Lambert, 1996). Pending the fact that SG genotypes have the greatest DM per plant, carbon exchange rate and grain filling, especially during the last two-to-three weeks of grain fills over senescent genotypes (Hageman and Lambert, 1996), nothing is documented for such facts in tropical maize. However, Bänziger and Lafitte (1997) concluded that while the LCC character may shed information on environmental variation, EPP would discriminate the high yield genotypes under low N. Another concern could pertain to the source of extra N when soil-N is limiting. The paucity of knowledge concerning the interaction of differential levels of leaf N and other metabolic activities at various stages of plant growth and development is another challenge to the maize breeder. Hageman and Lambert (1996) questioned the effect, role and interaction of N metabolism on the size of the plant in lengthening photosynthetic activity of leaves. It has also been difficult to associate physiological traits such as LCC and DMA with final yield. Experiments to address these challenges may be costly and so require precise yet economical equipment to quantify the LCC character over grain filling stages under low and high soil N. In addition to this, combining knowledge on other traits related to yield and calendar physiological maturity may help to address these challenges. Since yield is the ultimate product of many processes, yield would be the best estimate for superior physiological processes.

1.3 Generation mean analysis and transgressive segregation

1.3.1 Importance of generation mean analysis

In a generation means analysis (GMA), basic generations are derived from a cross of two parents with contrasting traits to detect and estimate genetic effects of the quantitative traits. This genetic method provides more detailed information than diallel mating. Other variance-based mating designs (Coates and White, 1998) such as epistasis, degree of dominance, and inferences could at least be forecasted for higher-order epistatic interactions, according to information embedded in assumptions underlying GMA that will be soon be covered. Populations for GMA provide generations that could be used directly in an applied breeding programme (Campbell et al. 1997; Coates and White, 1998; Smith et al., 2009). Campbell et al. (1997) and Kang (1994) asserted that with GMA the errors are inherently smaller when working with means (first order statistics), rather than with variances (second order statistics) compared to purely variance based mating designs. Smaller experiments in GMA (e.g. the few crosses normally used compared with other mating designs) are required to obtain the same level of precision with other mating designs (Hallauer and Miranda, 1988; Campbell et al., 1997). Generation mean analysis detects the dominance more perfectly than most of the available mating designs (Mather and Jinks, 1982; Azizi et al. 2006). However, Kearsey and Pooni (1996) counter-argued that GMA does not finely provide dominance. Moreover, GMA deals precisely with quantitative studies where there is obscurity and/or ambiguity in inheritance, as it estimates all genetic parameters to their finest details. Epistasis, defined according to Sprague et al. (1962) as any interaction between genes at non-homologous loci. The type of epistasis is determined when dominance and dominance x dominance effects are statistically significant (Mather and Jinks, 1982, 1977; Kearsey and Pooni, 1996; Azizi et al., 2006; Shashkumar et al., 2010). These authors have provided the importance of the signs and magnitudes of genetic effects under the GMA. The type of epistasis, whether complementary and/or duplicative, could be identified in the cross where they occur. However, in terms of the importance of epistasis in maize, Rahman et al. (1994) reported on the descending order from dominance, additive; and dominance x dominance, additive x dominance, to additive x additive. The sign and magnitude count and they may vary per this trend, depending on the trait and environment. Factors other than digenic epistasis, such as high order epistasis, linkage and G x E interaction, could be inferred within the limits of the study at hand.

However, the limitations of GMA are that heritability and response to selection become difficult to compute due to lack of variances (Kang, 1994), because the GMA confounds the genetic effects (Mather and Jinks, 1982). This could be counter-argued because variances within and between the generations of GMA have been used to estimate heritable and non-heritable genetic parameters; and this is consistent with the literature (Shashkumar et al., 2010; Smith et al., 2009; Azizi et al., 2006; Kearsey and Pooni, 1996; Dabholkar, 1992). Mather and Jinks (1982) concluded that neither method nor mating design is satisfactory in genetic and breeding experiments but the methods should be complementary.

The genotypes under GMA would help to infer and utilise the phenomenon of transgressive segregation. Transgressive segregation is ubiquitous in plants and this phenomenon creates superior phenotypes and, as a consequence, hybrids that are adapted to local stresses (Falconer and Mackay, 1996; Rieseberg et al., 1999). Rieseberg et al. (1999) reported that 65% of transgressive segregation in plants affects morphological traits and the rest are on the fecundity, biochemical compounds, physiology, life history and tolerance to local stresses. However, important questions have not been cleared regarding transgression. First, how frequent is transgression in crosses due to genetic distances (narrow vs. wide crosses)? Secondly, is transgressive segregation for a given cross or a given character predictable? Thirdly, since Rieseberg et al. (1999) reported that transgressive segregants are heritable, the magnitudes of this heritability are not clear across environments, particularly those under low N, as maize in SSA is produced under low N conditions. Generally, visual illustration of transgressive segregation for the point of inferring inheritance of the secondary traits in tropical maize under high and low N conditions has not been established. Frequency distributions observed by plotting P_1 and P_2 against their segregating generations (i.e. F_2, BCP_1 and BCP_2) may help to express comprehensive understanding into the phenomenon of transgressive segregation (Braden, 2005; Smith et al., 2009). Skewness of the distribution curve of the segregating generation towards either parent may indicate the parent which provided dominant genes. High population means and variances may warrant selection from either positive or negative segregants depending on the trait of interest as Lamkey et al. (1995), Smith et al. (2009) and Shashkumar et al. (2010) suggested. When transgression is implied in an experiment, Carson and Hooker (1981) working with phytopathology in maize asserted that selection could be done from the intermediate x intermediate in the F_2, whereas Lamkey et al. (1995) reported that F_2 and backcrossed generations had been used in the United States of America (USA) to develop inbred lines. Furthermore, Shashkumar et al. (2010) asserted that diallel mating or biparental mating designs could be used directly on the segregants or, the segregants could first be selfed or randomly mated. This would break linkage blocks to release the genetic variance that is embedded in the segregants and then selection done at later stages. This may suggest the need to investigate and use various statistical tools to infer and design breeding strategies, particularly in SSA, where genetic effects are compromised by many environmental factors such that breeders very often select under ideal and no-ideal conditions.

1.3.2 Assumptions of a generation mean analysis and their critique

According to Wright (1968), Mather and Jinks (1977) and Lande (1981), certain basic assumptions could be made when undertaking GMA experiments. These included:

i) all segregating genes are located in one parent,
i) responsible genes are not linked,
ii) all responsible genes have equal effects with respect to the character under study,
iii) there is no epistasis,
iv) there is no dominance, and
v) there is no genotype x environment interaction.

However, these assumptions have been falsified in the literature, as reported below:

i) the differences between F_1 and mid parents indicates dominance (Mather and Jinks, 1977, 1982; Fehr, 1991; Hill et al., 1998),

ii) skewness in the back-crossed progenies shows dominance (Shashkumar et al. 2010; Smith et al, 2009; Braden, 2005), in other words, this occurs when $BCP_1 \neq BCP_2$ implies presence of epistasis (Melchinger et al., 1988; Hill et al., 1998),

iii) transgressive segregation in F_2 denies the assumption of isodirection (Carson and Hooker1981), Kearsey and Pooni (1996) indicated transgression when F_2, BCP_1 and BCP_2 exceeded or were below the mid-parents or F_1,

iv) very wide crosses lead to a preponderance of dominance effects at the expense of additive effects (Kearsey and Pooni, 1996; Braden, 2005; Checa et al., 2006; Azizi et al., 2006), while much earlier, and to the contrary, Kelly and Bliss (1975) reported that lower differences between parents may result in low broad sense heritability. However, Kearsey and Pooni (1996) added that the means and dominance remain independent of dispersion effects.

v) dispersion and association affects the detection and estimation of genetic effects (Mather and Jinks, 1977, 1982; Kearsey and Pooni, 1996; Braden 2005), and

vi) standard errors reduce the size of estimated genetic effects such that the effects with low errors occur significantly more frequently in the model or experiment regardless of the magnitude of genetic effects (Braden, 2005; Smith et al., 2009). Under non-ideal experimental conditions, standard errors increase, whereby epistatic interactions increase relative to additive and dominance effects (Rahman et al., 1994; Ceballos et a., 1998). This increase of experimental errors may deny the assumption of lack genotype x environment interaction.

In the light of the above, according to Smith et al. (2009), the challenge to breeders has been lack of consistency in detecting and estimating genetic effects from GMA studies. For instance, heritability estimates and number of genes have not been measured precisely enough in GMA or other breeding and genetic studies. Ceballos et al. (1998) suggested use of the criterion of minimum standard error, while Melchinger et al. (1988) recommended use of experiments with high confidence interval and high R^2 values as an alternative to heritability estimates. Sources of errors are not necessarily environmental, as Mather and

Jinks (1977) reported that use of inappropriate germplasm for traits of interest may also be a source of error. Furthermore, experimental conditions may have different effects on segregating and non-segregating generations (Gomez and Gomez, 1984).

1.4 Heterosis and its role in maize under low soil nitrogen conditions

Heterosis is a measure of relative superiority of F_1 hybrids over best- and mid-parent(s) (Falconer and Mackay, 1996; Hill et al., 1998), but it is an arbitrary phenomenon (Hallauer and Miranda, 1988) and its root causes are not clear (Jinks and Jones; 1958; Hallauer et al., 1996). Heterosis is specific to the cross, trait, and environment (Hallauer and Miranda, 1988; Hallauer et al., 1996). Kang (1994) reported that the value of any foreign germplasm is to introduce new genes that are assessed by the presence of heterosis, which would a measure of adaptation to important local stresses. Being a relative measure of superiority, the phenomenon could for convenience and practical reasons be extended to cover popular commercial checks (local, best and mean of checks heteroses) and trial mean heterosis. Furthermore, according to Virmoni et al. (1982), this has been designated as standard heterosis.

However, the literature makes scant reference to the direct role of heterosis in improving maize yield in tropical Africa under stress conditions such as low N, since most studies on heterosis were conducted under optimum conditions. Due to the fact that the interaction between genotypes and levels of N are a reality, especially under small-scale production environments in SSA (Worku et al., 2007), this would justify a comparative study on heterosis under both low (LN) and high N (HN) conditions. Furthermore, the need for such comparative study is indicated by the literature above in section 1.2, which in summary demonstrates that the levels of N would affect the components of maturity and grain yield. Since the yield of genotypes under stress such as LN may be measured by the number of kernels plant (KPP), the number of ears per plant (EPP), harvest index (HI), anthesis-to-silking interval (ASI), and the index of kernel dry-down (Echarte et al., 2008), heterosis, a measure of adaptation for yield, would be crucial (Kang, 1994). Addy et al. (2010) reported HI, which is a final economic yield to be a good measure of NUE. The general trend has been that heterosis decreases linearly as the environment improves (Mather and Jinks, 1982) and, specifically with N, Meseka et al. (2004) reported higher heterosis under LN than under HN conditions.

Attempting a comparative study under LN and HN conditions is relevant in the SHZ, where, despite a rapid decline in soil quality (Bisanda et al., 1998), farmers continue to apply variably low rates of N to their maize fields (Lyimo et al., 2006).

Heterosis is one of the greatest scientific advancements to revolutionalise the maize industry. It has improved productivity of maize in the USA (Duvick, 1999). In addition to heterosis, better yields can be obtained by stress management or by breeding varieties tolerant to local stresses, such as low N in SSA, where maize is exclusively produced under LN (Bänziger et al., 1999, 2000). Lee et al. (2005) reported that the causes of final yield were not clear and Hageman and Lambert (1996) established final yield to be the result of complex components, which may include genetic, physiological, developmental and environmental factors. Therefore, heterosis for final yield would be a pertinent measure of local adaptation (Kang, 1994; Ahmadzadeh et al. 2004). However, under LN, not all lines or crosses may have enough seed to evaluate yield, so standard or relative heterosis will become relevant rather than variance-based methods (general and specific combining abilities i.e. GCA and SCA). Nevertheless, the implication of excluding GCA would be that the loss of favourable additive alleles in early generations might not be recovered by heterotic grouping and hybridisation (Hallauer and Miranda, 1988; Ipsilandis and Koutsika-Sotiriou, 2000). The remedy for this demise would be to identify key yield components through trait relationships and study their genetic effects in a target environment. This knowledge would help devise breeding strategies, whether to develop inbred lines or hybridisation. Elsewhere, the common agreement, in order to justify the need for heterosis as a measure of widening local adaptation of genotypes, has been to breed and test genotypes under both ideal and non-ideal environments (Bänziger et al., 2000).

1.5 Response of genotypes to low nitrogen stress

1.5.1 Low nitrogen as compounded by other stresses

Stress is any phenomenon or process that reduces the yield potential of a genotype (Hill et., 1998). Nitrogen does not only fulfil a regulatory role in metabolic processes, but it also regulates DMA and the uptake and utilisation of other mineral nutrients. In connection with this, Hageman and Lambert (1996) reported that variance of yield could be a good measure of a genotype’s response to N. Pending the crucial role of N in maize (Lafitte, 1994), tropical maize in SSA is produced under LN conditions, therefore this subsection reviews stresses related to low N and genetic characters that confer tolerance to these conditions. The relationships of such characters and their outward contribution to final yield are also reviewed.

The effects of drought, low N and high plant density stresses are severe at silking and grain filling stages in maize and these may cause barrenness in the case of drought and/or poorly developed ears and thick plant stands (Ribaut et al., 1996; Bänziger et al., 2000; Sangoi, 2001; Magorokosho et al., 2003; Lal et al., 2010). These authors agree on the compounding effects of (LN) with other stresses being short reduced plant stature, greater leaf area reduction of pre- and post-flowering, especially at R_1, enhanced floral protandry, lower pre- and post-leaf chlorophyll content, lower KPP, kernel weight, HI, and enhanced per plant-yield variability. However, Boomsma et al. (2009) and Lal et al. (2010) reported on the lack of genetic efforts to improve maize genotypes for tolerance to such multiple stresses and simultaneous focus on NUE and N stress tolerance. Detecting genotypes that reduce the effects of these stresses, such as continuous growth of reproductive parts, is crucial, and this would further translate into kernel set and their maintenance to physiological maturity.

1.5.2 Physiological basis of prolonged leaf chlorophyll concentration character

Extended leaf chlorophyll content (SG) is a function of the balance between demand by and supply to the grain of N during grain filling. Genotypes vary for this trait. Conceptually, such a trait has a time component and plant and soil N status, and is relevant at post-flowering growth stages. At the pre-anthesis stage, more N has been found to be proportionally allotted to leaves of non-senescent genotypes (SG) in a sorghum variety (Borrell and Hammer, 2000). The possible explanation could be leaf structure differences in both SG and senescent genotypes. Borrell and Hammer (2000) added that the leaves for SG genotypes are thicker than their senescent counterparts, so creating more demand for N at anthesis in SG genotypes. Essentially, senescence (due to normal ageing and/or N deficiency) is the result of more demand than supply of N from the plant source and the environment. Moisture stress, in fact, may accelerate senescence. A larger portion of N is remobilised, as opposed to being drawn from the soil. Delayed remobilisation from leaves prolongs photosynthetic machinery, which becomes a plus to yield (Subedi and Ma, 2005; Hawkins et al., 2007).

However, other studies indicate that SG genotypes have well ramified root systems to meet the further demands of N from the soil during grain filling periods, as opposed to senescent genotypes (Pan et al., 1986; Bänziger et al., 1999; Hawkins et al., 2007). Besides the advantages of the SG trait, its physiological and genetic bases are not clear, even in crops where it has been mostly studied, such as sorghum and horticultural crops.

The leaf N in SG genotypes remains higher than in non SG because in SG:
i) the leaf N level at anthesis was higher,
ii) N uptake during grain filling was higher, and
iii) the remobilisation of N from leaves of SG during grain filling was less (Borrell and Hammer, 2000).

Host-pathogen relationships at grain filling may influence the SG profiles but debate has been inconclusive as to whether the green colouration in that relationship is due to retention, regreening, and/or new synthesis. This argument has been reported mainly in fungal pathotypes in cereals (Scholes and Farrar, 1987). Basra and Goyal (2002) reported on the nitrate ions (NO_3^-) as a reservoir of leaf N, where excess NO_3^- stored in leaf vacuoles is remobilised when the N supply from the soil is depleted. Therefore, accumulation of NO_3^- in leaves during the vegetative phase under low N may act as a marker for selecting genotypes with enhanced yield potential under such conditions. Very little systematic research has been conducted to evaluate whether the SG character would still be beneficial during late season stress i.e. foliar diseases, low N and drought. It is also not clear whether the SG hybrids require additional N fertiliser, let alone in which growing conditions. A study on SG sorghum has indicated a penalty to yield and yield components at low N and drought stress environments (Borrell and Hammer, 2000). Although the SG trait in maize is crucial for grain filling and kernel maintenance, especially under late season stress, the inheritance of the trait, especially when measured at different growth stages, has not been established. Knowledge on the profiles of leaf chlorophyll content across kernel fill stages will help determine the rate of DMA and the specific stage in maize where N is needed the most.

Various studies support the SG character being a function of soil N. The late season test at ear leaf (around ¼ milk line) by Chlorophyll Meter (Model SPAD-502 Minolta Camera, Japan) has been proven reliable and economical in separating cultivars with adequate N from those that are deficient in N, also the same trend may apply for N sites (Piekielek et al., 1995). Although leaf N in maize stabilises with age (Coe and Nueffer, 1979; Binder et al., 2000), Piekielek et al. (1995) reported that the stability of leaf N with age was inherent to the genotype's leaf chlorophyll content, consequently affecting the late season SPAD-502 tests. However, Bertin and Gallais (2000) counter-argued by reporting that chlorophyll content was affected by N stress early in plant development. The general trend was that early season SPAD test would not be reliable due to soil temperatures and hybrid vigour imposed by selection. Crafts-Brandner and Poneleit (1987), Piekielek et al. (1995), Mahalakshmi and Bidinger (2002) and Hawkins et al. (2007) suggested that experiments on SG should involve plants with uniform phenology, sowing dates, and periods of maturity, since N varies according to genotype and environment. This suggests that experiments on SG should be designed to maximise precision, thereby enabling interpretation and practical application of the results.

The influence of growth hormones under soil N regimes may determine the SG trait and some yield components. Cytokinins have been reported to relate directly with the SG character and the KPP (Robson et al., 2001; Bertin and Gallais, 2000). Further, Bänziger et al. (2000) and Daynard and Duncan (1969) established that Abscic acid (ABA) regulates the number of kernels that reach maturity under multiple stresses, including low soil N. Cultivars that resist leaf photo-oxidation may have extended leaf chlorophyll concentration, thus adapting them to multiple stresses and they produce more dry matter than susceptible genotypes (Robertson, 1975; Britton, 1995; Ping et al., 2005; Joshi et al., 2007). High rates of cytokinin transported from roots to leaves result in SG cultivars, whereas such cultivars block transport of abscic acid from roots to shoots, thus retarding leaf senescence (Ping et al., 2005). Therefore, the physiological basis of extended leaf chlorophyll content in maize could be a function of the genotype, growth hormones, status of N in plant and soil, moisture, and sink-source relationship, among other factors.

1.5.3 Measurement of leaf chlorophyll concentration using the SPAD-502 meter

The Chlorophyll Meter (Model SPAD-502 Camera Minolta Co. Ltd., Japan) has been proven to quantify leaf chlorophyll concentration (Martinez and Guiamet, 2004; Subedi and Ma, 2005). Other studies indicate that SPAD readings and extractable chlorophyll content are strongly correlated (Dwyer et al., 1991; Subedi and Ma, 2005). The study by spectroradiometer on maize leaves also indicated that N and chlorophyll content are strongly correlated (Ercoli et al., 1993), but good results may be obtained at high and medium concentrations of N. This suggests that SPAD-502 is a reliable meter when it comes to quantifying leaf N, which also reflects on the status of soil-N and the health of the plant. In addition to the Chlorophyll Meter method and laboratory analyses for plant and soil-N, other methods of quantifying LCC include leaf area based indices, leaf area under curve and leaf green-colour scores. A combination of these techniques would help to check for consistency among these methods in response of genotypes to soil-N across management regimes. However, it is best to use the SPAD-502 to quantify plant (leaf) and soil N if a comparison is made between the meter’s perfect correlations and others recorded through different methods.

The precision of SPAD values is determined by factors such as genotype, environment, meter differences, and human dexterity when recording the LCC data. The lower the irradiance the higher the SPAD values, and vice versa (Hoel and Solhaug, 1998). However, plants adapted to high light intensities are less affected by irradiance variations compared to shade adapted plants. Chlorophyll meter readings may be affected by movements and varying orientations of chloroplasts. In low irradiance, chloroplasts are oriented along the upper and lower cell walls, thereby maximising light absorption, while in high irradiance, they are oriented mainly along the vertical walls parallel to incident irradiance (Hoel and Solhaug, 1998). Robson et al. (2001) reported that shade could retard the cytokinin growth hormone that also reduces photoreceptors phytochromes, so accelerating chlorophyll senescence. Dwyer et al. (1991) established that high temperatures may inflate SPAD readings.

In addition, leaf spectral properties per se were found to be affected by leaf age, leaf position, and region within a leaf (Dwyer et al., 1991; Earl and Tollenaar, 1997). Young, chlorotic, and senesced leaves have low SPAD values. In mature leaves, the variability of SPAD readings is small. Despite these setbacks, SPAD provides estimates of critical levels of N under both stressful and stress-free conditions (Earl and Tollenaar, 1997; Martinez and Guiamet, 2004; Subedi and Ma, 2005). Based on the various factors that affect SPAD readings, Piekielek et al. (1995) suggested the readings be normalised, where the standard N treatment could be considered as a reference to reduced N. Generally, experimental conditions to estimate LCC should be well controlled in order to minimise experimental error. For example, genotypes should be of relatively equal physiological maturity and equally treated.

1.5.4 Genetics of extended leaf chlorophyll content character

Information on genetic control of extended leaf chlorophyll concentration (SG) in maize is scanty. Few studies indicate that a single dominant gene governs the SG trait in maize (Thomas and Smart, 1993; Ceppi et al., 1987; Gentinatta et al., 1987). On the contrary, Ahmadzadeh et al. (2004) reported a preponderance of additive genetic effects for leaf carbon dioxide exchange rate (CER) late in the season. In sorghum, Kassahun et al. (2010) reported that the onset of senescence (declining LCC) is additively controlled, whereas the slow rate of senescence is governed by complete dominance, as opposed to fast senescence. Still with sorghum, (Walulu et al., 1994) asserted that the SG character is highly influenced by the environment but at the genetic level there are varying levels of dominance. The trait falls under polygenic control in sunflowers (Cukado-Olmedo and Miller, 1997). However, all of these studies might be asserting on the obscurity of the inheritance of the trait. Subedi and Ma (2005) added that the genetic and physiological bases of the extended leaf chlorophyll character are not clear. Other workers, to mention but a few, reported that the character is highly variable with the environment (Piekieleki et al., 1995; Robson et al., 2001; Martinez and Guiamet, 2004; Hawkins et al., 2007). Therefore, the inheritance of the character is likely to be unclear, but more work is required to clarify this, especially in tropical maize under low N conditions. The few reports are therefore inconclusive but further investigations may be recommended, such as the present study. Despite these challenges, Robson et al. (2001) believe that varieties that retain high LCC can be bred and they may also remain photosynthetically active.

1.6 Secondary traits and stability of grain yield and maturity

This section analyses the various secondary morphological and reproductive traits that are crucial in developing models that would simulate and mimic breeding for maize yield stability. However, Elings (2000) and Lizaso et al. (2003) reported limited studies to simulate and model yield in tropical maize, as compared to temperate maize. Furthermore, the rate and duration of grain filling and grain dry-down are reviewed. Grain moisture content and calendar physiological maturity are highly correlated (Duvick, 1999). Acquisition by and accumulation of dry matter in the kernel operate on the theory of displacement, therefore kernel dry matter-moisture relationships must be studied in order to classify maize according to their maturity classes and potential yield.

1.6.1 Leaf angle and number of leaves above the ear

The angle of leaves above the ear better determines yield than the leaves below the ears due to the fact that the lower leaves do not directly intercept solar radiation and so contribute little to yield (Mickelson et al., 2002). Leaf angle character is highly heritable (Schuetz and Mock, 1978). Studies on quantitative trait loci (QTL) have established the non-significance of epistatic interactions for leaf angle. In addition to leaf angle, Shaver (1983) reported that the number of leaves above the ear have been associated with maturity and are seen as a component of grain yield. Under favourable, drought and low N conditions, Elings (2000) found that the area of the largest leaf relative to the total leaf plant area was constant and this constancy was linear to the total number of leaves. However, the number of leaves referred to were total leaves rather than the number of leaves above the ear. Studies concerning the number of leaves above the ear for tropical maize under low N conditions have not been reported in the literature yet genetic control of this trait, even under favourable conditions, is inconclusive. Bonaparte (1977) reported partial dominance with large effects and little dominance gene action. Bonaparte and Brawn (1976) reported the inconsistency of the trait with the environment, which may suggest polygenic control. Hageman and Lambert (1996) asserted that the total number of leaves varies with the altitude. Higher altitude genotypes may have many leaves, which decrease as the attitude decreases. Shaver, observing the wide distribution of the F_2 for the trait, confirmed additive genetic effects, thus agreeing with Bonaparte and Brawn (1976). Furthermore, Shaver (1983) distinguished the genetic control of normal from leafy maize and found that the latter is governed by a single dominant gene. The disagreement concerning the genetics controlling the number of leaves above the ear may justify the need for a study to clarify its inheritance. Although leaf number above the ear is an important yield component and it may be used to classify genotypes across environments, its genetics and its relationship with other yield components under low N conditions in tropical maize is not clear.

1.6.2 Kernels per plant and anthesis-to-silking interval

The developmental processes that determine the number of kernels per plant (KPP) in maize can be divided into three consecutive stages:

i) initiation and differentiation of reproductive structures,
ii) maturity of the structures that engage in pollination, and
iii) fertilisation and kernel formation (Lizaso et al., 2003).

However, current simulation models skip steps i) and ii). The final KPP relate with the current supply of photosynthates or related characteristics, such as light interception or plant growth rate, the time of silking and reduced leaf senescence. Furthermore, KPP is related to DMA (Boras et al., 2009) and it is determined one week before to three weeks after silking (Lizaso et al., 2003). Capristo et al. (2007) associated increased KPP with physiological maturity of a genotype but the individual kernel growth rate decreased.

Since short anthesis-to-silking interval (ASI) is a function of synchronous pollination, selecting for reduced ASI leads to increased kernel number, reduced abortion rate and increased grain filling period, while the vegetative growth phase is shortened (Mock and Pearce, 1975; Lizaso et al., 2003). However, it is difficult to locate the cause of long ASI, since it may be affected from the early growth stages, for example, flooding during early vegetative growth will delay silking more than tasseling (Lizaso et al., 2003). Since the extension of ASI is due mainly to delayed silking stage, this stage could be timely targeted and used as a reference to improve the yield and maturity of maize cultivars.

Although ASI has been extensively used as a measure of stress and a reference for improving yield under stress, the trait is not reliable. Edmeades et al. (2000), Carova et al. (2003), Anderson et al. (2004), and Boras et al. (2009), among other researchers, criticise use of the trait.

a) The interval is subject to random errors since it involves two independent developmental stages,

b) Extremely very low or negative ASI values may in the long run compromise yield and male fertility,

c) The interval is only relevant to moderate stresses (i.e. not to severe stress or stress-free situations); Bertin and Gallais (2000), who worked on N-use efficiency, added that ASI should only worry a breeder under low N and not at optimal N input.

d) Under severe stresses, which may intensify with space and time yield, KPP, HI, and EPP could be used as indices of stress to replace ASI.

e) The interval may not convey comparable meaning for genotypes that differ in maturity[1]. For example, late genotypes may have the same ASI as early maturing genotypes.

1.6.3 Pollination synchrony on plant and ears

There exist asynchronies in pollination within and between ears such as:

1) the developmental dominance of basal kernels over apical kernels on the same ear (i.e. within ear asynchrony); and

2) the delayed development of sub-apical ears relative to apical ears (i.e. between ears asynchrony) (Lizaso et al., 2003).

The authors added that a fraction of kernel set decreases each day as the number of previously set kernels increases, such that the success of later-formed kernels decreases as the number of prior pollinations on the same ear increases. The number and timing of pollinations referred here are those under controlled situations by the breeder. Furthermore, prolonged pollinations could be undesirable, for instance, the accumulation of aflatoxin has been found to correlate with extended pollinations (Betran and Isakeit, 2004). Therefore, care must be taken on the timing and duration of pollination in order to have comparable conclusions among genotypes. However, Carova et al. (2003) and Carova and Otegui (2007) reported that shorter ears (i.e. those with reduced kernels per row) have convergence between silking at the base and at the tip, and conversely to long-eared hybrids. Carova and Otegui (2007) found a higher ratio between tip-to-base of the developing ear set; there were

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[1] Arnold Mushongi. Results from the miniproject trial. 2007. ACCI/CERU Tunnels, Faculty of Science & Agriculture, Pietermaritzburg Campus, University of KwaZulu-Natal.

many kernels per ear, with R^2 >90%. Thus, shorter-eared genotypes may enjoy a high floret fertility index (FFI, i.e. number of kernels ÷ number of pollinated silks) (Anderson et al., 2004). High FFI is a function of the balance of source: sink ratio. Nonetheless, early pollinations may have heavy kernel weight (Anderson et al., 2004), which may permit increased carbohydrate partitioning to those kernels and extend the filling period by a few days. It may therefore be suggested that if many KPE are desired as a yield component, the shorter ears may be more stable over suboptimum cropping inputs than long-eared genotypes.

1.6.4 Prolificacy and stability of grain yield

The prolificacy trait in maize derives from its progenitor, teosinte, thus a single eared trait in maize is suggested to be an artefact of artificial selection (de Leon et al., 2005). Increased prolificacy is usually associated with decreased ASI, because prolificacy typically indicates a reduction in apical dominance, leading to greater synchrony of male-female flowering (Saquis et al., 1998). Flowering dates are associated with the production of nodes during plant development, but less so with internodal elongation on the main stalk. This may suggest that plant and ear heights are independent of maturity. Prolific genotypes have higher ASI, ear height, and high sink strength (Brotslaw et al., 1988). De Leone et al. (2005) reported the existence of sufficient genetic variability and positive correlations between shorter ASI with total yield, yield of second ear, yield of top ear and increased development of bottom ear in prolific genotypes. Contrarily, significant reductions in ear width, row number, kernel weight, total kernel number and reductions in grain moisture are associated with prolificacy (Coors and Mardones, 1989) and are factors that may affect reduced yield. This could suggest that prolific hybrids have higher rates of kernel dry-down than non-prolific genotypes. The key breeding issue remains: how much the rate of grain dry-down correlates with yield, yield stability and traits that are related to maturity under high and low N, conditions under which tropical maize is typically produced.

The yield of the second ear is source-limited, whereas that of the primary ear among prolific and non-prolific maize is sink-limited (Saquis et al., 1998). The second ear often produces kernels only after the first ear has achieved its maximum possible kernel number (Varga et al., 2004). In maize leaves, maximum net photosynthesis and sucrose phosphate synthase

(SPS) enzyme activation occur at midday (Yadava, 1986), as with other plants. The sucrose phosphate synthase enzyme regulates photosynthesis and carbon partitioning between starch and sucrose. The enzyme is also an important determinant of source capacity. A defined interval of duration in pollinations may therefore offset the discrepancy in yield between ears that culminates in increase of mean yield. For more reliable comparisons among genotypes for maturity, synchronous pollination is important.

Because the yield of the second ear is highly variable (Saquis et al., 1998), the breeding goal would be to stabilise such yields as a fixed component of total yield. In order to complete its growth, the second ear must reach a minimum stage of growth before active grain filling begins on the first ear (Varga et al., 2004). This also corroborates the idea that maximum total yield per plant would be when both ears are pollinated at the same time (Varga et al., 2004). This suggests that in order to set the kernels and maintain them to physiological maturity, the source must be photosynthetically active.

Early prolific hybrids have been found to out-yield late non-prolific ones (Varga et al., 2004), possibly due to inflated KPP in the former hybrids. However, row number and 1000 kernel weight per primary ear and per plant are stable across seasons and input systems, whereas KPE and KPP are the yield components that mostly vary with cropping inputs. Genotypes with reduced leaf senescence would mitigate for such diverse stresses that appear at the grain-filling stage, thus maintain kernels to physiological maturity (Subedi and Ma, 2005; Robson et al., 2001). It is therefore suggested that breeding efforts should focus on KPP and KPE, which vary according to cropping inputs that are common in farmers' production environments.

1.6.5 Plant growth at flowering and grain yield

The number of kernels per plant (KPP) can be determined by relating them to the rate of plant growth at flowering. At a rate higher than 6.5 g carbohydrates per plant per day, a second ear forms in semi-prolific cultivars and KPP is described by two hyperbolas: plant growth rate at which kernels begin forming on a second ear (X*int*); and the number of kernels on the primary ear if kernels begin forming a second ear (Y*int*) (Elings et al., 1997). The X*int* and the Y*int* are model inputs that can be varied and which permit simulation of semi-prolific cultivars. Reduced growth rate at flowering widens ASI, thus negatively affecting KPP, because ASI and KPP are positively correlated. For instance, the model by Bolanõs and Edmeades (1993b) cited in (Elings et al., 1997), records that in both favourable and drought conditions, the ASI had no negative effect on later crop growth stages. Elings et al. (1997) also indicated that low leaf senescence results in higher potential grain-filling rates. Active ear growth would indicate that at high production levels, e.g. high N, grain filling is sink limited, such that greater KPP should increase yield.

The size of the maize plant (height and weight) correlates positively with the length of the vegetative phase and the partitioning of dry matter (Begna et al., 2000). The authors added that taller hybrids produce a great amount of dry matter but circulate it slowly, whereas dwarf hybrids yield low dry matter but circulate it quickly. Plant height also correlates strongly with the flowering date, since internodal formation stops at the floral initiation stage, indicating that earlier flowering genotypes are usually dwarf varieties (Troyer and Larkins, 1985; Vladulu et al., 1999). Spaner et al. (1996) established that dwarf genotypes may flower earlier and yield less than the taller, late flowering ones. It is also suggested that higher ear height increases the possibility of lower internodes bearing more ears (Flint-Garcia et al., 2003). Plant and ear heights may correlate well with yield and the rates of kernel dry-down. However, plant and ear heights vary widely with environment, such that their relationship with yield and maturity may not be easy to establish. Furthermore, the relationship between the LCC character, plant size, plant height and the grain filling stages found in tropical maize, under both high and low N, is obscure.

1.6.6 Dry matter accumulation and grain filling

The component processes of grain yield include dry matter accumulation (DMA) throughout the life cycle i.e. mostly at physiological maturity; other components include harvest index (HI), leaf area index (LAI), extended LCC, and leaf carbon exchange rate (CER) (Lee et al., 2005; Ahmadzadeh et al., 2004). Lee et al. (2005) reported that a large proportion of DMA in the kernel occurs during the grain filling period, i.e. mostly six weeks after silking. About 75% of genetic control of final yield per se has been reported to be a function of additive genetic variance (V_A), suggesting the efficiency of response to selection (Lee et al., 2005). Hallauer and Miranda (1988) disaggregated the relative genetic control of final yield and reported 61.2

and 38.8% for additive and dominance genetic effects, respectively. They further found that the relative contribution of additive-dominance effects to yield components proved to be negligible. However, there could be bias since the authors assumed absence of epistasis and linkage. Studies to dissect the genetic control of growth stages from kernel set and maintenance to physiological maturity would provide an indication on the growth stage at which a breeder would concentrate breeding efforts. Since yield potential may at least be predictable at grain filling stages, selection could be done at a specific filling stage before harvest of the final crop, thus reducing breeding cycles.

1.6.7 Environmental correlation and grain filling

The genotype x environment correlation imposed by breeders and farmers through selection (Falconer, 1989; Kearsey and Pooni, 1996) may influence grain filling rates and duration in maize. For instance, differential application of fertilisers may lead to staggered flowering and, as a consequence, different rates of physiological maturity in maize (Vladulu et al., 1999), even if the genotypes were the same in the same field. The differential effects in both dosage and time of application of N treatments on the physiological maturity of tropical maize are less systematically documented. Below et al. (2000) established that such studies could help determine the availability of N against physiological maturity and yield although, in his earlier study, Swank et al. (1982) reported that under low N, the grain filling period decreases, resulting in low yield. Mock and Pearce (1975) affirmed that both the rate and duration of grain filling are influenced by environmental and genetic factors, however the grain-filling duration would suffer the most from changes in plant density and ambient temperature. The authors could therefore be suggesting that it would be easier to breed for increased rate of grain filling than for the duration of grain filling. Monneveux et al. (2005) supported that the reduction of final grain weight under high plant density and other abiotic conditions are more attributable to reduction in grain filling period than growth rate. Contrarily, Kempton and Fox, (1997) showed that grain filling extends under stresses but the genetic, developmental and physiological mechanisms for that extension are not clear.

1.6.8 Rate and duration of grain filling

The inheritance of the rate and duration of grain filling in maize is not clear, although at least both general combining ability (GCA) and specific combining ability (SCA) have been reported to control the duration and rate of grain fill, with kernel weight the most affected (Wang et al., 1999). Despite the paucity of genetic information either on plant and/or on ear basis, hybrids with high kernel dry-down rates would be the best for grain filling. Cross and Kabir (1989) and Mock and Pearce (1975) established that kernel size may determine drying rates of kernels and it is correlated with kernel filling rate, suggesting that correlated response to selection exists among these traits. Genotypes that flower later dry down faster than those that flower earlier. The later flowering genotypes attain mid-silking more rapidly, but they have longer mid-silking to physiological maturity period, suggesting that they dry faster than those that flower earlier (Cross and Kabir, 1989). Therefore, later silking, if desired, could serve as a selection index for longer grain filling duration, however, penalties to yield and yield components are expected as ASI widens. While kernel number is controlled by the additive and dominance genetic effects, the grain filling rate falls under additive effects, suggesting that it is heritable (Cross and Kabir, 1989). The effects of the LCC and grain filling duration under N conditions that may suggest increased kernel density has not been studied in tropical maize. The relationship between the LCC and KDD with yield remains a controversial subject in the literature.

The rate and duration of grain filling affects stalk strength, kernel moisture content at harvest, plant height, HI, and physiological maturity (Hartung et al., 1989). The rate of dry matter accumulation (DMA) determines the number of kernels per plant (KPP), kernel weight, and overall HI, compared to the effective filling period duration (EFPD). However, hybrids with high rates of kernel fill, the trait determined by GCA (Hartung et al., 1989), are desirable in short season areas and indicate the possibility of response to selection for this trait. This would further confirm that the extended LCC character prolonging the EFPD may not be relevant in short season areas where extra early cultivars are required. In addition, kernel growth rate is controlled by dominance effects, suggesting the possibility of direct selection for EFPD without affecting physiological maturity. Hartung et al. (1989) reported that kernel weight decreases with direct selection for long EFPD, due to the correlated response of low DMA with long EFPD selection. The relationship between grain fill duration and other components of yield and physiological maturity under low N in tropical maize has never been studied.

1.6.9 Rate of grain dry-down

A maize cultivar whose grain dries down earlier may reduce costs incurred by farmers in drying maize grain, and avoid the risks of leaving maize in the field to dry under natural conditions, thereby falling prey to disease, damage by vermin and insects, and lodging (Zhang et al., 1996). Cross and Kabir (1989) pointed out that the rate of KDD is determined by pericarp thickness, husk cover, ear tip exposure, rate of husk senescence, hydrophilic compounds in the endosperm, and kernel moisture content at physiological maturity. Kernel dry-down rate is conditioned by GCA, thus pointing to the possibility of effective response to selection for the trait (Cross and Kabir, 1989; Zhang et al., 1996). However, Zhang et al. (1996) argued that KDD rates are controlled by both genetic and cytoplasmic factors, thus implying the need for reciprocal genetic analysis for the trait. There exists positive correlation between rates of ear moisture loss and grain filling (Kang et al., 1986), while KDD rate correlates negatively with yield components. For instance, relative grain moisture loss is negatively correlated with kernel depth, kernel size, number of kernel rows per ear, large ear diameter and ear length (Cross and Kabir, 1989). The challenge is to strike a balance between KDD, yield and reduced physiological maturity, which are the key desirable traits in tropical maize varieties. These relationships have not been studied under low N conditions prevalent where tropical maize is grown in Africa.

Hybrids that silk at an earlier date but have similar grain filling periods may begin losing moisture and attain physiological maturity earlier than those that silk later (Cross and Kabir, 1989). However, Chachalis et al. (2006) reported that ultra early planting had lower grain moisture content at harvest than early and normal plantings. In addition, it has been observed that hybrids have higher grain moisture content than inbreds, indicating that hybrids have longer grain filling and dry-down periods than inbreds (Betran et al., 2003b). Hallauer et al. (1996) reported on the difficulty of correlating inbred traits with hybrid performance and the need progeny tests.

The later maturing a variety, the higher the possibility that it will succumb to aflatoxin contamination. However, early-maturing varieties do not only yield less but may also have loose husk cover, which may predispose them to aflatoxin (Betran and Isakeit, 2004). Aflatoxin contamination may be higher in areas where maize is harvested earlier and forced to dry or in areas where maize matures earlier and coincides with high rains. Breeding for secondary traits such as rapid rate of ear and/or kernel dry-down might reduce the incidence of aflatoxin through escape mechanisms.

1.7 Molecular marker approaches to studying genetic effects

The mapping of quantitative trait loci (QTL) allows detection and estimation of genetic effects related to expression of quantitative traits (Sari-Gorla et al., 1999; Jansen, 2001; Witcombe and Virk, 2001; Liu et al., 2010). Regardless of challenges in the production environment and the quantitative nature of traits in tropical maize, the role of QTL research stands. Nevertheless repeatability of the QTLs over different environments has been questionable for most traits (Jansen, 2001; Tuberosa et al., 2002; Enoki et al., 2005) due to factors such as pleiotropy, linkage and environmental effects (Falconer, 1989; Tuberosa et al., 2002). These same factors may also influence correlation between traits. Putative QTLs that condition anthesis and silking dates, and plant height, depend on plant maturity and development which are controlled by pleiotropic alleles (Vladulu et al., 1999). Kassahun et al. (2010), working on sorghum, detected QTL for LCC and SG, which were overlapping.

Taking into consideration low N, which is a pervasive stress factor under which the tropical maize is produced (Zaidi et al., 2004; Bänziger et al., 1997), deliberate efforts may be needed to assess breeding strategies suited to such a production environment. Liu et al. (2008) conducted a QTL study on four traits in maize and found that low N increased starch relative to protein content. They also reported that QTLs were higher for yield under high N than low N conditions, but specific QTL for the two N regimes were evident, which may provide insight to the basis of the efficiency of N use. Yet Liu et al. (2010) identified three QTLs in maize with small R^2 between 18.07% and 20.96% for yield under high and low N, respectively. Furthermore, they reported positive additive and negative additive variances for yield under high and low N, respectively. The relative decrease of the magnitudes and lack of commonality under N regimes, whether based on QTLs or from conventional studies, is not confined to maize alone. In rice, Lian et al. (2005) reported that fewer QTLs were detected under both low and high N and most of the QTL for relative measurements under two N regimes was not consistent with the two separate regimes. This may indicate very little correspondence on the genetic basis between traits and relative effects of N under high and low N. The relative contribution of epistatic effects to total genetic variation for several traits was large, while QTL x environment effects were negligible (Lian et al., 2005). However, in an earlier study, Agrama et al. (1999), using restricted fragment length polymorphism DNA markers, found correspondence between several genomic regions with yield and yield components under low N conditions, suggesting the presence of QTL which is associated with NUE. Contradicting information concerning the lack or presence of correspondence on the genetic basis of QTL under N regimes may require further research.

The QTLs have also been used to investigate the role of epistasis and degree of dominance effects in controlling yield and secondary traits (Jampatong et al., 2002). While additive x additive effects have not been reported, the additive x dominance interactions were implicated in traits such as EPP (de Leone et al., 2005), thus opposing many studies which ignore the presence of epistasis in quantitative traits, particularly in maize. Dudley and Johnson (2009), working on S_2 lines of maize for kernel oil content, protein, starch and yield, found that R^2 was high between the predicted and observed means when the epistatic models were integrated with the single nucleotide polymorphism (SNP) molecular marker data. This suggested: i) the existence and value of epistatic interactions in quantitative traits of maize, and ii) the predictive power of incorporating epistatic models in marker assisted breeding. However, in support of the opponents of the existence of epistasis, Berke and Rocheford (1999) using QTL analysis, found epistatic effects for tassel characters to be non-significant, although this contradicted the findings of Lian et al. (2005) in rice. It may therefore be concluded that models which include epistasis should be explored. For example in maize heterosis can be partly attributed to positive epistasis (Mather and Jinks, 1982; Kearsey and Pooni, 1996) in addition to f dominance. Nonetheless, the role of epistatic effects under low N conditions in SSA has not been studied.

1.8 Relationships between grain yield and secondary traits

Correlations and heritability estimates are crucial tools available to the breeder as they measure the degree of association among traits. There are two common types of correlations in breeding. Genetic correlations measure association of breeding values (i.e. additive genetic variance) among the two traits (Falconer, 1989). Phenotypic correlations are a direct measure of the association between two traits and they comprise genetic and environmental effects (Hallauer & Miranda, 1988). Positively correlated traits suggest that the two traits can be improved, based on one trait, while separate breeding strategies are required for negatively associated traits (Falconer, 1989; Falconer and Mackay, 1996; Lynch and Walsh, 1998). Lynch and Walsh (1998) reported that the same character, measured in different environments, may be considered separate traits.

Heritability measures the degree of resemblance between parents and their progenies (Kearsey and Pooni 1996; Hill et al., 1998). Piepho and Möhring (2007) added that heritability is a measure of precision in the experiment. There are two types — broad sense heritability (H^2) is the ratio of total genotypic variance to phenotypic variance, whereas narrow sense heritability (h^2) is the ratio of additive genetic variance to phenotypic variance. In this case, H^2 is higher than h^2. Balzarini (2002) compared the correlation with heritability as the correlation between genotypes and phenotypes being equal to √heritability, although this refers to the upper-bound observed correlation. Thus breeding gains could be obtained from the two tools of correlations among traits and response to selection from heritability estimates. However, it has been difficult to breed for direct improvement for yield (Bänziger et al., 2000; Lee et al., 2005). The correlation and heritability values for secondary traits have been reported to be higher than that for yield under stress conditions. The survey of the literature indicates that regardless of tropical maize being produced under low N conditions, few studies to estimate and predict traits relationships s of secondary adaptive traits with themselves and to final grain yield have been established under such conditions.

1.9 Summary of the literature review

Research gaps in the objectives of the present thesis were identified as follows:

- The existence of genotype x N interaction observed in the literature suggests the need for embarking on breeding programme for tolerance to low N conditions to improve and stabilise grain yields that are already very low and erratic.
- The contribution of leaf chlorophyll concentration (LCC) to grain filling duration in maize is appreciated. However, only a few inheritance studies have been conducted under low and high N regimes.
- Low N reduces plant stature and negatively affects secondary traits, such as number of kernels per ear, and consequently grain yield and physiological maturity.

- Heterosis, being a measure of local adaptation, increased under low N than high N. However, relative grain yield loss due to low N and its effects on yield components and calendar physiological maturity have not been established. Research to fill this gap would enhance maize productivity under low N conditions.
- Relationships among the crucial adaptive traits have not been extensively studied especially under low N in tropical maize, including LCC vs. stay-green characteristics, kernel dry-down rate, kernel moisture content at harvest, effective grain fill duration and floret fertility index, ear prolificacy, synchronous pollinations and physiological maturity.

Surprisingly, only a few studies have been undertaken on genetic analysis of tropical maize grown in low soil N, since maize in Africa is typically produced under such conditions. Efforts are therefore needed to identify and study traits for the genetic control of crucial traits and the relationships among them under low N in order to improve yield. The grain yield penalty for producing maize under low soil N, and the negative effects on physiological maturity have not been established. Maize cultivars that are adapted to low soil N conditions would also be resilient to most of the stresses which are associated with low N, and commonly found under the production conditions in SSA.

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Chapter 2:

Farmers’ perceptions of production constraints and preferences for maize varieties and other supporting technologies in the intermediate altitude zone in Tanzania

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Abstract

In Tanzania, the bulk of staple maize comes from the intermediate altitudes, although farmers’ knowledge about crop improvement in that zone has been neglected. The present study was conducted to determine farmers’ perceptions of production constraints and their preferences for maize cultivars. A formal survey was conducted among 214 randomly selected farmers from two districts in the Mbeya region. Farmers also participated in an informal group discussion. Results indicated significant variation in background training and experience, access to new technology and preference for maize cultivars which impact on maize productivity. Only 29% of the farmers had agricultural training. The study revealed that 90% and 68% of the farmers had no access to credit and extension services respectively, which limited their access to essential agricultural inputs such as improved seed and fertilisers. Only 53% used improved seed varieties and 60% used pesticides. About 37% applied fertiliser and 87% thought it was not required. Early maturing cultivars were highly preferred (61-83%). With respect to satisfying agronomic requirements of farmers, there is also need to breed for adaptive traits, such as stay-green (66%) and quick kernel dry-down rate (61%). About 30% required large number of kernels per plant and per unit area in the preferred varieties. Additionally, the study indicated that other secondary traits, especially adaptive traits, should be seriously considered for improving grain yield under low N conditions, because only a few farmers applied adequate N to support the maize crop. Results also suggested that the development of drought-tolerant varieties would help improve not only the farmers’ socio-economic situation but also impact positively on grain yield, with equally positive multiplier effects on the rural economy in this important agricultural zone in Tanzania.

Keywords: intermediate altitudes, maize, production constraints, production opportunities, small-scale farmers, Tanzania, varietal preferences.

2.1 Introduction

Maize is a staple food and cash crop in Tanzania and the bulk of it comes from the intermediate altitudes (Bisanda et al., 1998). Production, however, is not adequate because, among other natural limitations, farmers' knowledge and practices concerning maize breeding have not been given due attention. Farmers know their agricultural environments better than anyone and how to adapt and make allowances for any undesirable change in the cropping system. In Tanzania, maize is mostly produced in the Southern Highlands Zone (SHZ) by small-scale farmers who do so under multiple stresses. Average maize production has been consistently below 1.5 t ha^{-1}, notwithstanding the production potential of about 8 t ha^{-1} for improved cultivars in the intermediate altitudes of SHZ (Lyimo, 2006). Environmental and socioeconomic challenges prompt farmers to demand early-maturing maize cultivars that are stable and widely adapted (Bisanda et al., 1998; Lyimo, 2006). Nevertheless, information on maturity for maize varieties in Tanzania is limited. Another obstacle is that of incorporating high grain yield into early maturing cultivars (Finlay and Wilkinson, 1963; Witcombe and Virk, 2001). Despite the potential contribution of farmers towards breeding new varieties, their know-how has generally been ignored by breeders in the Tanzanian programme.

Participation of farmers and breeders in the development of varieties would result in the use of new varieties that impact positively on grain yield. Farmers and maize breeders have different selection criteria (de Groote et al., 2002; Virk et al., 2005). A crop variety is a complex entity, with each farmer making his/her own choices and these are always difficult to quantify (Hu et al., 2007). Most disappointingly, farmers' selection criteria have not been systematically documented (Bänziger et al., 2000; de Groote et al., 2002). In such cases, agronomic features embedded in released cultivars might not have been defined adequately (Joshi and Witcombe, 1996), so most cultivars that reach farmers are inappropriate (Efisue et al., 2008). Partnerships between farmers and breeders, or client-oriented breeding (CoB) approach (Witcombe et al., 2003; Virk et al., 2005), has been known to work well. For instance, such an approach has reduced the breeding cycles of elite varieties in Thailand and India (Pandey and Rajatasereekul, 1999; Witcombe et al., 2003; Virk et al., 2005). There is potential for the approach to be applied in Tanzania (Nkonya et al., 1997), where the adoption of improved maize varieties in intermediate altitudes has been very low, compared with high altitude zones. Therefore, the adoption of improved maize varieties in intermediate altitudes would provide the potential to increase maize grain yields.

Newly released varieties should conform to farmers' production environments, so that farmers will choose to grow them and they should also impose no added input costs (Joshi and Witcombe, 1996). These varieties should be superior to existing ones in terms of maturity, grain yield, adaptability, stability and marketability (Witcombe et al., 2003; Virk et al., 2005; Derera et al., 2006). Very often such varieties are specific to culture, environment and socioeconomic aspects, as reported by Derera et al. (2006) in eastern Zimbabwe. Unfortunately, breeders working alone would not easily identify these traits. They should therefore engage with farmers in target environments.

The intermediate altitudes in Tanzania produce maize below their production potential due to multiple stresses that farmers can rank and thereby decide their breeding priorities. The present study therefore focused on that zone for greater impact to be realised by the maize breeding programme in the country. The study investigated farmers' perceptions and their implications to breeding for maize varieties that mature earlier and yield higher for intermediate altitudes in Tanzania. Special emphasis was placed on identifying traits that farmers prefer, and identifying production constraints and the need for widely adapted varieties.

2.2 Materials and methods

2.2.1 Study area

The study was conducted in Mbarali and Mbozi districts in the Mbeya region. Mbarali district lies between latitudes 7^0 and 9^0 S and longitudes 33^0 8' and 35^0 E. Mean annual rainfall ranges from 300-800 mm, with high, unpredictable distribution and the altitude ranging from 750 to 1200 metres above sea level (m.a.s.l.). Mbozi district lies between latitudes of 8^0 and 9^0 S and longitudes 32^0 7' and 33^0 2' E. The East African Rift Valley divides Mbozi district into lowland (Rift Valley or dry part) and highland (wet) areas. Mean annual rainfall ranges from 750 - 1200 and 1350 - 1550 mm for rift valley and highlands, respectively. The district lies between 900 and 2750 m.a.s.l. The rift valley (900 - 1400m.a.s.l) has deep, well drained volcanic soils, whereas the highlands (1400 - 2750 m.a.s.l.) have loamy and reddish soils with low natural fertility regeneration. The two districts have a unimodal rainfall, which falls between November and May.

The study sites comprised eight villages, four in each district and involved 214 maize farmers (Table 2.1). In order to record farmers' perceptions on agronomic and breeding aspects across the maize farming communities, the study was conducted in Mbarali in January, 2008, when the crop was at the vegetative stage, whereas in Mbozi, it was conducted in June, 2008, when the crop was at or near harvesting stage. For convenience and economic reasons the growth stages of these crops were considered, in order to gather data on crop management and on the aspects of grain yield and physiological maturity, and their components. All farmers were asked the same questions.

Table 2.1: Study sites and number of participants by gender

District	Village	Sex		Total
		Female	Male	
Mbarali	Itipingi	7	20	27
	Igomelo	5	16	21
	Utengule-Usangu	4	22	26
	Ruiwa	12	15	27
	SUB TOTAL	28	□□□1	101
Mbozi	Igamba	7	24	31
	Msia	11	10	21
	Msangano	17	14	31
	Chitete	8	22	30
	SUB TOTAL	43	70	113
	TOTAL	71	143	214

2.2.2 Criteria for selecting study sites

The criteria for selecting study sites were obtained jointly with district and community officials during a pre-survey tour. The following factors were considered:

- maize as an important crop, i.e. food, feed, and cash,
- intermediate altitudes (900-1600 m.a.s.l.), targeting a bit shorter maturity period compared with high altitude,
- logistical reasons, i.e. road accessibility, and

- rainfall of ≤1000 mm per annum.

2.2.3 Sampling procedures and experimental design

A pre-survey tour was conducted in each district before formal interviews were held to select study sites, gain an insight into situation analysis, test study tools, and get items for pair-wise matrix ranking. The districts and villages were obtained by purposive sampling. The sampling frame was maize farmers in the respective districts in which a total of 214 farmers selected from Mbarali (101) and Mbozi (113) were randomly sampled. Farmers were interviewed individually using questionnaires that constituted a formal survey. In each village, farmers who could be interviewed in the formal survey and those who could not were divided into three groups, based on their socio-economic status. Other criteria were not applicable, thus the poverty index was established as high, medium, and low income. Extension officers and community leaders assisted with the anonymous study, conducted with farmers at village offices. Grouping criteria included the ability to purchase agricultural inputs with the voucher system, the ability to educate their children, ownership of land, use of iron-sheet thatched houses, food security throughout the year and lenience in paying government levies and taxes, among others.

Items for cross validation were obtained from farmers and local officials after their views, obtained during the pre-survey stage of the study, were grouped into four categories and subjected to the three established socioeconomic strata above:

i) limitations to maize production,
ii) suggested solutions,
iii) uses of maize, and
iv) traits in the desired varieties.

Information from group discussions was evaluated across the three socioeconomic strata, using a pair-wise matrix sheet. The generated information was organised and used to validate the information from the formal survey. The information helped to check on the validity of individual responses, test the level of agreement, reliability, and repetition of the results from individual interviews. The scores given by respective social strata to the sub-factors was used to infer the agreement and disagreement among sub-factors. The closeness of scores among socioeconomic strata would imply some levels of agreement, whereas contrasting scores would suggest some disagreement among the socioeconomic strata. Furthermore, the validation helps to involve both quantitative and qualitative data, consequently maintaining the objectivity of the results of participatory rural appraisal (Clavarrino et al., 1995; Martin, 1995; Grenier, 1998; Bänziger et al., 2000). Very sensitive information would entail a low percentage in agreement (Clavarrino et al., 1985; Grenier, 1998). The native speaker to the respective communities was therefore included in a pre-survey team to deal with problems related to sensitive information.

2.2.4 Data collected

Farmers were questioned in Kiswahili, the national language, except in a few cases, where a native speaker to the community translated the survey items and the following data were collected during the formal survey:

- Socioeconomic characteristics of farmers;
- Farmers' training level;
- Crops grown in the study area, including the land utilised per crop;
- Categories of maize varieties;
- Agronomic features and crop management;
- Preferences of varieties by physiological maturity in months;
- Choice of characters and stage of crop growth considered important for earliness and high grain yield potential;
- The stay-green and rate of kernel dry-down characters;
- Accessibility to credit, extension and input services; and
- Major limitations to maize productivity and suggested solutions.

2.2.5 Data analysis

The questionnaire data was processed and analysed by the SPSS computer program (Version 15.0). Scores from group discussions were summarised from pair-wise matrix scores across all socio-economic categories in the eight villages and these were compared against SPSS output to validate the results of the entire study.

2.3 Results

2.3.1 Social features of respondents

Table 2.2 indicates socioeconomic features and the farming experience of the studied communities. Most of the respondents had a primary school education and their agricultural training was inadequate in equipping them with the necessary production skills. Male respondents were the most numerous. The results showed an average age of 41 years, with a mean farming experience of about 19 years.

Table 2.2: Socio-demographic characteristics and farming experience of the heads of households

Variable	Frequency	Percentage
Education		
None	7	3.3
Adult	7	3.3
Primary	184	86.0
O level	16	7.5
Total	**214**	**100.0**
Agric training		
Attended	61	28.5
No training	153	71.5
Total	**214**	**100.0**
Type of course attended		
Short course	58	95.1
Tour	1	1.6
Short and long course	2	3.3
Total	**61**	**100.0**
Sex of respondent		
Male	143	66.8
Female	71	33.2
Total	**214**	**100.0**
Mean age of respondents (41.1 years, No. = 214)		
Mean farming experience (18.7 years, No. = 214)		

2.3.2 Crops grown in the study area

Table 2.3 shows various crops that are grown and annual acreage (hectares) in the study area. Maize was a major crop, followed by rice, beans, groundnuts, coffee, sunflower, and sorghum. In terms of the area under crop, maize was, on average, second to coffee. A large area in the Mbozi district was under maize cultivation.

Table 2.3: Crops grown and mean annual acreage (hectares) in the study area

Crop per village or district	Percentage of crops grown	Mean annual acreage (acres)
Maize	31.4	0.84
Beans	17.5	0.46
Coffee	6.3	0.94
Sunflower	5.7	0.55
Banana	0.3	0.43
Cowpeas	0.6	0.20
Pumpkin	0.9	0.58
Sweet potato	0.9	0.46
Groundnuts	9.1	0.80
Onions	2.4	0.20
Tomato	1.6	0.14
Rice	17.8	0.78
Sorghum	4.4	0.75
Finger millet	1.0	0.32
Maize *district		
Mbarali		0.81 (47.2%)
Mbozi		0.88 (52.8%)
Total	100	0.84 (100.0%)

2.3.3 Type of maize cultivars grown, seeding rates and crop management

Table 2.4 shows that most farmers grew improved maize varieties. However, Mbarali district grew more unimproved varieties than Mbozi. Both districts used more or less improved varieties in equal proportions. At the village level, the use of both improved and local cultivars were reported only in Msangano and Chitete villages in Mbozi. The three villages of Igomelo, Igamba and Msia used improved varieties exclusively.

Table 2.4: Type of maize cultivar * district and village cross tabulation

		District										
		Mbarali					Mbozi					
		Village					Village					
		Igomelo	Untangle/Usangu	Itipingi	Ruiwa	Total (%)	Igamba	Msia	Msangano	Chitete	Total (%)	Overall total
Maize cultivar	Local (%)	0	5.1	8.9	7.5	21.5	0.5	1.4	6.5	5.1	13.6	35.0
	Improved (%)	9.8	7.0	2.8	5.1	24.8	12.6	7.9	4.7	3.3	28.5	53.3
	Local & improved (%)	0	0	0.9	0	0.9	1.4	0.5	3.3	5.6	10.7	11.7
Total village		9.8	12.1	12.6	12.6		14.5	9.8	14.5	14.0		100.0
Total district						47.2					52.8	100.0

Table 2.5 indicates the seeding rates per village and district. The seeding rates ranged from one to four seeds per hole. Utengule-Usangu, Ruiwa and Itipingi villages planted about an average of three seeds per hole, whereas in Igomelo, farmers consistently planted one seed. However, at the district level, Mbarali had a higher seeding density.

Table 2.5: Seeding rate by villages and districts

Village/district	No.	Minimum	Maximum	Mean	% of total No.	Std. deviation
Igomelo	21	1	1	1.00	9.8	0.000
Utengule -Usangu	26	1	4	2.50	12.1	0.648
Itipingi	27	2	4	2.52	12.6	0.643
Ruiwa	27	1	4	2.52	12.6	0.753
Igamba	31	1	2	1.42	14.5	0.502
Msia	21	1	3	1.90	9.8	0.436
Msangano	31	1	3	1.61	14.5	0.615
Chitete	30	1	3	2.17	14.0	0.461
District						
Mbarali	101	1	4	2.20	47.2	0.860
Mbozi	113	1	3	1.76	52.8	0.587
Total	214	1	4	1.97	100.0	0.759

Farmers in Mbozi applied twice as much fertiliser as those in Mbarali. Urea, Di-Ammonium Phosphate (DAP), Calcium Ammonium Nitrate (CAN) and Sulphate of Ammonia (SA) in that order were commonly used in basal application. Mbozi used DAP and Urea equally (about 27% each), followed by CAN. Mbarali, though, used less fertiliser, but the trend indicated that all fertiliser types were almost equally used. Organic manure was not in common usage in the study areas. Table 2.6 reveals that N fertilisers were commonly used in the study area. Table 2.7 summarises some factors on maize crop management. Whereas maize production in the study areas was predominantly rain fed, about 87% of farmers did not apply fertilisers. Farmers weeded their maize fields twice in both districts and about 66% of farmers applied pesticides.

Table 2.6: Percent of types of fertilisers used on maize in Mbarali and Mbozi districts

	Type of fertiliser (%)					
	DAP	CAN	SA	Urea	Organic	
District						Total (%)
Mbarali	6.8	8.4	5.8	11.5	2.1	34.6
Mbozi	27.2	11.5	0.5	26.5	0	65.4
Total	34	19.9	6.3	37.7	2.1	100

DAP = Diammonium Phosphate, CAN = Calcium Ammonium Nitrate, SA = Sulphate of Ammonia, Organic = manure.

2.3.4 Factors affecting maize productivity

Table 2.7 indicates the summary of some of the factors in the present study, which might affect maize productivity. Credit and extension services were found to be inadequate in the areas under study. Multiple linear regression (Table 2.7) indicated that age and sex of farmers, area under maize production, usage of fertilisers and fungicides were statistically significant but had negative coefficients. Education, agricultural training, plant spacing within rows, occurrences of pest and diseases, and access to credit and extension services had positive regression coefficients. However, education and pest infestation had higher standard errors for regression coefficients. Early maturity and plant spacing between rows had negative standardised beta coefficients. Only education, pest and disease occurrence, and access to credit and extension services had positive beta coefficients. Overall regression explained only 20% of the model.

Table 2.7: Factors affecting maize productivity in Mbarali and Mbozi districts by multiple linear regression and percentage

	Multiple linear regression				
Factor	Unstandardised coefficients		Standardised coefficients		
	β	Std. error	Beta	t	Sig.
Constant	22.498	5.617		4.005	0.000
Age	-0.061	0.031	-0.133	-1.978	0.049**
Sex	-1.405	0.847	-0.112	-1.659	0.099*
Education	0.313	2.094	0.009	0.150	0.881
Agricultural training	-0.617	0.920	-0.047	-0.670	0.503
Area under maize (hectares)	-1.357	0.310	-0.293	-4.373	0.000***
Type of maize variety	-1.780	0.914	-0.144	-1.947	0.053*
Spacing between rows (cm)	-0.003	0.022	-0.011	-0.156	0.876
Spacing between plants (cm)	0.006	0.029	0.014	0.193	0.847
Seed rate (no of seed per hole)	-0.199	0.593	-0.026	-0.336	0.737
Fertiliser use	-3.457	1.111	-0.283	-3.112	0.002**
Disease in the field	0.302	0.769	0.025	0.393	0.695
Pest occurrence in the field	0.656	2.359	0.018	0.278	0.781
Fungicide use	-1.401	0.858	-0.113	-1.632	0.104*
Earliness	-0.448	0.916	-0.036	-0.490	0.625
Access to credit	0.573	1.319	0.029	0.434	0.665
Access to extension service	0.854	0.866	0.068	0.985	0.326

Rainfed maize	87.4%
Animal power usage	81.8%
Twice weeding	81.3%
Fertiliser application	36.6%
No need of fertiliser	86.8%
Use of pesticides	66.4%
Trends of need of earlier cultivars	83.2%
No access to credit services	90.2%
No access to extension services	68.2%

$R_a^2 = 0.201$ ***, **, * = statistically significant at 1, 5, and 10%, No. = 214.

2.3.5 Need for early maturing and high grain yield varieties

Farmers were asked, according to their ecological zones, which type of maize varieties they would prefer based on maturity in months and adaptive traits (Table 2.8). About sixty-one percent of farmers preferred varieties that mature in 2 months, but this was more pronounced in Mbarali. Generally, Mbarali required earlier varieties than Mbozi. Mbarali required varieties that mature in 60 to 90 days, whereas Mbozi needed varieties of 60 to 150 days. Mbarali required varieties whose kernels dry-down faster; however, the overall need for maize varieties with this character was 61.2%. About 41 out of 66% of farmers who required varieties with a stay-green character were from Mbarali. Table 2.9 indicates that about 30% of farmers considered productivity of kernels per plant and per ear when selecting seed for planting in the next season. Almost the same percentage of the farmers selected seed at home-yard before shelling.

Table 2.8: Types of maize varieties farmers prefer by percent for calendar maturity, rapid kernel dry-down and stay-green character

District	Maturity (days)				Rapid dry-down			Stay-green		
	60 days	90 days	120 days	150 days	yes	no	NA	yes	no	Total
Mbarali)	34.1	10.7	2.3	0.0	32.2	14.5	0.5	40.7	6.5	47.2
Mbozi (%)	27.1	7.9	8.9	8.9	29.0	23.8	0.0	25.2	27.6	52.8
Total	61.2	18.7	11.2	8.9	61.2	38.3	0.5	65.9	34.1	100

NA = not applicable

Table 2.9: Characters and stage at which farmers selected for earliness of maize varieties and improved grain yield

Stage/character	Frequency	Percent
Before tasseling	1	0.5
Tasselling and silking	21	9.8
Fully grain fill	11	5.1
Harvesting day	26	12.1
Home-yard before shelling	71	33.2
After shelling	17	7.9
High ear and plant productivity	67	31.3
Total	214	100.0

2.3.6 Overall perceptions of farmers of different socioeconomic status

Results from the focused group discussion (Table 2.10) validated some of the information obtained from the formal survey. The overall cases were almost equal for limitations to maize production, uses of maize and the kind of traits in the varieties that farmers preferred. Respondents both agreed and disagreed on certain items across the socioeconomic strata. For instance, the respondents' responses tallied with some important sub-factors that commonly affect the entire maize farming community. With limitations to maize production, pests and lack of inputs were in common agreement across the three socioeconomic groups. Strengthening awareness was considered a common solution across the three groups. Regarding the uses of maize, cash, food and seed were in common agreement across socioeconomic groups. Early maturing genotypes were commonly cited as requirement in the new varieties that farmers desired. Furthermore, farmers' responses were associated with the level of income. For instance, consumer preferences increased with income, post-harvest pest resistance was less desired by the poorer group, varieties that used inputs efficiently was greatly needed by the poorer group, high yield was greatly needed by the wealthier group, credit and improved varieties were not greatly needed by the poorer group, whereas the problem of low soil fertility was very important among the wealthy group.

Poverty, drought, and lack of awareness were identified as the major limitations to maize production. The solutions were strengthening awareness, increasing the availability of improved, affordable and appropriate varieties, and early planting. Food, cash and seed were the most common uses of grain maize. Pest resistance in the field, high grain yield, and nitrogen use-efficient genotypes were the most desired traits in the varieties that farmers preferred.

Table 2.10: Overall perceptions of farmers from different socio-economic background

Factor	Sub factor	Scores/Socioeconomic category			
		Poor	Medium	Wealthy	Total
Limitations	Drought	40	31	30	101
	No inputs	22	22	20	64
	Low awareness	29	35	27	91
	Pests	14	15	14	43
	Poverty	40	33	40	113
	Soil infertility	10	5	16	31
	Unimproved varieties	13	27	20	60
	TOTAL	168	168	167	503
Solutions	Early planting	14	11	7	32
	Improved, affordable & appropriate varieties	11	15	15	41
	Credit union	3	6	7	16
	Strengthening awareness	20	16	19	55
	TOTAL	48	48	48	144
Use of grain maize	Cash	27	26	29	82
	Feed	1	4	2	7
	Food	43	40	43	126
	Food and cash	38	40	41	119
	Local brew	13	5	8	26
	Roasting/boiling	19	20	16	55
	Seed	27	31	29	87
	TOTAL	168	166	168	502
Desired varieties	Early maturity	26	22	25	73
	High grain yield	25	31	36	92
	Input use efficient	37	21	23	81
	Pests resistance (field)	33	36	27	96
	Pests resistance store)	18	22	23	63
	Standability	19	19	15	53
	Consumer qualities[a]	8	13	17	38
	TOTAL	166	164	166	496

[a] taste, pounding quality, aroma. Pest refers to disease, vermin, insects, weeds

2.4 Discussion

2.4.1 Socio-economy and importance of maize

From the results it is evident that maize is the source of livelihood for the communities in which the study was conducted. The importance of maize can be seen by acreage and the number of people engaged directly in maize production and usage of the crop for food and cash security. Therefore, any threat to this crop may negatively affect the livelihood of everyone in the study area and have an impact on the country at large. However, chronological and productive ages would be enough to supplement the inadequate agricultural training of the respondents. Despite divisions in socioeconomic strata in the study area indicated in Table 2.10**,** creating awareness about maize production and helping farmers to access appropriate and affordable elite maize hybrids would be pertinent solutions to raising maize productivity.

2.4.2 Preference of current varieties

Maize is mainly grown in the Mbozi district, where better use is made of improved varieties than in Mbarali. This is indicated by contrasts in other agronomic features related to maize production, such as higher seeding rate and lower utilisation of fertiliser in Mbarali than in Mbozi. These results would suggest differences in perceptions and requirements of crop varieties by the two districts, as was the case of maize in Zimbabwe (Derera et al., 2006) and rice in Mali (Efisue et al., 2008). The common application of the nitrogenous (N) fertilisers in the study area would suggest that N is a key nutrient in maize production, which agrees with previous reports (Lafitte, 1994). However, the different ecological and social set-up of the two districts would imply variety x N interaction, as Worku et al. (2007) and Bertin and Gallais (2000) reported in similar situations to the present study. The use of improved varieties in Mbozi and Mbarali, grown under low N conditions, would result in a low yield even if the varieties were improved. Problems in using seed from unimproved maize cultivars was covered in Table 2.10, where farmers from all socioeconomic groups indicated on the use of grain maize as seed.

The need for varieties with extended leaf chlorophyll concentration for the physiological maturity or stay-green (SG) trait was mainly expressed in Mbarali (Table 2.8) and, with low application of N in the same district in mind, this calls for the need to breed for such a trait and make varieties available in Mbarali and other areas experiencing similar conditions. Maize cultivars with the SG character have been reported to be tolerant to random late season abiotic stresses (Bänziger et al. 2000; Subedi and Ma, 2005). Their kernels have high N content at maturity and the forage may have high feed quality for ruminant livestock. They are therefore dual purpose cultivars. The SG varieties have extended effective grain filling duration, which would increase grain yield in short-season and intermediate altitude areas (Hunter, 1980), as in the sites of the present study.

The higher seeding rate recorded in Mbarali than in Mbozi suggests that maize may be more vulnerable in Mbarali, since planting many seeds may reduce the risk of losing plant stands from random stresses. Furthermore, farmers in the present study reported that local varieties were more resilient to local stresses, due to their lack of uniformity, than the improved ones, which are more uniform by definition. This perception would confirm the existence of multiple stresses in farmers' production environments in tropical maize. Varieties that tolerate or escape drought, which is a random stress, are important to the studied communities (Table 2.10). Breeding efforts should therefore consider traits that mitigate most multiple stresses and random stresses in particular. This suggests that stabilising final grain yield is a crucial challenge to maize breeders, since in most cases farmers chose grain yield and resistance of varieties to pests and diseases in the field. This is in agreement with Barah et al. (1981) and Witcombe (1988) who reported that the only factor relevant to the farmers' choice of seed is the stability of a variety. However, stability and adaptability may not necessarily imply high performance (Barah et al., 1981), while stability has a time component, adaptability connotes a spatial component.

The results from group discussion partly tallied with the results from the formal survey. This was evident in the usage of inputs such as seeds of improved varieties, chemical inputs, uses of maize and need for training, among others. Although the group discussion exercise of the present study indicated some agreement for items across the poor, medium, and wealthy strata (Table 2.10), Heisey and Edmeades (1999) summarised several studies and found contrary results. The authors found them inconclusive across crops and across regions of the globe when economic levels were associated with gradients of marginality of geographical areas.

Previous studies indicated that farmers in the SHZ preferred flint varieties with white kernel types, which were easy to pound and mill (Lyimo, 2006). However, the present study (Table 2.10) found that the choice of kernel type was not relevant to the study areas, suggesting that consumer preference would be relevant where grain yields were stabilised. Furthermore, results in the same table indicated that the choice for kernel pounding and milling qualities in varieties that farmers preferred decreased with the farmers' income level. Kernel productivity per ear and per plant were both important criteria for selecting varieties with high grain yield potential in the study area. Ear size (i.e. kernel numbers per row, row numbers per ear, big kernel size) were common characteristics considered by farmers in variety selection. Large cobs and prolificacy were also among the criteria for selection of maize varieties in the SHZ. Preference for varieties with big and heavier kernel weight in the SHZ, as Gibson et al. (2005) reported, could probably be because such varieties sell well, as they quickly fill the weighing containers. In the absence of balances, grain is usually sold by volume on the local market. These findings should be incorporated in variety design and selection criteria for the maize improvement programme in Tanzania.

2.4.3 Need for earlier maturing varieties

Maize varieties that are early maturing and locally adapted are in great demand in the SHZ. Table 2.8 indicated that the demand for such varieties decreased as the maturity period of the variety increased. More than 80% of farmers reported that the trend for the demand for early maturing cultivars increased in space and time (Table 2.7). However, farmers reported in previous studies that early maturing cultivars had small grain size (Gibson et al., 2005), which required improvement. Because grain size is a grain yield component, small grain size will be a penalty to final grain yield. This supports the results of the present study, where most farmers demanded varieties with large and many kernels which dry-down fast (Tables 2.8 and 2.9). Cross (1985) and Cross and Kabir (1989 found that fast rate of kernel dry-down was inversely correlated to grain yield components. The early maturity trait was found to be a priority elsewhere (de Groote et al., 2002). In Kenya, grain yield components were recorded as a second criterion to earliness, followed by breeding for stress tolerance and desirable general plant morphology. Similar results and trends have been found in maize in eastern India (Virk et al., 2005; Witcombe et al., 2003), in rice in Mali (Efisue et al., 2008) and maize in the SHZ (Lyimo, 2006). However, the large number of requests by farmers in Mbarali for varieties with fast kernel dry-down and SG characters suggests that short season varieties are appropriate in this area. It is anticipated that the ruminant livestock would benefit from increased forage due to the SG character. This supported the high demand by farmers in the Mbarali district for early maturing varieties than by farmers in Mbozi (Table 2.8). The breeding challenge would be to increase and maintain high kernel numbers into early maturing maize cultivars in areas where the seasons are becoming shorter such as the present study areas.

2.4.4 Use of fertilisers and pesticides

Only 37% of farmers interviewed applied fertilisers to maize fields but at Mbozi fertilisers were applied twice as much as in Mbarali. However, about 87% of farmers reported that fertilisers were not needed. The fact that maize was about 90% rain-fed and about 80% of farmers, on average, weeded their maize twice (Table 2.7), suggest that soil N, which is the key nutrient in maize production, is limited in the study area. Both the application of fertilisers and pesticides were statistically significant. They displayed negative beta regression coefficients, which showed that if these inputs were not used, they could reduce maize productivity. Also, the type of variety would determine the response to agricultural inputs, since the variety was statistically significant. This is in agreement with Echarte et al. (2008) who reported that improved varieties were more responsive to chemical input than unimproved cultivars. However, the findings of the present study suggest that current perceptions of maize farmers with regard to the application of fertilisers may continue to compound the problem of low maize productivity in Tanzania. Nkonya et al. (1997) reported that the rate of application of N for maize in Tanzania is inversely related to the size of the farm land. This would further be worsened by the very nature of N, which is highly volatile in the farmers' production environment. The need for fertiliser and improved varieties was high in villages in the relatively elevated altitudes of Msia and Igamba in the escarpment in Mbozi district and also in Igomelo village in the Mbarali district, where maize is irrigated (Table 2.4). However, the present study (in a later finding), could not establish reasons why only Igomelo village in Mbarali district, which irrigates its maize, used more seeds from improved varieties.

2.4.5 Access to credit and extension information

The results of this study indicated that farmers had limited access to credit and extension services (Table 2.7). Previous studies under similar situations to the present study reported low productivity of agricultural systems. Communities with adequate training may be willing to try new technologies, provided such innovations do not confuse farmers and introduce additional costs to the existing farming system (Sall et al., 2000; Huan et al., 2005), whereas an oversimplification of technology may diminish the intended benefits (Hu et al., 2007). Although farmers have been known to accept additional costs for profitable innovations, the literature barely supports this assertion. The ability of communities in the present study to improve maize productivity is questionable, considering the inadequate training of farmers (Table 2.2). Although improved technologies should not fully replace existing technology (Hu et al., 2007), they might, in the communities under the present study, due to lack of credit, awareness (Table 2.7), and training (Table 2.2). Communities that are unaware may have no choice of new technology. Communities that are aware of unnecessary external inputs could reduce these without affecting the profitability of the current farming system (Huan et al., 2005). In China, large application of N fertilisers on rice resulted in low N-use efficiency and minimal environmental pollution with susceptibility of the crop to insect and disease pests, plus lodging, thereby creating a dependence on pesticides (Lu et al., 2004; Hu et al., 2007). Where farmers had alternative sources of income or their livelihoods depended essentially on a particular commodity in relation to newly developed technology, the adoption of the new technology was destined to fail (Hu et al., 2007). Hu et al. (2007) partly corroborated the present study in Msia and Igamba villages, where coffee is a cash crop, and in Ruiwa village, where rice is a cash crop (results not shown). Credit availability may promote the purchase of seeds of improved varieties and complementary inputs (Hu et al., 2007). Put simply, adoption may correlate positively with the experience of the farmer and available information.

2.4.6 Multiple linear regression

The results from Linear Regression Analysis, after screening for a few important factors (Table 2.7) showed that land under maize cultivation, fertiliser use, and age of respondents influenced maize productivity significantly ($p \leq 0.05$). The type of maize variety and the gender of the respondents significantly affected maize productivity ($p \leq 0.10$), with use of fungicides having slight influence on maize productivity. Nevertheless, all these statistically significant factors had negative regression coefficients, which indicate that an increase in such factors could positively affect maize productivity. Access to extension and credit services, use of varieties that are resistant to pests and diseases, and increased education might improve maize productivity, since these factors had positive regression coefficients. However, the adjusted coefficient of determination (R_a^2) was only 20.1%, indicating that only approximately 20% of the total variation could be explained by the variables specified in the model of the present study. This might be attributed to the presence of multicolinearity among the many factors that influenced maize productivity in the farmers' production environment (Lafitte, 1994). The targeting of breeding strategies in these areas may be complicated by the prevalence of multiple stresses and the reduction of inputs may reduce rates of adoption of new technologies (Lorenzana et al., 2008; Carena et al., 2009; Romay et al., 2010). In such production systems, Simmonds (1991) suggested low regressions, as it was found in the present study.

2.4.7 Conclusions and Implications for maize breeding

The results of the present study indicated that farmers require from maize breeders traits of a quantitative nature, such as the components of grain yield, physiological maturity and adaptation in the varieties they prefer. Traits related to grain yield components involved number of kernels per ear, number of kernels per plant and number of ears per plant. Calendar physiological maturity, especially of the extra early category, was highly required in the study areas. There was a big demand for adaptive traits, such as stay-green and rapid rate of kernel dry-down in Mbarali, demonstrating that this district has a harsher maize production environment than that of Mbozi. The preferred traits in the study areas were needed most where farmers used unimproved varieties, applied anything from suboptimal amounts to no amount at all of fertiliser, especially N, and experienced drought, thus posing challenges to the maize breeder when it came to providing suitable cultivars for the study areas.

Overall results indicated that maize productivity could be enhanced by improving both genetics (varieties) and management of the crops by investing in fertiliser, credit schemes, and training and education among other factors. The 'idealised' variety identified in the study is the one that yielded higher, matured earlier (≤140 days), stayed green longer and whose kernels dried down faster upon physiological maturity, and were tolerant to local stresses.

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Chapter 3:

Generation mean analysis of leaf chlorophyll concentration from mid-silking to physiological maturity in three tropical maize inbred lines under low and high nitrogen conditions

Abstract

Genetic control of leaf chlorophyll concentration (LCC) in tropical maize has not been established, especially at different reproductive growth stages under low and high N. This study was therefore conducted to identify the genetic effects that govern LCC, from mid-silking to physiological maturity under high and low N conditions. The F_1 crosses between contrasting inbreds (T20 with low LCC; C58 and N_G8 with high LCC) were advanced to F_2 and back-crossed to both parents. All generations of each cross were evaluated under 120 (HN) and 60 kg N ha^{-1} (LN) with two replications, in Tanzania. Mid-parent heterosis for LCC increased with growth stages under both N conditions but it was more pronounced under LN. Generally, genetic effects for LCC were easily estimable under HN than LN. A general trend observed was that additive gene effects decreased with stage of grain filling, whereas dominance effects increased, irrespective of the genotype and environment. Overall, additive effects accounted for about 79% and 41% of total genetic variation in the cross T20 x C58 under HN and LN, respectively. In sharp contrast, additive and dominance effects contributed about 40% each in the cross T20 x N_G8 under HN, whereas dominance effects were preponderant (70%) under LN in the cross T20 x N_G8. Additive x dominance digenic epistatic effects were great in the cross T20 x N_G8 under both N regimes. Overall, the ratio of fixable (additive plus additive x additive) to the non-fixable (dominance plus additive x dominance, and dominance x dominance) genetic effects was 74% to 26% under HN, and 35% to 65% under LN for the cross T20 x C58, while for the cross T20 x N_G8, the ratio was 37% to 63% under HN, and 20% to 80% under LN. The trend observed suggest that fixable effects are preponderant under HN in one cross, while non-fixable effects prevailed under the LN in the other cross.

Keywords: genetic effects, grain filling stages, leaf chlorophyll concentration, nitrogen regimes, tropical maize

3.1 Introduction

The relationship between the leaf chlorophyll concentration (LCC) and final grain yield in maize is appreciated, yet genetic control of this character has not been resolved, especially under contrasting N conditions in tropical maize. The leaf chlorophyll concentration relates directly to the amounts of plant and soil nitrogen (N) (Hawkins et al., 2007). Thus genotypes which retain high LCC until physiological maturity have been recognised to contain high N and water in their leaves (Thomas and Smart, 1993), such that leaf chlorophyll would be a direct measure of the stay-green (SG) character. If the inheritance and genetic control of the SG character were unravelled, a strategy to breed new maize cultivars that used N efficiently could be developed. Such cultivars, if identified, would be really useful, particularly in Tanzania, where the area under maize production related inversely to the amount of fertiliser applied by farmers (Nkonya et al., 1997; Lyimo, 2006). In the Southern Highlands Zone (SHZ), where soils are deteriorating fast (Bisanda et al., 1998), continuous application of suboptimal fertiliser rates on impoverished soils might alter the amounts of nutrients available per plant, thus perpetuating poor grain yields. Despite N being a key nutrient in maize production in the SHZ and the significance of maize to the livelihoods of Tanzanians, no systematic breeding studies have been reported that incorporate key traits that condition the use of N in maize improvement. Worku et al. (2007) reported that small-scale maize farmers in sub-Saharan Africa apply less than 20 kg N ha^{-1}. It is therefore crucial to comprehensively study the inheritance of the LCC character in tropical maize genotypes, especially under low N conditions, because farmers apply less N to maize, soil N is volatile, the soils are inherently deficient of N, and prices for N fertilisers are higher than farmers can afford.

Research questions to bridge the paucity of information on the inheritance of the LCC character would be aimed at confirming whether maize varieties that stay-green for a longer period require additional N nutrients or not. A similar question had been posed by previous researchers (Joshi et al., 2007) though without a conclusive research answer. Such a correlation would be important in light of improvements made to maize cultivars that use N efficiently. Random stresses that affect maize at and/or shortly before and after the grain-filling stages require use of the functional extended LCC (SG) character and the traits therein. However, studies of the genetic control of traits under the SG character for such periods to physiological maturity have not been reported. Put simply, the relationship between the SG trait and the LCC character in tropical maize has not been studied before.

Nitrogen that is used for kernel development in maize comes from the soil and re-mobilised vegetative tissues and supports the stay-green trait in highly productive cultivars. The SG phenotypes exhibit higher water and chlorophyll contents in the leaves at maturity According to Robson et al. (2001), the N content of kernels is higher in SG genotypes than senescent cultivars. However, with regards to such genotypes, no studies have confirmed correlation between grain yield and quality of forage for use as feed by ruminant livestock. Robson et al. (2001) reported such cultivars to have a higher grain yield than senescent ones, especially in stressful environments. The SG cultivars have strong root systems that allow prolonged N uptake during grain filling (Rajcan and Tollenaar, 1999), pointing to tolerance to late-season random stresses. These cultivars have been reported to minimise foliar diseases (Robinson, 1996) and heat and drought stresses, when compared to senescent cultivars (Earl and Tollenaar, 1997; Bänziger et al., 2000; Joshi et al., 2007). Lyimo (2006) reported that in Tanzania, the intermediate maize is hit hard by more stresses than the high-altitude maize. Therefore, maize cultivars with the SG character would help improve yield and general stress tolerance in the intermediate-altitude maize.

The SG trait would be important in tropical maize that is produced under harsh conditions. In tropical areas, the seasons are getting shorter and the rains are becoming unpredictable. Maize genotypes in shorter seasons are source limited such that incorporating the SG character in these genotypes would improve grain yields for these areas. In tropical areas where drought and low soil-N are rampant and erratic, leaf senescence is severe at grain filling stages (Khanna-Choppra and Maheswari, 1998; Bänziger et al., 2000; Subedi and Ma, 2005), and this may compromise grain yield. In an earlier study, Tollenaar and Daynard (1978a) counter-argued that green husk cover might negate the effects of leaf defoliation by supplying the developing ear with carbohydrates. Other researchers reported on the switching role of stalk to supply the sink with the assimilates when random stresses cause leaf senescence in maize (Duncan et al., 1965; Cavalieri and Smith, 1985; Willman et al., 1987) but still grain yield is compromised once green leaf area has been reduced before grain filling has been completed. The SG character in maize is highly influenced by genotype x environment interaction (Robson et al., 2001). More specifically, it has been found that genotype x N interaction is due to variation in low N and that N metabolism plays a more major role in low N than it does in optimum N conditions (Bertin and Gallais, 2000), this being the root basis of G x N interaction. Mahalakshmi and Bidinger (2002), working on sorghum, indicated that the SG trait in sorghum is highly variable and care must be taken when comparing varieties that widely differ in flowering dates, especially under severe stress. Time series following flowering affect LCC due to differential accumulation of dry matter. However, Subedi and Ma (2005) reported on the limited knowledge of the physiology and inheritance of the extended functional LCC (SG) trait. This study therefore aims to establish the role of additive and non-additive gene effects governing LCC trait under LN and HN conditions in tropical maize genotypes.

It is also crucial to establish whether different gene effects control LCC at different growth stages under stress and non-stress conditions. The knowledge would be useful in devising strategies for improving this trait in tropical populations under LN and HN conditions. Breeders, for example, would like to know the stages at which the trait is highly heritable, to focus selection — or whether selection would be effective at any stage between silking and physiological maturity. A survey of the literature does not yield any results regarding the actual mode of inheritance at different stages. However it has been shown that LCC stabilises more from the periods of silking to physiological maturity than during the vegetative stages (Martinez and Guiamet, 2004; Subedi and Ma, 2005), suggesting that breeders should select during this period. Elsewhere, the chlorophyll concentration, as established by readings from the Chlorophyll Meter (Model SPAD-502 Camera Minolta Co. Ltd., Japan), have been reported to correlate highly with the status of N in plants and soils, indicating that inheritance of the trait might also be different under different soil N conditions. However, SPAD-502 gives only total chlorophyll (a and b) (Martinez and Guiamet, 2004) for both stressed and stress-free N conditions. Alternative methods to SPAD values based on silking stage as reference point include: leaf areas of Montigomery (1911), Daynard (1972), and Khanna-Choppra and Maheswari (1998); leaf area under greenness of Joshi et al. (2007); and leaf score for greenness of Bänziger et al. (2000). The high and significant correlations of SPAD values with leaf N indices using such alternative methods to estimate LCC validate the use of SPAD-502 Chlorophyll Meter to quantify plant and soil-N. The meter is a relatively recent invention, and is a non-destructive technology that quickly, efficiently and economically estimates the LCC character. It was therefore used in the current study.

When measuring LCC, all experimental conditions and instructions for use of the chlorophyll meter should be observed in order to obtain precise SPAD values. If these conditions are observed, the meter readings and results of plant and soil N status should tally. This should be noted and followed, because chlorophyll pigment is sensitive to handling conditions before and during analysis (Yadava, 1986; Martinez and Guiamet, 2004). Martinez and Guiamet (2004) and Binder et al. (2000) added that the SPAD-502 readings may be influenced by irradiance, leaf water status, time of measurement (i.e. morning or afternoon), genotype differences, leaf diseases, stage of plant growth, and meter differences. In maize, the same field conditions, whether under high or low management regimes, should be the only variable while the non-treatment variables are kept optimum (Hawkins et al., 2007). This demonstrates that chlorophyll stability could be highly influenced by genetic, developmental and environmental factors. Thus one must be cautious when taking and interpreting the SPAD values (Piekielek et al., 1995). The meter is an effective and precise tool for measuring LCC and since it is non-destructive, it allows for the collection of other data on the same plants.

Considering that little is known about the inheritance of the LCC character, the small amount of literature that does exist supports the polygenic nature of the character, but the estimation of individual genes cannot be relevant for the character. Therefore, genetic methods that detect and estimate the amount and type of genetic effects rather than the individual genes become relevant. Generation mean analysis (GMA) has been used to detect the genetic effects of quantitatively inherited traits in maize and other crops (Azizi et al., 2006; Checa et al., 2006; Smith et al., 2009; Shashkumar et al., 2010). Generation mean analysis is effective in partitioning the non-additive effects into dominance and different types of epistasis. Knowledge of different types of epistasis is crucial in crops like maize, where heterosis is an important phenomenon in improving grain yield.

The present study was begun to determine the inheritance of LCC in maize genotypes at different growth stages, from mid-silking to physiological maturity, under low- and high-N conditions. The hypotheses tested were that the N level does not affect the gene action and inheritance of LCC at:

i) 50% silking,

ii) milk,
iii) dent,
iv) physiological maturity stages, and
v) average across these growth stages.

3.2 Materials and methods

3.2.1 Germplasm and generations involved in the study

Three inbred lines contrasting for the LCC character were selected during screening in season 2007/08. The F_1 crosses were made as follows: T20 (low LCC) by C58 (high LCC) i.e. (*UYL 15-11-1-8-5)* by *MAS[MSR/312]-117-2-2-1-B*5/MAS[202/312]-86-1-3-1-B*4)*), and T20 (low LCC) by N_G8 (high LCC) i.e. *(UYL 15-11-1-8-5)* by *(TZE-Y Pop Co S6 Inb 62-3-3*)). The F_1 from the two contrasting parents for the LCC character was advanced to F_2, and then back-crossed to the respective parents to generate BCP_1 and BCP_2 to constitute six generations that were used in the study. Inbred T20 was a common female parent to both crosses. This parent is a locally adapted, high yielding commercial inbred line with high combining ability for early maturity. Inbred lines C58 and N_G8 are exotic, confirmed to have high SPAD values during screening phase of the present study. The three inbred lines are resistant to foliar diseases that may affect the SPAD values.

3.2.2 Experimental design and management

The trials were conducted at the Inyala Agricultural Training Institute (slope <1%; 1520 m.a.s.l.; S08°51.011' and E033°38.227') in Mbeya Rural district in Tanzania. Two crop harvests were obtained with supplemented irrigation water in dry and wet seasons in 2009. The same field plots were maintained throughout the study. The site is generally free from diseases so the Southern Highlands Maize Improvement Programme uses it for trials of grain yield potential. The two environments were 120 and 60 kg N ha^{-1}, which mimic the recommended fertiliser rates (or high N) and reduced fertiliser rates that are close to those amounts of N applied by farmers, respectively. Phosphorus was applied at the rate of 30 kg P ha^{-1} in the form of P_2O_5. Six generations of each cross were planted in two replications under two N regimes in two-row plots for non-segregating generations (P_1, P_2, and F_1) to make 36 plants, and 7 row plots for segregating generations (F_2, BCP_1, and BCP_2) to make 126 plants if all plants were available during data collection (Table 3.1). Seeds of all the generations were planted in 5.1 m plots at 75 cm between and 30 cm within rows. The experiments were laid out in the field in a randomised complete block design, with two replications. Prior to planting, the soils were sampled and subjected to full standard analysis at the ARI-Uyole Soil Science Laboratory which enabled to locate study sites for high and low N. Irrigation water was also tested.

Table 3.1: Number of plants evaluated in each generation over two seasons and two N regimes in Tanzania

Cross	Season	N regime	Replication	Number of plants in each generation					
				P_1	P_2	F_1	F_2	BCP_1	BCP_2
T20 x C58	WS09	HN	1	30	27	36	125	126	125
			2	35	33	36	126	125	123
			Total	65	60	72	251	251	248
		LN	1	34	30	36	123	125	119
			2	30	35	36	118	123	123
			Total	64	65	72	241	248	242
	DS09	HN	1	27	32	31	122	119	122
			2	28	33	31	120	121	122
			Total	55	65	62	242	240	244
		LN	1	30	32	36	120	124	126
			2	30	33	31	126	123	120
			Total	60	65	67	246	247	246
T20 x N_G8	WS09	HN	1	32	22	35	114	122	111
			2	30	33	33	115	126	120
			Total	62	55	68	229	248	231
		LN	1	31	22	36	118	122	117
			2	30	27	34	112	110	90
			Total	61	49	70	230	232	207
	DS09	HN	1	25	32	33	117	112	121
			2	29	30	30	113	120	114
			Total	54	62	63	230	232	235
		LN	1	25	33	34	109	114	125
			2	28	29	30	109	113	119
			Total	53	62	64	218	227	244

WS09 = wet season year 2009, DS09 = dry season year 2009, HN = high nitrogen fertiliser application rate (120 kg N ha^{-1}), and LN = low nitrogen fertiliser application rate (60 kg N ha^{-1})

3.2.3 Measuring the traits

The data were collected for the LCC character at four intervals of 14 calendar days. These intervals were considered as separate traits since the LCC character is affected by time. The mid-silking calendar days (SD) stage was set as a reference point to collect the LCC data in SPAD values. Silking was considered when the silk had extruded by 0.5-1.0 cm. The data were recorded on individual plants for each replication after each generation had reached that stage using the Chlorophyll Meter (SPAD-502 Camera Minolta Co. Ltd., Japan) (see Figure 3.1) from the second leaf below the flag leaf and the means of the SPAD values were obtained from replications. Such a leaf was assumed to be relatively free from mechanical injury and shading that would affect SPAD values. The data were taken between 0930 and 1130 a.m. The leaf was cleared of dust and water film before the recording of SPAD data. The instrument was protected from direct sunlight as recommended by the manufacturer. The data were recorded at three points: top, middle and at the base of the leaf, and averaged to get a single value per plant. The SPAD values were recorded following the six reproductive stages in maize denoted as R with each stage regarded at a seven-day interval (Lafitte, 1994; Hawkins et al., 2007), which meant after every two weeks with regards to this study. The SPAD values at the mid-silking stage reflected R_1 and were recorded as LCC_1), R_2 = blister stage, R_3 = milk stage (LCC_2), R_4 = dough stage, R_5 = dent stage (LCC_3) and R_6 = physiological maturity, thus LCC_4 was recorded in the 7th week from the mid-silking stage. The SPAD values at the four growth stages and an average of the values for these stages were considered as five separate traits for the LCC character for generations of crosses T20 x C58 and T20 x N_G8.

Figure 3.1: Chlorophyll Meter (Model SPAD-502 Camera Minolta Co. Ltd., Japan)

3.2.4 Data analysis

Genetic assumptions for generation mean analyses were observed as follows:

i) all segregating genes are located in one parent,
ii) responsible genes are not linked,
iii) all responsible genes have equal effects with respect to the character under study,
iv) no epistasis,
v) no dominance, and
vi) no genotype x environment interaction (Lande, 1981; Mather and Jinks, 1977; Wright, 1968).

The data were analysed in SAS using the PROC GLM and PROC REG procedures. First, the overall model was subjected to analysis to determine the significance of main and interaction effects:

Y = replication + generation + nitrogen + season + generation * nitrogen + generation * season + generation * nitrogen * season + error.

Where Y = overall mean for trait.

The replications and seasons were considered random, while generations and nitrogen levels were fixed effects. The generation mean analyses were then performed in SAS, using

PROC GLM and PROC REG codes as described by Kang (1994). The following model was used:

$$Y = m + \alpha a + \beta d + \alpha^2 aa + \alpha\beta ad + \beta^2 dd$$

Where Y = generation mean, m = mean of the F∞ generation and intercept values defined by Van der Veen (1959) as representing all effects common to every genotype, such as the effects of genes that are monomorphic and fixed, the common effects of the environment and the average effects of genes showing polymorphism,

a = pooled additive effects (homozygote loci),

d = pooled dominance effects (heterozygote loci),

aa = additive x additive (homozygote x homozygote) interactions,

ad = additive x dominance (homozygote x heterozygote) interactions, and

dd = dominance x dominance (heterozygote x heterozygote) interactions.

α, β, α^2, β^2 = coefficients associated with genetic effects a, d, aa, ad, and dd, respectively.

Separation of means was performed using the LSD procedure for pair-wise mean comparisons ($p \leq 0.05$) (Steel and Torrie, 1980; Neter et al., 1996) in SAS. Frequency distribution histograms of the traits for the segregating generations (F_2, BCP_1 and BCP_2) were performed in MS Excel 2007 software to check and confirm whether the traits were under polygenic control. The recurrent parents (P_1 and P_2) were plotted against the segregating generations to illustrate existence of transgressive segregants for the traits.

3.3 Results

3.3.1 ANOVA, mean separation of generations and mid-parent heterosis

The nitrogen regime was statistically significant across all the grain filling stages. The generation means were significant for LCC under all N regimes (Table 3.2). The R^2 values were less than 50% at maturity stage in generations of cross T20 x C58 and at 50% silking and dent stages in cross T20 x N_G8. Both parents (P_1 and P_2) were not significantly different ($LSD_{0.05}$) at all grain filling stages under low N for both generations of crosses T20 x C58 and T20 x N_G8, hence the data were not submitted for GMA, as the criteria for contrasting parents was not met under LN conditions. High N trials had statistically significant parents for all growth stages, except at the physiological maturity stage in generations of cross T20 x C58.

Generally, the HN trial had higher mean values than LN. The SPAD values peaked at the milk stage and began to drop at the dent stage and became smaller at maturity than at the dent stage, regardless of generations of crosses and N conditions. However, the values started to drop earlier at the milk stage under LN conditions in T20 x C58. The differences for SPAD values between P_1 and P_2 faded with age and occurred earlier under LN, compared with HN. Mid-parent heterosis built up with grain filling stages, increasing sharply at the dent stage and was highest at the maturity stage in all conditions, although this was more pronounced under LN than in HN. Parent one was consistently lower throughout the experiments, except at the maturity stage for T20 x C58 in LN, and at the dent stage in LN where it equalled P_2 for T20 x N_G8.

Table 3.2: ANOVA summary on the statistical significance (Pr>F) of grain filling stages for maize crosses T20 x C58 and T20 x N_G8 over two regimes of N and two seasons

		Cross T20xC58					Cross T20xN_G8				
		Grain filling stage					Grain filling stage				
		50%SD	Milk	Dent	Maturity	Average	50%SD	Milk	Dent	Maturity	Average
Source	DF	Pr>F					Pr>F				
Nitrogen	1	0.052	0.0018*	0.0029*	0.0017*	0.0004*	0.0417*	0.0056*	0.0095*	0.0025*	0.0005*
Generation	5	0.0115*	0.003*	0.0016*	0.0167*	0.0005*	0.0051*	0.0001*	0.0017*	<0.0001**	<0.0001**
Season	1	0.389	0.351	0.763	0.649	0.607	0.142	0.0138*	0.777	0.305	0.345
Gen x N	5	0.662	0.306	0.665	0.311	0.343	0.155	0.375	0.242	0.344	0.109
Gen x season	5	0.766	0.698	0.671	0.321	0.735	0.438	0.912	0.305	0.847	0.597
Gen x season x N	6	0.0532	0.0339*	0.0838*	0.0825	0.0295*	0.158	0.588	0.845	0.896	0.769
Error mean square	22	26.59	22.09	40.02	43.85	19.07	26.42	26.59	37.82	37.50	16.90
R^2(%)		76.6	69.8	67.1	32.1	73.5	43.8	91.2	30.5	84.7	59.7
CV (%)		13.57	12.06	17.86	24.78	12.54	13.50	12.75	16.58	21.47	11.40

**, *, Data statistically significant at p≤0.0001, 0.05, respectively. Source = source of variation, SD = silking date, D.F = degrees of freedom

Table 3.3: Least significant differences in pair-wise means comparison tests and heterosis for leaf chlorophyll concentrations across the grain-filling stages of six generations for maize crosses T20 x C58 and T20 x N_G8 evaluated over two regimes of N

a) Cross T20 x C58

Cross T20 x C58														
50% Silking stage			Milk stage			Dent stage			Maturity stage			Average of stages		
Generation	Mean		Generation	Mean		Generation	Mean		Generation	Mean		Generation	Mean	
						High N								
P_2	46.2	A	P_2	45.8	A	BCP_2	44.0	A	BCP_2	34.2	A	BCP_2	40.9	A
BCP_2	40.7	AB	BCP_2	44.7	A	F_1	41.1	A	F_2	32.8	A	P_2	40.4	A
F_1	39.5	AB	F_2	42.6	A	F_2	40.2	A	F_1	31.8	A	F_2	38.8	A
F_2	39.2	AB	F_1	41.5	A	P_2	39.2	A	BCP_1	31.7	A	F_1	38.5	A
BCP_1	38.9	AB	BCP_1	40.9	A	BCP_1	36.3	AB	P_2	29.9	AB	BCP_1	36.9	A
P_1	32.5	B	P_1	32.8	B	P_1	30.0	B	P_1	20.4	C	P_1	28.9	B
MPH (%)	0.3			5.6			27.1			26.4			11.2	
						Low N								
BCP_2	41.3	A	BCP_2	45.4	A	BCP_2	40.7	A	F_1	29.9	A	BCP_2	39.2	A
P_2	38.2	AB	P_2	38.8	AB	F_1	37.4	AB	BCP_2	29.2	AB	F_1	35.5	AB
BCP_1	36.6	AB	F_1	37.7	AB	F_2	33.0	ABC	BCP_1	24.1	AB	BCP_1	31.4	BC
F_2	35.6	AB	F_2	34.2	B	BCP_1	32.5	ABC	P_1	19.4	AB	P_2	30.5	BC
F_1	35.6	AB	BCP_1	32.5	B	P_2	26.4	BC	P_2	18.6	B	F_2	30.3	BC
P_1	31.3	B	P_1	30.9	B	P_1	24.1	C	F_2	18.5	B	P_1	26.4	C
MPH (%)	2.4			8.1			61.2			57.6			24.7	

Means with the same letter in the same column for respective N regime and generation are not significantly different. α= 0.05, 17 error degrees of freedom.
MPH = mid parent heterosis = $[(F_1\text{-MP})/ (\text{MP})]*100$

b) Cross T20 x N_G8

Cross T20 x N_G8

50% Silking stage			Milk stage			Dent stage			Maturity stage			Average of stages		
Generation	Mean		Generation	Mean		Generation	Mean		Generation	Mean		Generation	Mean	
						High N								
P_2	46.0	A	P_2	47.3	A	F_1	46.6	A	F_1	41.4	A	F_1	44.6	A
F_1	43.1	AB	F_1	47.3	A	P_2	41.2	A	BCP_2	35.9	AB	BCP_1	40.6	AB
BCP_1	42.9	AB	BCP_2	45.9	A	F_2	40.8	A	BCP_1	34.7	AB	P_2	40.6	AB
F_2	37.6	ABC	BCP_1	42.9	AB	BCP_1	40.5	A	F_2	30.6	B	BCP_2	39.2	B
BCP_2	36.0	BC	F_2	39.4	B	BCP_2	39.0	A	P_2	27.7	B	F_2	37.1	B
P_1	32.3	C	P_1	33.3	C	P_1	29.5	B	P_1	18.8	C	P_1	28.7	C
MPH (%)	10.1			17.4			31.8			78.0			28.9	
						Low N								
F_1	40.8	A	BCP_2	46.6	A	F_1	43.4	A	F_1	38.1	A	F_1	41.5	A
BCP_2	40.4	A	F_1	43.7	AB	BCP_2	40.7	AB	BCP_2	27.3	B	BCP_2	38.7	A
P_2	38.1	AB	P_2	37.5	ABC	F_2	33.0	BC	F_2	25.5	BC	F_2	32.3	B
F_2	34.6	AB	F_2	35.9	BC	P_2	30.5	C	BCP_1	22.7	BC	P_2	31.3	B
BCP_1	33.8	AB	BCP_1	34.4	BC	BCP_1	30.4	C	P_1	20.2	BC	BCP_1	30.3	B
P_1	31.1	B	P_1	30.7	C	P_1	29.4	C	P_2	19.0	C	P_1	27.8	B
MPH (%)	17.9			28.0			45.0			94.6			40.4	

Means with the same letter in the same column for respective N regime and generation are not significantly different. α= 0.05, 17 error degrees of freedom.

MPH = mid parent heterosis = $[(F_1\text{-}MP)/ (MP)]*100$

3.3.2 Estimates of genetic effects

Genetic effects were only estimated when the data showed a clear significant difference of P_1 from P_2 means based on LSD values at 5% probability in Table 3.3 a and b, as required by GMA model assumptions. Thus an estimation of genetic effects was only made under HN conditions.

Generations of cross T20 x C58

Table 3.4a indicates the genetic effects for generations of cross T20 x C58. Only positive additive effects were significant under all grain filling stages, including mean LCC. The R^2 values were marginally greater than 50% at all growth stages except at 50% silking and dent stages.

Generations of cross T20 x N_G8

In Table 3.4b (cross T20 x N_G8), the R^2 values were ≥70% at the milk and mean LCC. All the grain filling stages had significant positive additive genetic effects. The dominance genetic effects were statistically significant and positive at the milk stage. The epistatic genetic effects of negative additive x dominance type were statistically significant at 50% silking, dent and average of growth stages, while the positive epistatic genetic effects of additive x additive nature were significant at the milk stage.

Table 3.4: Estimated genetic effects of leaf chlorophyll content for two maize crosses under high nitrogen conditions

a) Generations of cross T20 x C58

Growth stage	Genetic effects						Statistics of the models	
	m	a	d	aa	ad	dd	R^2 (%)	CV (%)
				HN				
50%silking	36.54±15.23**	6.75±2.11***	5.08±35.53	1.91±14.62	-11.96±9.48	-2.52±21.97	38.65	2.49
Milk	37.92±13.68**	6.56±1.83**	21.22±31.69	5.45±13.02	-5.68±8.45	-13.52±19.61	51.85	2.05
Dent	34.32±16.04**	4.72±2.07**	20.28±37.26	2.91±15.35	5.65±9.84	-10.23±23.08	46.06	2.71
Maturity	28.26±19.99	4.65±2.32*	34.26±46.63	2.29±19.26	-3.12±12.11	-23.05±28.80	54.34	5.17
Mean stages	35.66±12.89**	5.81±1.70**	16.97±29.96	2.57±12.34	-4.19±7.94	-10.26±18.53	53.70	2.25

***, **, * indicates the term is significant at p≤0.0001 p≤0.05, p≤0.1, respectively; R^2= coefficient of determination, CV= coefficient of variation.

b) Generations of cross T20 x N_G8

Growth stage	Genetic effects						Statistics of the models	
	m	a	d	aa	ad	dd	R^2 (%)	CV (%)
				HN				
50%silking	40.20±15.29**	6.77±2.15**	-1.32±35.75	2.47±14.69	-32.33±9.55**	7.82±22.21	52.15	2.56
Milk	20.57±10.15**	7.05±1.39***	48.52± 23.84*	19.55±9.71**	-8.87±6.45	-21.70±14.89	71.49	1.486
Dent	38.93±17.43**	5.79±2.25**	-19.23±40.10	-10.48±16.65	-20.62±10.41*	20.43±24.82	54.10	2.85
Maturity	-4.54±16.96	4.13±1.97*	89.05±39.78**	27.34±16.11	-5.33±10.70	-42.12±25.11	69.34	4.20
Mean stages	22.89±8.88**	5.85±1.17**	34.32±20.75	11.27± 8.49	-14.97±5.54**	-12.83±12.94	76.85	1.50

***, **, * indicates the term is significant at p≤0.0001 p≤0.05, p≤0.1, respectively; R^2 = coefficient of determination, CV = coefficient of variation.

3.3.3 Relative contribution of genetic effects

All genetic effects were shown because of small sums of squares that consequently contributed little to the total sums of squares of the models.

Generations of cross T20 X C58

In the generations of cross T20 x C58 (Table 3.5a) under HN conditions, the additive genetic effects were about 40% and above at all growth stages, whereas the dominance effects predominated more at maturity, dent and average of growth stages, in that order. The maximum epistatic effects was about 15% and was derived more from additive x dominance (mid-silking stage), and additive x additive (maturity stage). The additive effects predominated the mean LCC to about 80%, followed by only about 16% from the dominance effects. With LN, the additive effects contributed similarly about 60% only at the mid-silking and milk stages. Dominance effects predominated at the dent and physiological maturity stages. Digenic epistatic effects of dominance x dominance nature were more important at all stages, also with significant share from additive x dominance (mid-silking), and additive x additive (physiological maturity stage). On average, dominance genetic effects predominated, followed by dominance x dominance and additive effects under LN. Under HN, the fixable effects were predominant at all grain filling stages, however, the fixable and non-fixable effects were equal at the physiological maturity stage. Under LN, both effects predominated at all stages, except that the fixable effects were negligible at the dent stage. The mid-silking and milk stages had equal proportions of fixable and non-fixable effects. Overall, the ratio of fixable to non-fixable effects was 73.90:26.10 under HN and 35.10:64.67 under LN.

Generations of cross T20 x N_G8

With cross T20 x N_G8 (Table 3.5b), under HN over 70% of the genetic contribution at maturity was from the dominance effects, whereas over 65% of the contribution at milk stage came from the additive effects. About 45% of genetic contribution at the dent stage came from the dominance effects of LCC and 70% from the additive x dominance epistatic effects; these effects also provided more than 20% genetic contribution at the dent stage. However, at the mean LCC, the additive and dominance effects contributed about 42% each to the total genetic effects for LCC in cross T20 x N_G8 under HN. Under LN, dominance effects contributed most of the genetic effects to all stages. The contribution was highest at physiological maturity and dent stages, and was consistent over the N regimes and crosses. The additive effects contributed highly at the mid-silking stage. Regarding epistatic effects, the additive x dominance had similar contribution of about 20% at the milk and dent stages. The dominance x dominance effects were higher at physiological maturity. Under HN, the additive x dominance effects contributed about 70% of the total genetic variation at the mid-silking stage. Still under HN, the fixable effects were only high at the milk stage, at about 71%; the rest i.e. about 30% of the non-fixable effects predominated at other growth stages. Under LN, apart from the mid-silking stage, other generations had larger non-fixable effects than the fixable genetic effects. Overall, the ratio of fixable to non-fixable effects was 37.43:62.57 for HN and 19.97:80.11 under LN. The general trends in Tables 3.5a and b indicate that additive genetic effects decreased as growth stage increased, while dominance effects increased at later growth stages, irrespective of genotype and N regime.

Table 3.5: Relative contributions of genetic effects to the models' total sums of squares over growth stages in maize under high and low nitrogen conditions
a) Generations of cross T20 x C58

	Genetic contribution (%)					Fixable	Non-Fixable
	a	d	aa	ad	dd		
Growth stage	High N						
50% silking	84.31	0.61	0.00	14.95	0.12	84.31	15.68
Milk	87.50	5.44	1.24	2.96	2.86	88.74	11.26
Dent	63.05	32.15	1.29	2.11	1.41	64.34	35.67
Maturity	39.74	44.39	11.32	0.55	4.00	51.06	48.94
Mean grain fill stages	78.73	15.58	2.30	1.70	1.69	81.03	18.97
	Low N						
50% silking	61.47	4.41	1.26	0.01	32.86	62.73	37.28
Milk	59.39	5.15	3.36	17.27	14.84	62.75	37.26
Dent	3.06	69.29	1.00	7.26	19.38	4.06	95.93
Maturity	1.53	46.18	20.52	5.32	26.44	22.05	77.94
Mean grain fill stages	23.28	41.21	1.14	8.46	25.91	24.42	75.58

Fixable genetic effects (i.e. additive main effects and additive x additive epistasis), Non-fixable genetic effects (i.e. dominance, additive x dominance and dominance x dominance epistasis)

b) Generations of cross T20 x N_G8

		Genetic contribution (%)					
	a	d	aa	ad	dd		
Growth stage			High N			Fixable	Non-Fixable
50% silking	19.82	4.84	5.94	68.66	0.73	25.76	74.23
Milk	65.60	19.27	5.38	4.78	4.98	70.98	29.03
Dent	27.88	44.51	0.20	23.45	3.96	28.08	71.92
Maturity	18.83	72.57	0.51	0.77	7.32	19.34	80.66
Mean grain fill stages	41.50	42.35	1.47	12.94	1.74	42.97	57.03
			Low N				
50% silking	56.43	32.13	6.69	3.86	0.88	62.12	36.87
Milk	17.49	51.23	0.82	21.58	8.88	18.31	81.69
Dent	0.97	71.72	2.78	23.78	0.75	3.75	96.25
Maturity	0.11	84.31	0.00	1.57	14.01	0.11	99.89
Mean grain fill stages	11.62	69.44	3.92	14.56	1.86	15.54	85.86

Fixable genetic effects (i.e. additive and additive x additive), Non-fixable genetic effects (i.e. dominance, additive x dominance and dominance x dominance)

3.3. 4 Frequency distributions

Transgressive segregants were observed for LCC character across the grain filing stages (Figures 3.2 and 3.3) with similar trends for the two crosses. Only the frequency distribution curves for mid-silking LCC for the two crosses are presented here whereas the curves of the other growth stages revealed common trends as summarised below. It was observed in both crosses that post-mid-silking LCC was unstable under low N environments and/or N starved genotypes, regardless of the three segregating generations i.e. F_2, BCP_1 and BCP_2. The general trend for the frequency distribution curves of the LCC for the generations of the two crosses can be described as follows:

i) low regimes of N had consistently lower SPAD values,

ii) N deficient generations associated with inferior positions on the curves with the grain filling age,

iii) all the inferior parents under both low and high N regimes and the superior parent in low N regimes pooled towards lower tails at the dent, physiological maturity and average of stages for the two crosses; all the inferior parents and BCP_1 maintained positions at the lower tail, while the superior parent under low N tended to BCP_1 as growth stage increased, whereas the superior parent in high N was maintained at the higher tail,

iv) the milk stage was the cut-off point where the changes in trends of the SPAD values were most pronounced,

v) transgressive segregants built up with age, reaching maximum at physiological maturity stage for backcrossed progeny of the superior parent, and

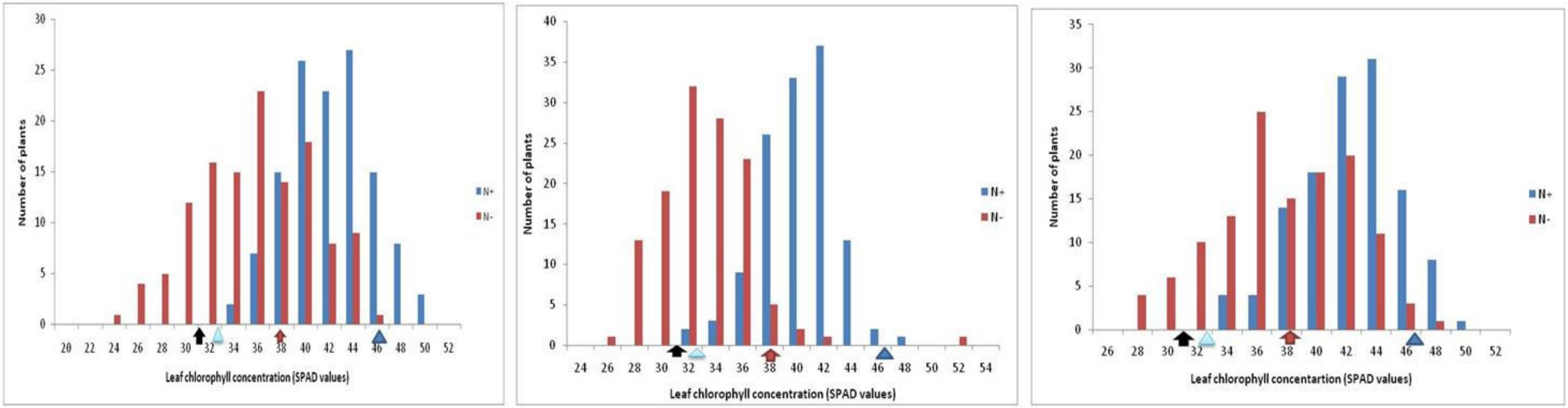
vi) LCC varyied with the environments under which LCC was measured, and genotype.

The segregating generations of both crosses T20 x C58 and T20 x N_G8 exhibited continuous and normal distribution curves. Frequency curves of both N regimes for F_2 at mid-silking and milk stages in generations of cross T20 x C58 overlapped. The BCP_2 at mid-silking stage in the same cross were skewed to the right-hand side under both N conditions, whereas distinct normal curves for each N regime were evident at maturity stage. In cross T20 x N_G8, the mid-silking and milk stages, BCP_2 was skewed to the right-hand side for both N conditions. At the dent stage, F_2 and BCP_2 were skewed to the left under low N. At maturity, F_2 was skewed to the left under both N conditions, whereas BCP_2 in low N was skewed to the right. With the mean LCC under low N, the frequency curve of BCP_2 was skewed to the right.

In the generations of cross T20 x C58, the F_2, BCP_1 and BCP_2 at mid-silking stage, F_2 and BCP_1 at the milk, dent and mean LCC stages, and F_2 at physiological maturity had negative segregants under only low N while there were positive segregants under both N conditions. The BCP_2 at milk, dent and mean LCC stages had no negative segregants under low, whereas positive segregants were observed under both N regimes for this generation and BCP_1 at physiological maturity.

In cross T20 x N_G8, negative segregants predominated under low N, while positive segregants existed under both N conditions for generations BCP_2 at the mid-silking stage, F_2 and BCP_2 at dent stage, and in all the three segregating generations at physiological maturity and mean LCC. Negative and positive segregants were observed under both N conditions for F_2 at mid-silking stage and BCP_1 at dent stage. Generation BCP_1 of the mid-silking stage had negative segregants under both N regimes, while positive segregants were observed only under low N. Negative segregants predominated under low N, while positive segregants were evident under low N at the F_2 of milk stage. At the same stage, generation BCP_1 had negative segregants under low N, while positive segregants were seen at high N. In contrast, generation BCP_2 at the same stage had no negative segregants under either N condition, whereas positive segregants were observed under both N conditions.

Generally, in both crosses, low N frequency distribution curves had more wide segregants than high N curves, but in terms of magnitude, high N was higher than the low N curves.

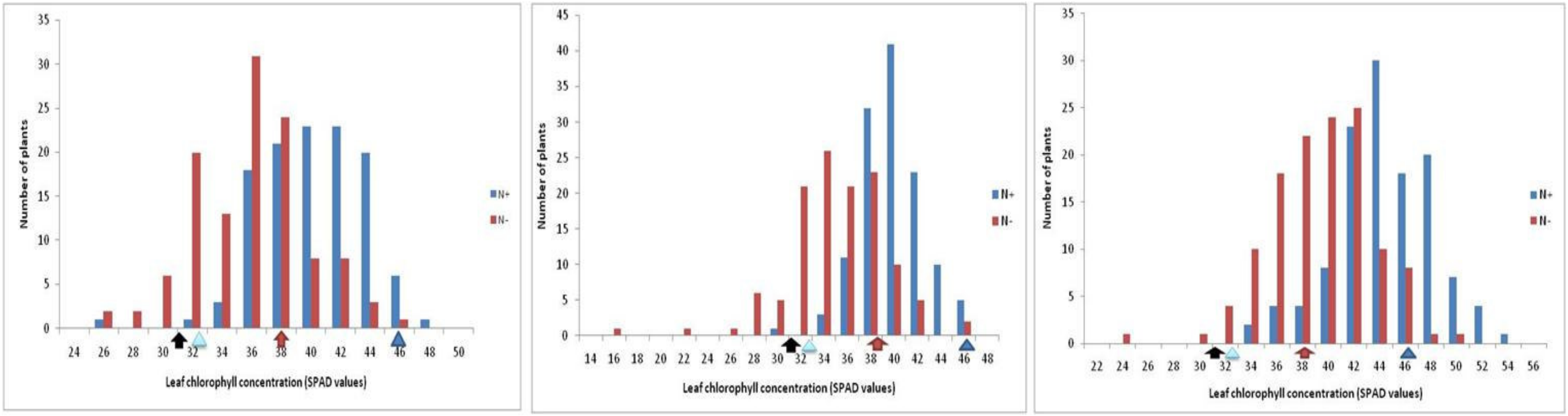


F_2 BCP_1 BCP_2

Figure 3.2: Frequency distributions of segregating generations for leaf chlorophyll concentration (SPAD values) at 50% silking stage for cross T20 x C58 across high and low N regimes

△ = high N regime (filled blue = superior parent i.e. C58; filled grey = inferior parent i.e. T20)

⇧ = low N regime (filled red = superior parent i.e. C58, filled black = inferior parent i.e. T20)



F_2 BCP$_1$ BCP$_2$

Figure 3.3: Frequency distributions of segregating generations for leaf chlorophyll concentration (SPAD values) at 50% silking stage for cross T20 x N$_G$8 across high and low N regimes

△ = high N regime also indicated as N+ (filled blue = superior parent i.e. N$_G$8; filled grey = inferior parent i.e. T20)

⇧ = low N regime also indicated as N- (filled red = superior parent i.e. N$_G$8, filled black = inferior parent i.e. T20)

3.4 Discussion

3.4.1 Mean separation of generations

Nitrogen regimes and generations except at 50% silking date in cross T20 x C58 were statistically significant (Table 3.2). The requirement for GMA of contrasting parents for the LCC character (P_1 and P_2), based on the mean separation test (LSD $_{0.05}$) was only met under high N for all grain filling stages in both crosses (Tables 3.3a and b). Therefore, the differences between parents were statistically significant under HN conditions for the LCC character across the grain-filling stages for both crosses. In terms of magnitude, the differences between P_1 and P_2 decreased at the grain-filling stage and they began earlier under LN compared with HN. This may suggest that genotypic differences for the LCC character could be clearer under HN than LN conditions. The results revealing LCC peaking at milk stage, irrespective of cross and N conditions, would suggest that leaf N is needed the most in maize at this growth stage. This may be called the linear graining-filling stage; any stress at this stage would result in irreparable effects, since DMA would be reduced and result in a low final yield. Genotypes that maintain higher levels of LCC at and past the milk stage under HN and LN would therefore translate into a higher yield than that of genotypes which quickly lose LCC at similar conditions. However, the stalk switches roles and assumes the function of leaves during random stresses that occur at the grain-filling stage (Duncan et al., 1965). Also, N remobilising from shanks and husk to the growing kernels (Beauchamp et al., 1976; Weiland and Ta, 1992) would not compensate for the effects of loss of leaf N if such stresses come at the milk stage, because this is the linear phase of kernel growth. However, the SPAD values began to drop earlier at milk stage under LN than HN in generations of cross T20 x C58, which was not the case for cross T20 x N_G8, suggesting genotypic differences for the LCC character even at the same growth stage. This may further demonstrate the effect of N on the genotypes.

The change in position of generations with superior genotypes for LCC in replenishing declining LCC with grain-filling age could demonstrate both adaptive and performance phenomena of the LCC character. This was the case between the superior parents, back-crossed progenies to superior parents for the trait, and F_1 generations. The changing positions of the generations could be used as markers or indications that grain-filling is taking place, further demonstrating the response of genotypes to N conditions and/or the status of soil N when other factors are held constant. Furthermore, the preponderance to the topmost positions of BCP_2 under LN in early grain-filling stages and to later stages under HN — also F_1 in later stages under both conditions and superior parents (i.e. inbred lines C58 and N_G8) in early growth stages under HN — demonstrate the evidence of N in maize adaptability.

Heterosis could be a measure of adaptability, as suggested by Kang (1994). The increase of heterosis with grain-filling ages and poor environments (i.e. low N) would prove the predominance of dominance genetic effects at physiological maturity and as the environment got impoverished. Mather and Jinks (1982) reported that heterosis levelled off linearly as the production environment improved, thus partly confirming the findings of the present study. Regarding the shift of heterosis with age from low at the early grain-filling stages (implying negligible non-allelic interactions), to high heterosis at later growth stages, which suggest a prevalence of non-allelic genetic effects, no literature is available to strongly support this finding. The consistently lower SPAD values and position of the inferior parent, which is a common female parent to both crosses, would suggest that the SPAD-502 Chlorophyll Meter was the appropriate and precise technique to quantify the trends of LCC from kernel set and maintenance until physiological maturity. It further demonstrates that the N regimes used for evaluation of the LCC character were ideal, since HN had consistently higher SPAD mean values than LN. This was irrespective of the many factors that may influence the SPAD-502 readings as they have been reported to prevail under field conditions (Yadava, 1986; Martinez and Guiamet, 2004; Scharf et al., 2006; Zhang et al., 2008).

The predominance of the F_1 in cross T20 x N_G8 could probably be because the parents were genetically and/or geographically distantly related, thus expressing heterosis. The F_1 occupied the topmost position from dent and physiological maturity stages, and also at the average of growth stages under HN, and except at the milk stage where BCP_2 was top the F_1 and dominated top positions in all stages under LN. Checa et al. (2006) reported that very distantly related genotypes had high mid-parent heterosis, which could be expected where there was little difference between the P_1, BCP_1 and F_1 generations. Moreover, in generations of cross T20 x C58 at the physiological maturity stage under HN, F_1 was topmost and this was not associated with high MPH, since the dent and physiological maturity stages had similar values for MPH. This appears to contradict the general trend observed in this study that heterosis increased with the grain-filling stage, although it partly agrees with Mather and Jinks (1982) that heterosis is low under optimum conditions.

3.4.2 Estimates of genetic effects

Genetic estimates are discussed only in crosses where there is a clear separation of P_1 and P_2 based on LSD value at 5% level of probability. The genetic effects were therefore estimated only under high N conditions, which obeyed the assumption of contrasting parents as a cardinal assumption of GMA for both crosses.

Generations of cross T20 x C58

With cross T20 x C58, only the additive genetic effects conditioned the LCC character at all the grain filling stages under high N regime. Predominance of additive effects implied that recurrent selection (RS) or any form of cyclic selection could be effective for the character at all growth stages. This appears to support the idea that the genetic potential of a genotype is well exploited under ideal production conditions. This may suggest further that only inbred lines could be generated at all grain-filling stages from segregating generations for the LCC character under high N. Furthermore, significant differences for only additive genetic effects for LCC character under HN contradicts Elings et al. (1997), Bänziger and Lafitte (1997), Bänziger et al. (2000) and Worku et al. (2007), who reported a preponderance of additive genetic effects in secondary traits, especially under low N. The LCC character has been associated with the physiological maturity of genotypes in that the greater the time it takes to reach maturity, the more the LCC character becomes relevant. On the other hand, shorter maturing genotypes may lose LCC earlier and faster under similar conditions. In short-season areas, therefore, the high LCC character would translate to high yield (Tollenaar and Daynard, 1978a, b), as opposed to long season or varieties with extended growth. This suggests that early-maturing or short-season cultivars are source-limited, such that extended LCC may increase DMA and so result in improved grain yield.

Generations of cross T20 x N_G8

Both fixable (additive, and additive x additive) and non-fixable (dominance, additive x dominance, and dominance x dominance) genetic effects for cross T20 x N_G8 were preponderant under HN. This may suggest that reciprocal recurrent selection (RRS) could be employed for the LCC character. Epistasis was observed for cross T20 x N_G8, and it could be defined as any interaction between genes at non-homologous loci (Sprague et al., 1962). A greater preponderance of epistatic interactions in cross T20 x N_G8 than in cross T20 x C58 supported the hypothesis that epistasis is real in maize, although it is specific to the cross, trait and environment, as opposed to many researchers who ignore such phenomenon. This further suggests that positive epistasis could have contributed significantly to heterosis, which was common in cross T20 x N_G8. The milk stage, which was earmarked as the most sensitive grain-filling stage, was more important for cross T20 x N_G8 in HN (Table 3.4b) but this was not the case for cross T20 x C58, implying the effects of the genotypes and N. This stage (i.e. 14 days after the 50% silking) has been also considered by maize physiologists as a linear grain-filling stage. Significant additive effects for the milk stage in both crosses and significant dominance effects for only cross T20 x N_G8 may demonstrate that the genetic effects for this trait are not easy to determine but it can simply be inferred that the trait is only estimable under high N. Generally, the R^2 values were slightly above 50% and about 70% for crosses T20 x C58 and T20 x N_G8, respectively suggesting that the genetic effects for LCC character would be more easily detected in and estimated for the latter, rather than the former cross.

In both crosses and growth stages for high N regime the sign of the genetic effects refer to the relative position of the parents to the mid-parent for the case of dominance effects including associated epistatic effects. In short, it refers to heterosis (Shashkumar et al., 2010) and for the case of the present study, high LCC values are desired, and vice versa. For the dominance effects, the sign is related to relative positions of F_1 from the mid-parent, and this was consistent with results from the mean separations. With regards to the additive genetic effects and related epistatic effects, the signs imply which parent was chosen as superior or inferior for the trait of interest (Azizi et al., 2006). The positive sign observed for additive genetic effects per growth stage would therefore imply that the choice of parents was appropriate during mating since the individual growth stages for the LCC character were considered as separate traits. And the converse would have been the case if negative signs for the additive effects were observed. Furthermore, in standard regression terms, the signs would be associated with regression coefficients (β) for individual effects, whereby either sign depends on the trait and breeding objective. Positive β is desired for traits where positive values are wanted, whereas negative β values are relevant where the trait should be decreased.

The preponderance of epistasis in T20 x N_G8 under HN (Table 3.5b) contradicts the findings by Perkins and Jinks. (1971), Jinks et al., (1973), Wolf and Hallauer (1977) and Ceballos et al. (1998), who reported that epistasis is more common in stressed conditions than in stress-free areas. These researchers added that the inherent phenomenon of epistasis is about specificity such that at/or near the extremes of the normal distribution curves of genotype responses, specific genetic interactions are expected compared to the middle part of the curves. The absence of duplicate epistasis for the LCC character would increase the response to selection (Iqbal and Nadeem, 2003). However, at these growth stages, heterosis breeding could still be effective due to the predominance of non-fixable effects (Table 3.5b). Viana (2006) reported that the additive x additive and additive x dominance to be inestimable, such that their relative importance is difficult to assess. This assertion could further confirm the unknowns on the inheritance of the LCC character in maize. The results of the present study appear not to be wholly in support of other research because no systematic work has been done to quantify the LCC character across grain-filling stages under high and low regimes of N in tropical maize.

Generally, the present study suggests that only RS procedures can be effective for the LCC character under high N conditions for population T0 x C58. The preponderance of both additive and non-additive genetic effects for all growth stages demonstrates the efficacy of RRS for the LCC character for population T20 x N_G8. Therefore, the inheritance of the LCC can be determined only under high N, although there are genotypic differences under N regimes, thus affecting the inheritance of the LCC character. The extended LCC (SG trait) that has been studied in many crops but not so much in maize was under the control of additive genetic effects in both crosses but the dominance effects were also statistically significant in one cross. Therefore, the inheritance of the SG trait is not fully understood but an inference can be made that it may be estimated only under ideal N conditions.

3.4.3 Relative contribution of genetic effects

Contrary to the estimation of genetic effects only at HN, in order to fulfil the assumptions of GMA, the relative contribution of genetic effects were estimated under both N regimes since the genetic component sums of squares contributed little to total sums of square for the models.

Generations of cross T20 x C58

The separation of total genetic effects into individual genetic effects for the LCC character during the grain-filling stages in maize seem not to be documented in literature. In cross T20 x C58 under HN, the predominance of additive genetic effects of about and above 40% (Table 3.5a) demonstrated the efficacy of the RS procedure for all the grain-filling stages. However, the RRS procedure could be used where both additive and dominance, and epistatic effects associated with these main genetic effects, existed. And this could be the case at the mid-silking, dent and physiological maturity stages. Dominance genetic effects prevailed at maturity, dent and mean LCC in that order. However, the epistatic effects of about 15% from additive x dominance for mid-silking and of additive x additive effects for physiological maturity appear to contradict previous results of the present study. Generally, additive effects predominated early growth stages and mean LCC as dominance effects prevailed in later growth stages. This may suggest that inbred lines could be developed at early growth stages, whereas hybridisation could be deferred to later growth stages for the LCC character under high N conditions.

Under LN, the additive effects contributed about 60% at the mid-silking and milk stages, whereas dominance genetic effects contributed more at the dent and physiological maturity stages. The dominance x dominance genetic effects contributed more than additive x dominance effects at all growth stages except at milk stage where these effects almost equalled. There was also a significant share from additive x dominance effects at the mid-silking stage and additive x additive effects at the physiological maturity stage, thus agreeing with findings under HN. Generally, the present study found that dominance effects predominated, followed by dominance x dominance and additive genetic effects under low N. The preponderance of the dominance effects observed under LN in the present study agrees with Azizi et al. (2006) that while additive genetic effects are stable, the dominance effects vary with environment (population density regimes), genotype and trait. The milk and mid-silking stages had similar upward magnitudes to the additive genetic effects under low N. The prevalence of the dominance effects under LN contradicted literature that supports the additive effects of secondary traits, including the LCC character (especially the SG trait) under low N conditions (Lafitte and Edmeades, 1995; Bänziger et al., 2000). In maize and other crops, the total genetic effects that condition the extended LCC character have been reported to be polygenic in sunflower (Cukador-Olmedo and Miller, 1997), polygenic in sorghum but with varying levels of dominance at the genetic level (Walulu et al., 1994), and single dominant gene in maize (Ceppi et al., 1987; Gentinatta et al., 1987; Thomas and Smart, 1993). However, all of these studies assert on the obscurity of the inheritance of the extended LCC character in literature.

Generations of cross T20 x N_G8

The results indicated a contribution of over 70% for dominance genetic effects at physiological maturity, over 65% additive effects at milk stage, about 45% dominance at dent, about 70% from additive x dominance, which also provided over 20% at dent, while the additive to dominance ratio was about 42% at mean LCC under HN (Table 3.5b). Therefore, relative contribution of the genetic effects has established that RRS could be effective for the LCC character across the grain filling stages in cross T20 x N_G8 under HN. Hybrids could be developed at the dent and maturity stages due to a preponderance of non-additive genetic effects. Under LN, while additive effects contributed highly to mid-silking, dominance genetic effects were high at all growth stages but mostly pronounced at the physiological maturity and dent stages. This was consistent over N regimes and crosses. In this environment, RRS could be effective in all stages, except at physiological maturity (with LCC now considered as a stay-green trait) where hybridisation would be the only option. All these results implied the complexity of the inheritance of the SG trait, which corroborates Thomas and Smart (1993) in maize and recently in sorghum (Kassahun et al., 2010). However, from the two crosses and N regimes, it could be suggested that the additive effects are important in conditioning the LCC character in early growth stages and which allows RS early in breeding cycles. Furthermore, prevalence of dominance effects at the physiological maturity stage in all crosses, regardless of the environment, may warrant a hybrid breeding procedure for the SG trait in the two regimes of N. Generally, the study has established that different gene effects govern LCC at the different grain-filling stages studied and this could be a function of the genotype, N regimes, time of maturity and the interaction of these factors. However, these results failed many of the assumptions of requirements of the GMA, highlighted in the methodology section. Some of the assumptions of GMA are lack of dominance and epistasis, which prevailed in this study.

Furthermore, in cross T20 x C58, the ratio of fixable to non-fixable effects of 73.90 to 26.10% under HN and 35.10 to 64.67% for LN suggested that fixable genetic effects govern the LCC character under high N, although dominance genetic effects prevailed under low N. There was a ratio of fixable to non-fixable genetic effects of 37.43 to 62.57% for HN and 19.97 to 80.11% for LN in cross T20 x N_G8. These ratios did not agree across the two crosses and N regimes. In cross T20 x C58, it was found that fixable genetic effects predominated at HN whereas non-fixable genetic effects were large under LN while in cross T20 x N_G8, non-fixable effects prevailed under both regimes of N but with the magnitude of non-fixable effects increasing at LN relative to HN. This may further indicate the difficulty in breeding for the LCC character, including the SG as Subedi and Ma (2005) and Hawkins et al. (2007) suggested. The general trend for both crosses and N regimes suggested that additive and dominance genetic effects for the LCC character decreased and increased respectively at the grain-filling stage. However, the profiles and trends of the inheritance of the LCC character to attain the LCC measured at physiological maturity (SG trait) in tropical maize may not be as conclusive as this study established.

3.4.4 Frequency distributions

The frequency distribution curves illustrated that transgressive segregants existed for the LCC character. These curves were normally distributed and continuous, which may suggest the preponderance of the additive genetic effects identified in this study. The higher variance for a low N regime (Figures 3.2 and 3.3) than high N would provide room for selecting from both negative and positive segregants. Where transgressive segregants exit, inbred lines could be developed through diallel or biparental mating designs or by first selfing the segregants and selection deferred to a later stage as Smith et al. (2009) and Shashkumar et al. (2010) suggested, and as was the case for LCC character in the present study. This study established that inbred lines could be developed from the F_2 and backcrossed progeny for superior LCC (i.e. BCP_2) under both N conditions, due to a preponderance of negative and positive segregants.

Environment and genotypes starved of N and their converse as grain-filling stages increased indicated that the post-50% silking LCC character was unstable (Figures 3.2 and 3.3). This may demonstrate that genotypes grown under low-N environments and/or genotypes with inherently low N would lose leaf N faster than under high N sites and genotypes with high leaf N concentration. In the former situation, it may imply that under low-N conditions, genotypes with low LCC may have a high rate of grain filling, but with short duration of grain filling and as a consequence, low grain yield in low N sites, plus genotypes susceptible to low N stress. This finding partly corroborates Edmeades et al. (1997) that low-yielding landraces that are earlier maturing are found under stressed environments such as drought and low soil N. However, the degree of instability of post mid-silking LCC character would increase if P_1 and P_2 differ in physiological maturity such that the generations may be segregating for maturity, thus influencing SPAD values as was the case for cross T20 x N_G8, compared with cross T20 x C58. Furthermore, the change in SPAD values with growth stage implied that N is exhausted with grain-filling age. This change could be monitored and used as an indicator or a marker towards breeding maize cultivars for tolerance to low soil N. The gross influence of differences in physiological maturity of genotypes on the trends of LCC has been at least reported in maize (Hageman and Lambert, 1996; Martinez and Guiamet, 2004). However, this has not been reported across grain-filling stages under high and low N conditions in tropical maize. Hawkins et al. (2007) reported that N deficiency at later grain-filling stages, particularly the dough and dent stages, related to response of maize genotypes to N than in early stages, although this study could not give the genetic basis of the assertion. These authors contradicted Lafitte (1994), Lafitte and Edmeades (1995), Bänziger et al. (2000) and Worku et al. (2007) who reported that the predominance of high positive transgressive segregants at later grain-filling stages may prove the suitability of the trait for tolerance to late-season multiple stresses that are random and erratic. Such stresses leave the breeder and/or farmer without the choice of replanting, since they come later in the season. Generally, the present study established that the post mid-silking LCC character would be a measure of N stress index, such that a genotype with high and stable SPAD values in space and time would be resilient over low-N-stress regimes.

3.4.6 Conclusions and implications to maize breeding

The following conclusions and implications could be drawn:

1) The proportions of additive to non-additive genetic effects for LCC character differed between the two crosses and between HN and LN. Generally the trend was that the fixable gene effects were predominant under HN, whereas the non-fixable effects prevailed under LN, although the magnitude of these effects varied with genotypes.
2) Generally, the additive genetic effects predominated for LCC at mid-silking and milk grain-filling stages, whereas dominance effects were highest at dent and maturity stages. Mid-parent heterosis increased with growth stage and this was expressed better under low N than under high N, pointing to the possibility of taking advantage of heterosis for LCC under low N conditions. The best stage for selection for LCC to develop breeding populations (e.g. inbred lines) would be at the early grain-filling stages.
3) The frequency distributions that considered the relative positions of recurrent parents to their segregating generations demonstrated observation of transgressive segregants under both HN and LN. These curves also tended to normal and continuous distribution, which implied that many genes were involved in the inheritance of LCC character. The positive segregants can be exploited in a breeding programme.

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Chapter 4:
Generation mean analysis of four secondary traits in crosses involving five tropical maize inbred lines under low and high nitrogen conditions

Abstract

Under stress conditions, the knowledge of the inheritance of secondary traits rather than grain yield *per se* may help to breed for improved yield in maize. However, the inheritance of such traits in tropical maize, especially under low soil nitrogen (N) conditions is not fully studied. This study was conducted in order to estimate the genetic effects controlling plant height and ear height, the number of leaves above the ear, and the number of kernels per ear in different crosses under 120 and 60 kg N ha^{-1}. The F_1 crosses between two contrasting parents were advanced to F_2, and back-crossed to both parents to generate BCP_1 and BCP_2. All the generations were evaluated in a randomised complete block design for two seasons. The data were then subjected to a generation mean analysis. The generations were statistically significant ($p<0.05$) across the N regimes and seasons, whereas the generation x N, generation x season, and generation x N x season interactions effects were non-significant. The main effects for genetic and digenic interactions varied with cross, trait and N regime. The mid-parent heterosis for all the traits were not consistent over traits, crosses and N conditions. Non-fixable (dominance, additive x dominance, and dominance x dominance) effects predominated in plant and ear heights under low N. The fixable (additive and additive x additive) and non-fixable effects for kernels per ear were large under high and low N, respectively. Additive effects contributed about 80% to genetic variation for the number of leaves above the ear. Positive duplicate epistasis conditioned leaf number above the ear under HN conditions. Significance of both fixable and non-fixable gene effects implied that reciprocal recurrent selection could be used to improve the traits under both N regimes.

Keywords: Generation mean analysis, gene effects, low N, maize, secondary traits.

4.1 Introduction

Farmers in sub-Saharan Africa (SSA) typically produce maize under low soil nitrogen (N) due to declining soil N, high prices of N fertiliser, lack of awareness on the benefits of N fertiliser, and use of seed of unimproved varieties. Except for the last factor, which may be a sustainable solution for farmers, the other factors are costly. It has been observed in SSA that only a few varieties (if any) have been released in the region that are based on tolerance to abiotic stresses, particularly low soil N (LN). Improving and creating varieties suited to conditions would help make a positive impact on livelihoods of the local people due to the fact that maize is a food security crop and a source of income in the region. Nitrogen is a key nutrient in maize (Lafitte, 1994), although the soils in SSA are impoverished of N (Lafitte and Edmeades,1995). However, small-scale farmers in SSA produce maize under low N (Worku et al., 2007), which is partly responsible for maize grain yield of < 2 t ha^{-1} (Elings et al., 1997; DeVries and Toenniessen, 2001). Breeding for grain yield per se is difficult under low N, which varies with space and time in SSA. Lee et al. (2005) reported that the genetic control of final yield is not clear, even under stress-free conditions. It has been suggested that, grain yield could be improved by targeting key component traits whose genetic effects improve with stress (Bänziger et al., 2000; Sawkins et al., 2004). Knowledge of the genetic effects that govern some of secondary traits such as the number of kernels per ear, the number of leaves above the ear, and plant and ear heights would be crucial in devising a breeding strategy for LN environments in SSA.

Among crucial secondary traits that determine final grain yield is the number of kernels per ear. This secondary trait is highly variable across management regimes and this could be the underlying reason as to why grain yields are highly variable among farmers' production conditions in SSA. The number of kernels per ear (KPE) is an adaptive trait and may define the floret fertility index (FFI) i.e. the number of kernels set and maintained until physiological maturity (Andrade et al., 1999). The FFI is a key grain yield component that could be considered a measure of stress index (Williman et al., 1987; Monneveux et al., 2005; Ojo et al., 2006). Because KPE is highly affected by input management regimes at the farmers' level, high FFI would suggest resilience of maize genotypes to local stresses. The trait has been considered to reflect ear size (length, diameter), all of which are highly affected by regimes of N (Costa et al., 2002). In addition, Chachalis et al. (2006) reported that the yield penalty observed in ultra-early planting was due to reduction in KPE rather than kernel rows. This trait regulates the source: sink relationships and eventual final yield, thus it could be referred to as a rate-limiting phenomenon (Dodd, 1977; Crafts-Brandner and Poneleit, 1987; Costa et al., 2002; Basra and Goyal, 2002). The silking stage should be targeted to improve yield, since every silk that is available, if conditions allow, produces a kernel, however small (Moss, 1962; Hunter, 1980; Weiland and Ta, 1992). Therefore, increasing KPE is a breeding objective in tropical areas where multiple stresses are common. However, being an adaptive trait, its inheritance, stability and response to selection under low N has not been fully studied.

In tropical maize, the genetic control of the number of leaves above the ear appears to be less studied than the total number of leaves per plant. If the number of leaves above the ear of the genotype remains photosynthetically active and maintains at least near physiological maturity, they will accumulate high dry matter and consequently improved grain yield. In addition, it can be hypothesised that the number of leaves above the ear is associated with the component traits for physiological maturity and final yield. The number of leaves has been associated with relative physiological maturity and the components for physiological maturity, such as flowering, kernel moisture content at harvest, and growing degree days (Chase and Nanda, 1967; Bonaparte and Brawn, 1976; Bonaparte, 1977). Later maturing varieties have been reported to have high leaf numbers above the ear (Shaver, 1983). The number of leaves above the ear correlates with improved grain yield as they are involved in direct trapping of incident radiation (Costa et al., 2002). The authors added that varieties with increased source, such as extra leaves above the ear, may relate to N-use efficiency. Therefore, if the genetic control of the number of leaves above the ear is studied and confirmed, it will be easier to improve yield and physiological maturity at the same time. This is a major challenge in tropical maize, where the varieties suited to this region are source limited.

Plant and ear heights are routine characters that are measured in any maize breeding programme but they have been barely reported in the literature, especially under LN conditions. These heights would reflect the standing ability of genotypes, maturity and flowering dates that impact on grain yield. However, both traits have been reported to reduce under stress. Bertin and Gallais (2000), Bänziger et al. (2000) and Worku et al. (2007) reported that the size of plant morphological characters, including plant and ear heights, is reduced under low-N conditions. These traits correlate positively with final yield, hence their reduction under low N may further reduce yields. In SSA, where maize is produced under low N, breeders have had to select genotypes bred on N-impoverished soils. Bertin and Gallais (2000) reported that the heritability for plant morphological traits has been found to be lower in N-stressed plants than for optimum N areas, especially for plant and ear heights. Since the genetic control of plant and ear height has not been fully studied, a breeding method to investigate their inheritance is suggested.

In hybrid maize, where improved final yield and other performance traits come mostly from heterosis, it would be worthwhile to study the detailed genetic control of some of the component traits that improve yield. Nevertheless, the genetic factors that control final grain yield are not yet fully known (Lee et al., 2005). This conjecture, however, may be inherent to the genotypes and/or production environments. The relative contribution of epistasis to final grain yields of tropical maize, especially under LN conditions, is not known. Yet, if epistasis is present and is significant, it would affect the breeding progress (Gorsline, 1961; Hallauer and Miranda; 1988; Lamkey et al., 1995; Dudley and Johnson, 2009). In most cases, the genetic effects that govern some of the inheritance of key traits that condition yield are polygenic. It would therefore not be possible to identify a single gene for these traits; rather the detection and estimation of genetic effects governing the inheritance of such traits would be an appropriate breeding investment. Genetic models that partition non-additive effects into dominance and different types of epistasis have been used in a generation mean analysis (GMA). The effects of epistasis on the number of kernels per ear, the number of leaves above the ear, and plant and ear heights, has been hardly reported in the literature, yet if these factors are significant, they have serious implications for breeding strategy. The present study thus was set out to involve both the high and low-N conditions to determine the mode of inheritance of these traits.

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4.2 Materials and methods

4.2.1 Germplasm

Five inbred maize parents contrasting for plant and ear heights, leaf number above the ear and kernels per ear were obtained from the screening of germplasm, which was conducted during the 2007/08 season. Plant and ear heights were studied in a cross between T20 (tall) and N_G8 (dwarf) (i.e. *(UYL 15-11-1-8-5)* by *(TZE-Y Pop Co S6 Inb 62-3-3)*). The number of kernels per ear (KPE) were studied in a cross between T20 (few kernels) and C58 (many kernels) (i.e. (*UYL 15-11-1-8-5)* by *MAS[MSR/312]-117-2-2-1-B*5/MAS[202/312]-86-1-3-1-B*4)*). The number of leaves above the ear was studied in a cross between N_G2 (many leaves) and C3 (few leaves) (i.e.(*TZE-W Pop X 1368 STR S6 Inb 2)* by *[Early*

Mid1/KatumaniSR]-#-169-2-4-B-1-#-BB). The two inbred parents (P_1, P_2), were crossed to generate F_1 which was advanced to F_2 then this F_1 crossed-back to both parents to generate BCP_1 and BCP_2, to constitute six generations of each cross for respective traits.

4.2.2 Experimental site and management

The design and management the experiments was conducted as explained in section 3.2.3 of chapter three in this study. The total number of plants involved in the study for the four respective traits is as indicated in Table 4.1.

The traits were measured on an individual plant basis for each generation and then averaged over replications. The number of leaves above the ears, and plant and ear heights were recorded when, on average, all genotypes were at the 50% silking stage. Plant height was measured as height from the base of the plant to the node of the first insertion of the branch of the tassel. Ear height was measured from the base of the plant to the node bearing the ear (upper most ear for a prolific plant).

The number of kernels per ear was calculated according to Khanna-Choppra and Maheswar (1998) as:

Number of filled kernels in a row x total kernel rows on a cob.

Table 4.1: Number of plants of two maize crosses evaluated in each generation over two seasons and two N regimes in Tanzania

Cross	Season	Nitrogen regime	Replication	Number of plants in each generation					
				P_1	P_2	F_1	F_2	BCP_1	BCP_2
T20 x C58	WS09	HN	1	30	27	36	125	126	125
			2	35	33	36	126	125	123
			Total	65	60	72	251	251	248
		LN	1	34	30	36	123	125	119
			2	30	35	36	118	123	123
			Total	64	65	72	241	248	242
	DS09	HN	1	27	32	31	122	119	122
			2	28	33	31	120	121	122
			Total	55	65	62	242	240	244
		LN	1	30	32	36	120	124	126
			2	30	33	31	126	123	120
			Total	60	65	67	246	247	246
T20 x N_G8	WS09	HN	1	32	22	35	114	122	111
			2	30	33	33	115	126	120
			Total	62	55	68	229	248	231
		LN	1	31	22	36	118	122	117
			2	30	27	34	112	110	90
			Total	61	49	70	230	232	207
	DS09	HN	1	25	32	33	117	112	121
			2	29	30	30	113	120	114
			Total	54	62	63	230	232	235
		LN	1	25	33	34	109	114	125
			2	28	29	30	109	113	119
			Total	53	62	64	218	227	244
N_G2 x C3	WS09	HN	1	34	35	36	121	122	122
			2	36	36	35	116	124	121
			Total	70	71	71	237	246	243
		LN	1	27	35	35	112	114	115
			2	30	32	34	115	123	115
			Total	57	67	69	227	237	230
	DS09	HN	1	27	35	29	115	120	118
			2	26	33	34	119	122	119
			Total	53	68	63	234	242	237
		LN	1	22	32	34	115	119	124
			2	27	31	36	116	117	113
				49	63	70	231	236	237

WS09 = wet season year 2009, DS09 = dry season year 2009, HN = high nitrogen application rate (120 kg N ha-^{1}), and LN = low nitrogen application rate (60 kg N ha^{-1})

4.2.3 Data analysis

The data were analysed using SAS computer package as described in section 3.2.4 (see Chapter 3).

4.3 Results

4.3.1 ANOVA, mean separation of generations and mid parent heterosis

The generation effects were highly significant for all traits studied, but their interactions with seasons and N were not significant (Table 4.2). Thus the experiments were analysed for N separately because N was the fixed effect for this study. However, the ear height had significant main effects. All traits had R^2 values of about and over 60%.

Table 4.2: ANOVA summary on the statistical significance (Pr>F) of four secondary traits for generations of maize crosses T20 x C58, N_G2 x C3, and T20 x N_G8 over two N regimes and two seasons

		T20 x N_G8		N_G2 x C3	T20 x C58
		P ht (cm)	E ht (cm)	Leaf No.	KPE
Source of variation	DF	Pr>F			
Nitrogen	1	0.134	0.0009**	0.268	0.554
Generation	5	0.0001*	<0.0001**	<0.0001**	0.0227*
Season	1	0.390	0.0001*	0.102	0.657
Gen x Nitrogen	5	0.595	0.302	0.302	0.850
Gen x Season	5	0.787	0.0782	0.457	0.649
Gen x Season x Nitrogen	6	0.800	0.208	0.478	0.546
Error mean square	22	268.49	60.94	0.26	8468.97
R^2 (%)		78.9	86.3	82.3	59.9
CV (%)		13.2	13.4	7.8	24.8

**, *, = statistically significant at ≤0.0001, ≤0.05, respectively. Gen = generation, P ht = plant height, E ht = ear height, Leaf No. = number of leaves above the ear, KPE = kernels per ear

Table 4.3 shows pair-wise mean comparisons of the six generations and mid-parent heterosis (MPH) for plant and ear heights, the number of leaves above the ear, and the number of kernels per ear. In cross T20 x N_G8, both parents (P_1 and P_2) were significantly different based on LSD $_{(\alpha = 0.05)}$ for plant height in both conditions of N, while the ear height P_1 was only significantly different from P_2 under LN (LSD $_{(\alpha = 0.05)}$). The F_1 maintained the top-most position in both regimes of N for plant and ear heights, whereas P_2 (the inferior parent) was at the bottom. Regarding the number of leaves above the ear in cross N_G2 x C3, P_1 was significantly different from P_2 (LSD $_{(\alpha = 0.05)}$) under both N conditions, whereas in magnitude, BCP_1 was top, while P_1 was at the bottom under LN and HN, respectively. Back-crossed progenies (BCPs) were associated with their recurrent parents and F_1 approached F_2 under both conditions. The two parents were not significantly different for the KPE in cross T20 x C58 under both N conditions. Magnitude wise, P_1 equalled P_2 under HN.

Generally, HN had higher mean values than LN for all traits. Some exceptions were means for KPE. Within the N regime, the KPE for both inferior and superior parents complied, whereas P_1 almost equalled P_2 across high and low N conditions. Both plant and ear heights had significant MPH of about 50% under HN, which declined tremendously for plant height under LN but was maintained for ear height. Mid-parent heterosis was only 16% under HN, but it declined non-significantly to negative values in LN for the number of leaves above the ear. The KPE had an MPH of about 50% under HN, although it declined tremendously under LN.

Table 4.3: Least significant differences pair-wise means comparison tests and heterosis of four secondary traits of six generations for generations of maize crosses T20 x N_G8, N_G2 x C3, and T20 x C58 evaluated over two regimes of N

Cross T20 x N_G8						Cross N_G2 x C3			Cross T20 x C58		
Plant height (cm)			Ear height (cm)			Leaf number			Kernels per ear		
Generation	Mean		Generation	Mean		Generation	Mean		Generation	Mean	
						High N					
F_1	160.1	A	F_1	76.3	A	P_1	7.6	A	BCP_2	469	A
F_2	140.4	AB	BCP_1	67.5	AB	BCP_1	7.3	A	F_2	403	AB
BCP_1	137.4	B	F_2	66.1	AB	BCP_2	5.9	B	F_1	387	AB
P_1	123.4	BC	BCP_2	64.0	AB	F_2	5.9	B	BCP_1	375	AB
BCP_2	112.6	CD	P_1	55.6	BC	F_1	5.8	B	P_2	369	A
P_2	92.5	D	P_2	46.9	C	P_2	5.5	B	P_1	270	B
MPH (%)	48.3			48.8			16.2			47.0	
						Low N					
F_1	141.8	A	F_1	67.4	A	BCP_1	7	A	BCP_2	462	A
F_2	126.3	A	F_2	60.4	A	P_1	6.9	A	F_1	391	AB
BCP_1	124.5	A	P_1	58.1	A	F_1	6.1	B	P_2	371	AB
BCP_2	122.0	A	BCP_1	54.0	A	F_2	5.9	B	BCP_1	345	B
P_1	120.8	A	BCP_2	52.3	A	BCP_2	5.6	B	F_2	313	B
P_2	87.0	B	P_2	32.6	B	P_2	5.6	B	P_1	295	B
MPH (%)	36.5			48.5			-2.0			17.3	

Means with the same letter in the column are not significantly different. α = 0.05, 17 error degrees of freedom. MPH = mid parent heterosis = $[(F_1\text{-MP})/ (\text{MP})]*100$; HN = high nitrogen application rate (120 kg N ha-^{1}), and LN = low nitrogen application rate (60 kg N ha^{-1})

4.3.2 Estimates of genetics effects

Genetic effects were estimated only for models where P_1 and P_2 were significantly different, based on LSD at 5% level of probability (Table 4.3). Since the assumption of contrasting parents under GMA of $P_1 \neq P_2$ for traits under study was not met for ear height in cross T20 x N_G8 under high N and for KPE in cross T20 x C58 under both N conditions, genetic effects were therefore not estimated for these traits under respective N conditions.

Table 4.4 indicates the results of various genetic effects for the traits under the study whose $P_1 \neq P_2$. In cross T20 x N_G8, the plant height was under the control of additive, additive x additive, and dominance x dominance effects under HN, whereas the additive effects were under LN. With ear height in the same cross, only the additive effects were significant under LN conditions. All the genetic effects were significant except the additive x dominance for the number of leaves above the ear in cross N_G2 x C3 under HN, whereas the additive and the additive x dominance effects were significant for the trait under LN. Regarding the signs of the genetic effects which were significant, plant height had negative additive and additive x additive effects, positive dominance x dominance effects in cross T20 x N_G8 under HN. Under LN in the same cross, the additive effects were negative for both plant and ear heights. The number of leaves above the ear in cross N_G2 x C3 had significant negative additive and dominance x dominance effects, and positive dominance and additive x additive effects under HN; the additive and additive x dominance effects were negative under LN. Positive dominance and negative dominance x dominance effects for the number of leaves above the ear in cross N_G2 x C3, under HN, indicated positive duplicate epistasis.

Table 4.4: Estimates of genetic effects of three secondary traits for maize crosses T20 x N_G8, T20 x C58, and N_G2 x C3 under high and low N conditions

Cross	Trait	Genetic effects						Model	
		m	a	d	aa	ad	dd	R^2 (%)	CV (%)
					High N				
T20 x N_G8	Plant height	149.41±39.21*	-15.67±4.84**	-135.28±89.56	-70.45±37.49*	-28.03±22.74	117.36±55.23**	80.64	1.09
N_G2 x C3	Leaf no.	3.56±1.34**	-1.07±0.19***	6.22±3.16*	2.72±1.29*	-0.61.±0.86	-4.34±1.96**	75.30	3.45
					Low N				
T20 x N_G8	Plant height	93.46±42.07**	-15.28±5.27**	34.67±98.05	-6.78±40.54	25.41±25.56	-2.24±60.75	65.53	1.28
T20 x N_G8	Ear height	61.59±32.24*	-9.86±3.77**	-41.64±74.30	-30.96±31.15	14.10±18.71	35.82±45.74	62.51	3.43
N_G2 x C3	Leaf no.	4.37±1.13**	-0.64±0.16**	3.64±2.66	1.65±1.09	-1.53±0.72**	-2.12±1.65	68.72	2.96

***, **, * indicates trait is significant at $p \le 0.0001$ $p \le 0.05$, $p \le 0.1$, respectively, R^2= coefficient of determination, CV= coefficient of variation. HN = high nitrogen application rate (120 kg N $ha-^{1}$), and LN = low nitrogen application rate (60 kg N ha^{-1}).

4.3.3 Relative contribution of genetic effects

Calculation of relative contribution of genetic effects included all traits and crosses under both N regimes even to parents that were not significantly contrasting because the relative contribution of genetic effects to the total sums of squares for the respective models was small. The trend showed that additive and dominance genetic effects contributed more to the studied traits under both N conditions, although the additive effects had more contribution (Table 4.5). Both additive and dominance effects were large, but the epistatic effects, ranging between 0 and 8%, for plant height in cross T20 x N_G8, were small under both N conditions. On the contrary, the dominance effects were larger than the additive effects (about 10%), while the epistatic effects were not significantly different from zero under HN and LN conditions for ear height in the same cross. Although the dominance effects were larger under LN conditions, the additive effects accounted for about 40% under these conditions. Epistatic effects for KPE of dominance x dominance and additive x dominance effects were predominant under LN, whereas the additive x additive effects were preponderant under HN in cross T20 x C58. Both additive and dominance effects were predominant for KPE, although the additive effects were greater. For the number of leaves above the ear in cross N_G2 x C3, the additive effects were higher than the non-additive effects under both conditions of N. The dominance and dominance x dominance effects in HN, and additive x dominance effects under LN were similar to the nearest 10%. For plant height (cross T20 x N_G8), the non-fixable genetic effects (dominance, additive x dominance, and dominance x dominance) were larger but the fixable genetic effects (additive and additive x additive) were still reasonable. The same trend existed for ear height in the same cross, except that there were negligible fixable effects under HN. The fixable effects were large at LN, whereas the non-fixable effects were high at HN for KPE in cross T20xC58. The fixable effects predominated under both N regimes for the number of leaves above ear, while, the non-fixable effects were negligible under LN in cross N_G2 x C3.

Table 4.5: The relative contributions of genetic effects to the total sum of squares of the generations in maize crosses T20 x N_G8, T20 x C58 and N_G2 x C3 for four traits

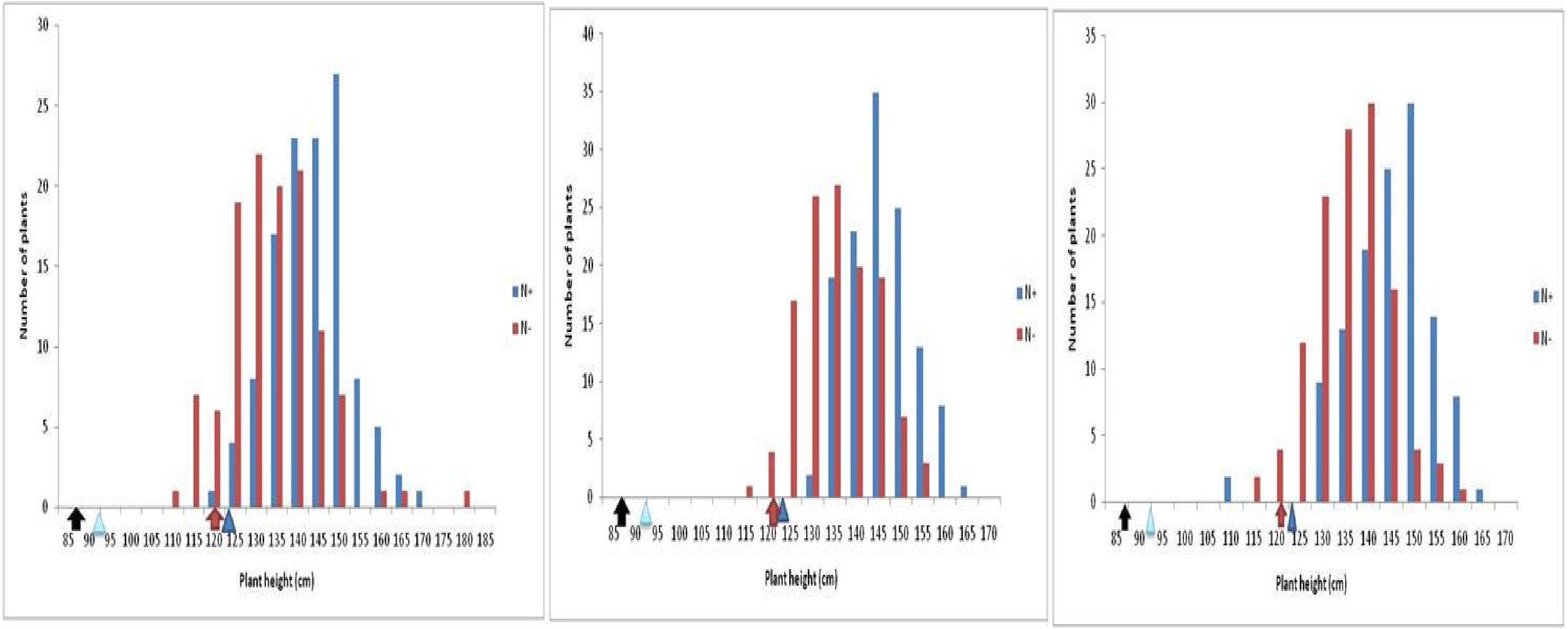
Cross	Trait	N regime	Genetic contribution (%)						
			a	*d*	*aa*	*ad*	*dd*	*Fixable*	*Non-fixable*
T20 x N_G8	Plant height	HN	37.72	50.72	0.01	3.66	7.89	37.73	62.27
		LN	34.18	61.77	0.70	3.34	0.00	34.88	65.11
	Ear height	HN	9.76	89.34	0.00	0.10	0.80	9.76	90.15
		LN	39.77	53.79	1.84	2.13	2.47	41.61	58.39
T20 x C58	kernel number	HN	48.04	25.02	17.95	3.13	5.86	65.99	34.01
		LN	33.32	20.96	3.35	10.44	31.93	36.67	63.33
N_G2 x C3	Leaves above ear	HN	76.99	12.74	0.21	0.53	9.54	77.20	22.81
		LN	80.85	1.12	2.20	11.31	4.53	83.05	16.96

Fixable genetic effects (i.e. additive and additive x additive), Non-fixable genetic effects (i.e. dominance, additive x dominance and dominance x dominance), HN = high nitrogen fertiliser application rate (120 kg ha^{-1}), LN = low nitrogen fertiliser application rate (60 kg ha^{-1})

4.3.4 Frequency distributions

The frequency distributions (Figures 4.1-4**)** provide evidence for transgressive segregants for the traits under the study for both regimes of N. Considering the relative positions of the two parents for the crosses, the trend indicated that positive segregants were more evident under high N than under low N, and the backcrossed superior progeny for the trait had larger positive segregants. Both parents under N regimes had equal KPE in cross T20 x C58 (Figure 4.2). High N had more positive segregants in BCP_1 and BCP_2 for KPE trait, whereas the F_2 curves almost fitted for the same trait under HN and LN. The general trend for KPE was BCP_2>BCP_1>F_2 for positive segregants. The frequency distributions for ear height in cross T20 x N_G8 in BCP_1 (Figure 4.3**)** demonstrated some skewness, with a long tail towards the right-hand side for high N and a long tail towards the left-hand side under low N conditions. For the number of leaves above the ear in cross N_G2 x C3, the BCP_1 was superior (Figure 4.4). The mean values for all traits were lower for LN than under HN in all the frequency distributions. All the frequency distributions were almost normal and continuous, except for the number of leaves above the ear that exhibited discrete distributions.

Plant height in cross T20 x N_G8 had no negative segregants under either N condition for the three segregating generations. However, positive segregants were observed under both N conditions for the three generations of plant height trait. For the number of kernels per ear in cross T20 x C58, F_2 had negative segregants under both N regimes, whereas BCP_1 and BCP_2 had negative segregants only under low N. Positive segregants were obvious at both N conditions for the three segregating generations. Negative segregants were not significant under both N conditions for ear height in cross T20 x N_G8. However, positive segregants were observed under both N conditions in the same cross. The number of leaves above the ear in cross N_G2 x C3 had negative segregants at high N in F_2 and in BCP_2 under both N regimes, whereas BCP_1 had no negative segregants under either high or low N, nonetheless, positive segregants were only observed under low N in generation BCP_1.

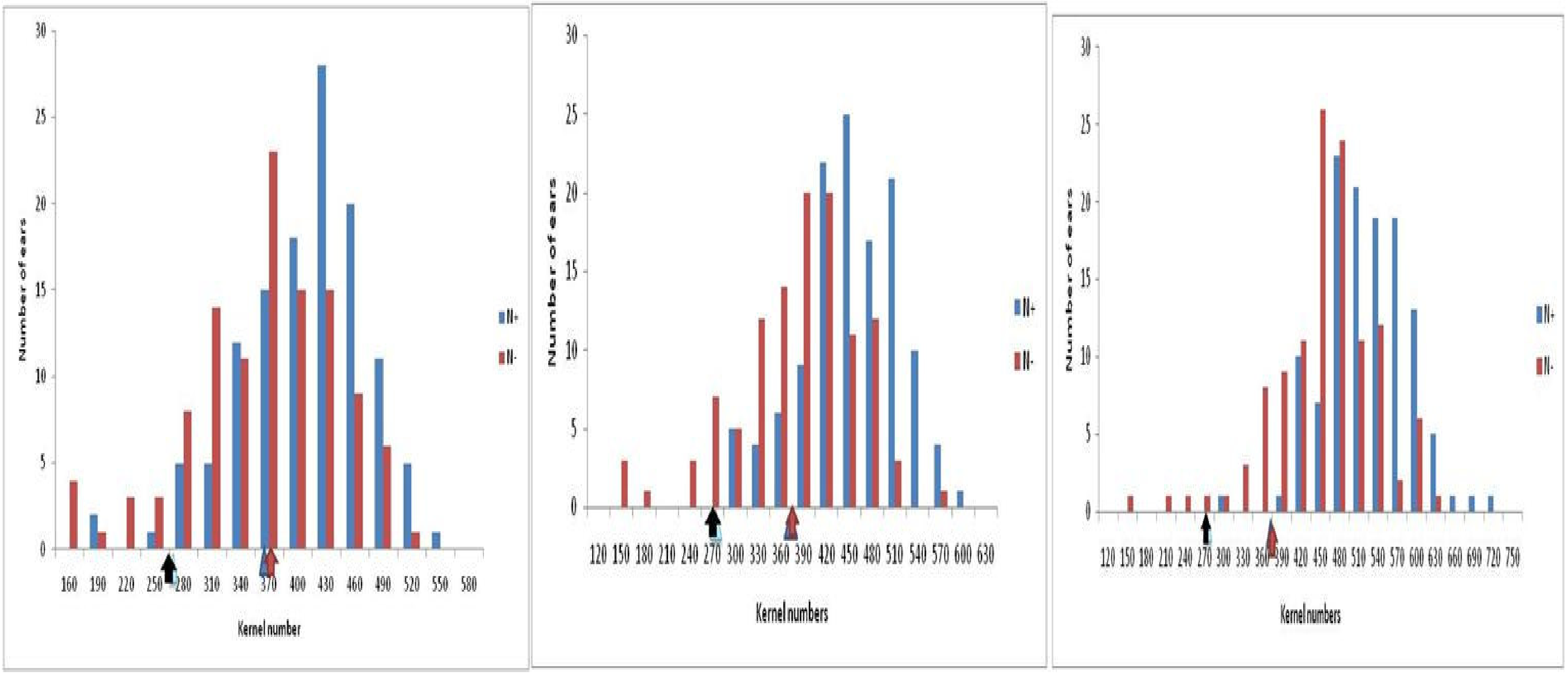


F$_2$ BCP$_1$ BCP$_2$

Figure 4.1: Frequency distributions of plant height in the segregating generations of cross T20 x N$_G$8 over N regimes

△ = high N regime also indicated as N+ (filled blue = superior parent i.e. T20; filled grey = inferior parent i.e. N$_G$8)

⇧ = low N regime also indicated as N- (filled red = superior parent i.e. T20; filled black = inferior parent i.e. N$_G$8)

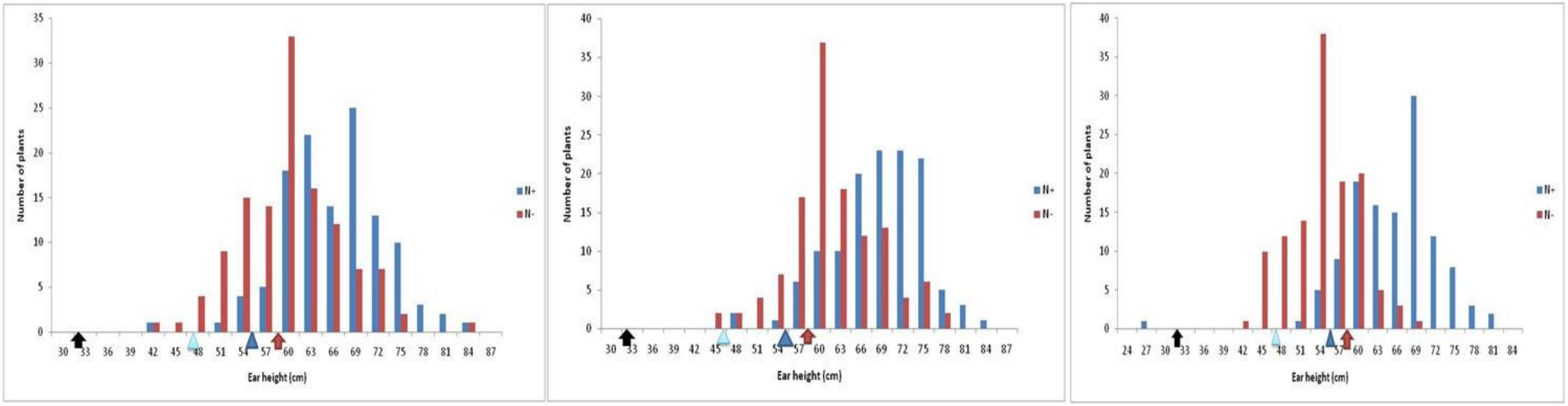


F_2 BCP_1 BCP_2

Figure 4.2: Frequency distributions of kernels per ear in the segregating generations of cross T20 x C58 over N regimes

△ = high N regime also indicated as N+ (filled blue = superior parent i.e. C58; filled grey = inferior parent i.e. T20)

⇧ = low N regime also indicated as N- (filled red = superior parent i.e. C58; filled black = inferior parent i.e. T20)

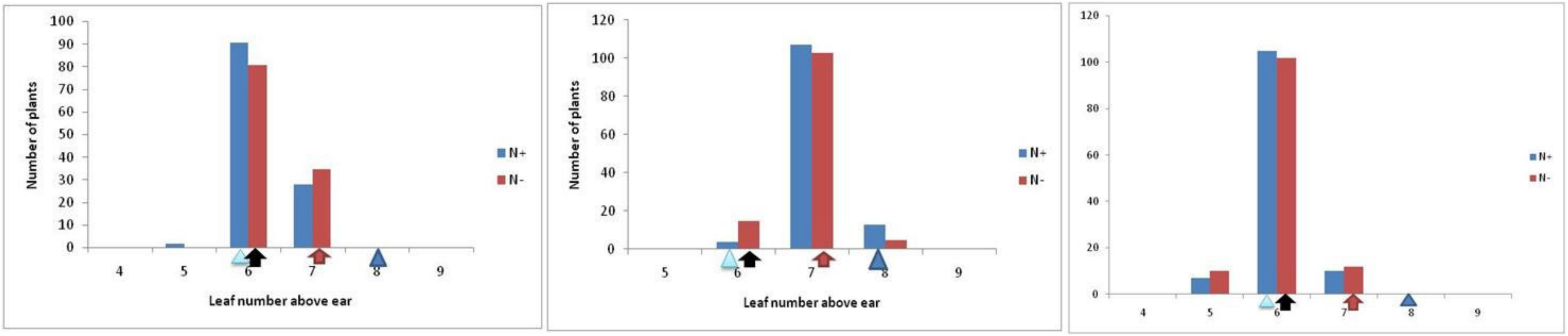


F$_2$ BCP$_1$ BCP$_2$

Figure 4.3: Frequency distributions of ear height in the segregating generations of cross T20 x N$_G$8 over N regimes

△ = high N regime also indicated as N+ (filled blue = superior parent i.e. T20; filled grey = inferior parent i.e. N$_G$8)

⇧ = low N regime also indicated as N- (filled red = superior parent i.e. T20; filled black = inferior parent i.e. N$_G$8)



F_2 BCP_1 BCP_2

Figure 4.4: Frequency distributions of leaf number above the ear in the segregating generations of cross N_G2 x C3 over N regimes

△ = high N regime also indicated as N+ (filled blue = superior parent i.e. N_G2, filled grey = inferior parent i.e. C3)

⇧ = low N regime also indicated as N- (filled red = superior parent i.e. N_G2, filled black = inferior parent i.e. C3)

4.4 Discussion

4.4.1 Separation of means

Results in Table 4.3 demonstrated that for the number of leaves in cross N_G2 x C3, the mean values of the back-crossed progenies were similar to the means of their recurrent parents, regardless of N regimes, which suggest that the two parents were contrasting for the studied traits, thus meeting the requirements of the GMA (Mather and Jinks, 1977; Checa et al., 2006). The assumption of $P_1 \neq P_2$ observed for plant height in cross T20 x N_G8 under both N regimes and for ear height only under LN in the same cross, and for the number of leaves above the ear in cross N_G2 x C3 under both conditions of N, complied with assumptions of GMA (Mather and Jinks, 1982; Kearsey and Pooni, 1996). Predominantly highest magnitudes of F_1 progenies for plant and ear heights in cross T20 x T20 x N_G8 under both N regimes may suggest preponderance of the dominance effects, which warranted the significant mid-parent heterosis (MPH) that was observed for these traits. However, the reduced MPH for plant height observed in LN could be due to the equality of BCP_1 and BCP_2 (Table 4.3), as they indicate the absence of non-allelic interactions, as Melchinger et al. (1988) asserted. These results demonstrated the possibility of improving yield by considering the plant and ear heights under low conditions of N.

The number of leaves above the ear was eight for HN and seven for LN for the superior parent (P_1), while the number of leaves did not change with the N regime for the inferior parent (P_2) (Table 4.3). Thus, the effect of N was in the range of ±1 number of leaves above the ear for the superior parent. These findings partly complied with Chase and Nanda (1967), Bonaparte and Brawn (1976), Bonaparte (1977) and Shaver (1983), who reported the stability of the number of leaves per plant across environments. These authors added that this trait may help to classify maize genotypes for maturity because it was stable over environments. However, these researchers, except Shaver (1983), reported the total number of leaves per plant, while the current study evaluated the number of leaves above the ear. None of the previous studies worked for the number of leaves above the ear under high and low N regimes. Since the stability of the number of leaves above the ear established by this study agrees with the literature, it may be concluded that this trait could also be used as a measure to classify maize genotypes. Breeding for number of leaves above the ear could be via recurrent selection as additive effects could be inferred from the situations of F_1 = F_2

implying no dominance, and F_2 nearing mid-parent, which Hayman (1958) called a complementary cross, and BCP_1 equalling BCP_2 (Melchinger et al. 1988).

However, the higher MPH at HN and its slight improvement in LN demonstrated a complementary cross as F_2 neared the mid-parent (Hayman, 1958). It has been suggested that in the absence of epistasis, the F_2 is expected to be superior for the elite x elite cross for kernels per ear in maize (Lamkey et al. 1995). The rising number of kernels per ear for the inferior local parent (P_1) under LN would suggest adaptability of the inbred line to such conditions. This could imply the possibility of creating inbred lines from the segregating generations, into which inbred line T20 is involved, in order to improve genotypes that tolerate low N, as it was practiced for cross B73 x Mo17 in the USA (Hallauer et al., 1996). This is possible, as this study demonstrated, since the segregating generations and the F_1 ranked highly for number of kernels per ear. Furthermore, improvement for the number of kernels per ear may depend on whether transgressive segregants were significant for this trait to warrant selection under high- and low-N conditions.

4.4.2 Genetic effects

To satisfy the requirement of GMA of contrasting parents for the traits under study, the genetic effects were only estimated when P_1 and P_2 were significantly different, based on LSD at a probability level of 5% (Table 4.3). Thus, genetic effects were not estimated for ear height in cross T20 x N_G8 under high N and for the number of kernels per ear in cross T20 x C58 under both N conditions. Although plant and ear heights are logically related, they appear to be controlled by different genetic systems under N regimes. Under high N, plant height was governed by additive, additive x additive and dominance x dominance effects (Table 4.4). Although the results contradict Yun-Kuei (1949) who reported plant height to be controlled by dominant effects, Bauman (1959) reported ear height to be conditioned by epistatic effects, but they did not specify the experimental conditions. Further disagreement with the present study concerns the control of ear height under low N by additive genetic effects. Seemingly, most previous studies were conducted under optimum N conditions. Preponderance of both additive and non-additive genetic effects for plant height under HN suggests reciprocal recurrent selection (RRS) for the trait. However, under LN, both plant and ear heights are the only additive effects that could be improved upon by any kind of cyclic selection that is practiced early in the selection cycles in the breeding programme. These results are consistent with Bänziger et al. (2000) and Bertin and Gallais (2000), who reported the effect of N regimes in maize. It could be suggested that raising the ear height would rationally increase the probability of an additional ear to the internodes below the primary ear, thus improving grain yield by increasing the standability of the plant to bear added cob.

The signs of estimated genetic effects were considered only when the associated effects were statistically significant, as Mather and Jinks (1977), Kearsey and Pooni (1996), Azizi et al. (2006), Smith et al. (2009) and Shashkumar et al., 2010 suggested. Statistically significant positive signs for dominance effects indicate the directional dominance of the increasing allele (Kearsey and Pooni, 1996). This was the case for the number of leaves above the ear under HN. The directional dominance indicates heterosis i.e. the relative position of parents or F_1 to the mid-parents. The sign of significant additive effects implies the choice of the parent in the cross (Cukador-Olmedo and Miller, 1997). The superior parent was P_1 for plant and ear heights, and the number of leaves above the ear and the additive effects were negative, regardless of N regimes (Table 4.4). However, Mather and Jinks (1982) and Kearsey and Pooni (1996) reported on the uncertainty of associating the genetic basis of the trait in question with the signs of genetic effects. The significant negative additive x additive effects that were observed for plant height suggest dispersion of alleles in the two parents for the respective cross (Braden, 2005). However, the positive sign for the additive x additive genetic effects that was observed for the number of leaves above the ear under high N has not been clearly explained in literature, although positive additive genetic effects would be associated with the possibility of improving the traits further. However, Azizi et al. (2006) reported dispersion of alleles in the cross when the additive x additive and additive x dominance effects had opposite signs, and that the same sign would imply association between alleles. However, this was not observed in the present study, suggesting that the dispersion and association of genetic effects is debatable.

Regarding the number of leaves above the ear, both N regimes could be amenable to RRS for the trait, due to the significance of all genetic effects except additive x dominance under high N and the preponderance of additive and additive x dominance effects under low N.

Bonaparte (1977) reported partial dominance for total leaf number per plant, but with large additive effects in one hand and the single dominant gene on the other. Lack of consistency of leaf numbers over environment and years made Bonaparte and Brawn (1976) conclude that the trait was under polygenic control, thus contradicting the findings of the present study — unless the authors specified otherwise the number of leaves and their position on the plant. Shaver (1983) asserted a quantitative inheritance (additive effects) for the trait in normal maize, as he observed a wide spread distribution of the F_2, whereas for leafy maize, the author reported a single dominant gene. This literature partly supports the present study, since additive effects were significant under N regimes (Table 4.4), although the frequency distributions in Figure 4.4 do not agree with this finding. Furthermore, the frequency distributions described in the present study may suggest few genes of discontinuous nature, thus contradicting previous research. Table 4.4 therefore suggests that the number of leaves above the ear was conditioned by a few major genes with some modifiers that exhibited some levels of additivity. The present findings established the existence of digenic epistasis of dominance x dominance and additive x dominance nature for the trait under HN and LN conditions respectively, although epistasis had not been reported previously. Kearsey and Pooni (1996) described the type of epistasis based on the direction of dominance and dominance x dominance effects. The equal positive sign refers to complementary epistasis between dominant increasers; the equal negative sign refers to complementary epistasis between dominant decreasers. The positive sign for dominance effects and negative dominance x dominance effects suggests duplicate epistasis between dominant increasers and, conversely, to duplicate epistasis between dominant decreasers. The positive duplicate epistasis observable for the trait under HN conditions does not only prove the preponderance of epistasis for the trait but it would also limit gains from selection for the trait. Kearsey and Pooni (1996), Iqbal and Nadeem (2003), Parvez et al. (2006) and Smith et al. (2009) reported that duplicate epistasis reduces genetic variation for the trait, thus reducing response to selection. However, this could still permit hybrid breeding, as Kearsey and Pooni (1996) asserted.

4.4.3 Relative contribution of genetic effects

The relative contribution of genetic effects was considered for all traits, crosses and N regimes, since the sums of squares of genetic effects contributed little to the total sums of squares of the models. Generally, the additive and dominance genetic effects had significant contribution to the total genetic variation of all traits, although additive effects had a higher contribution (Table 4.5). Regardless of N regimes, the additive and dominance effects contributed highly to plant height, thus agreeing with Yun-Kuei (1949). For ear height, the same trend was observed, although the additive effects were negligible with a HN regime. Dominance effects predominated, whereas additive effects contributed about 40% to the total genetic effects under low N. The preponderance of additive and dominance effects suggests that RRS could be employed to improve the traits. The epistatic genetic effects, which were suggested by Bauman (1959) to control the ear height were not the case with the present study, as Table 4.5 indicates only a contribution of a range of 0-8% by epistasis to both plant and ear heights under both regimes of N. The absence of epistasis for plant and ear heights could suggest that breeding strategies could be designed successfully to improve maize via plant and ear heights for both regimes of N.

The additive and dominance effects contributed greatly to total genetic effects recorded under both regimes of N for the number of kernels per ear, although the low magnitudes for the two effects were evident under the low N regime. Kernels per ear had additive x additive effects of about 18% under HN and additive x dominance effects nearing 10% and dominance x dominance effects of about 32% under LN. This suggests further that dominance effects were important under low N conditions. The importance of epistasis supports Worku et al. (2007), who reported on concerns pertaining to dealing with the interaction of maize cultivars x low N particularly in sub-Saharan Africa, where farmers apply as less fertiliser as 20 kg N ha^{-1} to their maize fields. Furthermore, an equal proportion of additive and dominance x dominance effects suggest RRS to improve the trait under LN but the 63% contribution of non-fixable genetic effects (Table 4.5) would support the strength of hybrid breeding. The number of kernels per ear could be targeted, as it is a measure of stress index (Monneveux et al., 2005) and Varga et al. (2004) added that the trait varies widely across regimes of inputs, especially in tropical maize. This could be the reason for the high variability of maize grain yields at the farmers' level in SSA. These findings could therefore support the concept that an ideal cultivar must perform consistently better under both ideal and non-ideal N regimes (Bänziger et al., 2000; Basra and Goyal, 2002), however, the hybrids may respond differently to any chemical input such as soil N, regardless of same kernel numbers per ear (Varga et al., 2004), as it was observed in Table 4.3 of the present study.

Regardless of N regimes, the additive genetic effects for the number of leaves above the ear were important to total genetic variation for the trait. Clearly, the non-additive effects of dominance and dominance x dominance under HN and additive x dominance in low N were less than 10%. This implies that recurrent or any form of cyclic selection procedure would be appropriate for the trait under two regimes of N. The prevalence of epistasis for kernels per ear under LN agrees with four studies conducted by Gorsline (1961), Perkins and Jinks (1971), Jinks et al. (1973), Wolf and Hallauer (1977), and Ceballos et al. (1998), who reported that epistasis could be a common phenomenon in non-ideal environments, although this was not the case with the number of leaves above the ear (Table 4.5).

Results indicate that epistasis conditions kernels per ear under low N conditions. This supports Wolf and Hallauer (1977), who reported epistasis as an important genetic component for the inheritance of grain yield components. Overall, Stuber (1969) reported existence of epistasis of ≤10% based on grain yield per se, the number of ears per plant, and time to tassel, which corroborated the present study. The prevalence of additive genetic effects agrees with Hallauer and Miranda (1988) and Lee et al. (2005), who reported a preponderance of additive genetic effects for most of the traits in maize.

The fixability of genetic effects varied with cross, trait and N regime (Table 4.5). Non-fixable effects predominated, although the fixable effects were still reasonable for plant height, so for ear heights, except that these effects were negligible under HN. While RRS could be practiced for plant height at both high and low N, ear height could only be improved through hybrid breeding under LN. Kernels per ear had higher fixable effects at LN than under HN. Hybrids could be improved under HN, while inbred lines could be developed at LN for the number of kernels per ear. With the number of leaves above the ear, fixable effects predominated at both N regimes, whereas non-fixable effects were negligible at LN. Recurrent selection could be important for the number of leaves above the ear under both N conditions. Bing and Guthrie (1991) reported that where dominance predominates in the F_1 genotype, improvement could be made with a back-cross approach, whereas high additive effects suggest the possibility of accumulation of desirable alleles at a number loci, so a high response to selection and h^2.

4.4.4 Frequency distributions

Transgressive segregants were observed in frequency distributions for all traits and crosses under both N regimes. All frequency distributions were continuous and normally distributed for all traits, except the number of leaves above the ear, which had discrete frequency distribution (Figures 4.1-4). Back-crossed superior progenies had higher positive segregants under both N conditions. As expected, the mean segregants for HN were higher than those under LN. The frequency distributions of segregating generations relative to their recurrent parents may help to practically strengthen the understanding of the phenomenon of transgressive segregation (Braden, 2005; Smith et al., 2009). The discrete frequency distribution for number of leaves above the ear suggests that the trait is conditioned by a few genes. A range of ±1 leaf number with environmental regimes was reported by Chase and Nanda (1967) and this is consistent with the present study, as Table 4.3 and Figure 4.4 demonstrate. The normal to near-normal frequency distributions demonstrated by plant and ear heights and the number of kernels per ear would demonstrate the polygenic inheritance of these traits. Also, the closeness of the back-crossed progenies to their recurrent parents would suggest that the traits being studied were conditioned by dominant genes. Furthermore, the long tail of the segregating generations away from the recurrent parent would indicate an absence of dominance. The parents had an equal number of kernels per ear, suggesting the absence of additive gene action (Hill et al., 1998). The frequency distributions also fitted each other across generations, demonstrating equal room for selection under both regimes of N. Equal opportunity for selection from both sides of the curves was clear for F_2 but positive selection could be evident in BCP_1 and BCP_2 for the number of kernels per ear. Also, the F_2 frequency distributions for plant and ear heights and the number of kernels per ear fitted for both regimes of N. This would justify the chance of selecting for genotypes that were better for both regimes of N, when selection was done in the centre of the curves where they merged. Contrasting inbred lines could also be developed from extreme tails of frequency distribution curves. Evidence of only positive segregants for plant and ear heights under both N conditions for all the three segregating generations (F_2, BCP_1 and BCP_2) suggest that inbred lines could be improved for these traits under such conditions. The positive segregants for the back-crossed progeny of the superior parent (i.e. BCP_1) for the number of leaves above the ear under low N suggest that inbred lines could be developed under such situations.

4.4.6 Conclusions and implications to maize breeding

1) Mid-parent heterosis was important for plant and ear heights in the cross T20 x N_G8, but it declined for plant height under low N. However it did not change for ear height under both N conditions. Both additive and non-additive genetic effects were significant for plant height under high N, whereas only additive effects were significant under low N. Additive effects were also significant for ear height under LN. Heterosis was not important for the number of leaves above the ear in the cross N_G2 x C3. Additive genetic effects were significant under both N conditions with high R^2 for the models of about 70%. However, positive duplicate epistasis was observed under high N which may compromise response to selection for this trait. The frequency distribution of the progenies was discrete, which may suggest that a few major genes which acted additively were involved in governing the number of leaves above the ear.
2) In cross T20 x C58, mid-parent heterosis for the number of kernels per ear was important under high N but it declined under low N. This was mainly controlled by genes with non-additive effects under both conditions of N.
3) With plant and ear heights and number of kernels per ear, frequency distribution curves were continuous and demonstrated transgressive segregation and polygenic inheritance for these traits under both low and high N regimes.

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Chapter 5:

Heterosis of hybrids among local and exotic inbred maize lines under low and high nitrogen conditions in Tanzania

Abstract

Despite the key role of nitrogen (N) in boosting maize production, tropical maize is still produced under low soil N, which compromises grain yield, especially in sub-Saharan Africa, because current varieties are not adapted to these conditions. This study was conducted to estimate relative grain yield loss from using reduced N dosage, determine effects of low N on secondary traits such as calendar physiological maturity and leaf chlorophyll concentration, and to assess adaptation of maize hybrids involving local and exotic germplasm lines via standard heterosis for grain yield under high and low N conditions. Fifty-four hybrids involving 48 F_1 experimental hybrids among exotic inbreds and local hybrids, plus six checks, were evaluated in a 9 x 6 alpha lattice design with two replications under 120 (HN) and 60 kg N ha^{-1} (LN), respectively, over three seasons, in Tanzania. There was a relative grain yield loss of about 30% for hybrids due to reduced N dosage. Results indicated superiority of local inbred maize lines to exotic germplasm for grain yield under both N conditions. At least six of the best 10 hybrids involved local inbred lines under HN and LN. In addition, results revealed three groups of hybrids with outstanding heterosis for yield across N regimes (four hybrids), under low N (four hybrids) and under high N (two hybrids). Most of these hybrids involved local lines underscoring the importance of exploiting local germplasm to enhance adaptation. The crosses between inbred sets exhibited higher standard heterosis under low N, compared to high N conditions. The study also suggests that earlier-maturing hybrids with appreciable yield could be developed by incorporation of exotic germplasm lines from West Africa (IITA) and Southern Africa (CIMMYT) in the maize breeding programme in Tanzania. This could be effectively exploited by improving adaptive traits such as leaf chlorophyll concentration, quick kernel dry-down rate and long effective grain filling period. It is derived from the study that grain yield can be enhanced by promoting application of fertiliser, and deploying hybrids under both LN and HN conditions.

Key words: adaptation, grain yield, maize, physiological maturity, soil nitrogen, standard heterosis.

5.1 Introduction

In sub-Saharan Africa (SSA) region, maize is typically produced by small-scale farmers under low- nitrogen (N) conditions. Farmers in this region produce maize under low N conditions for various reasons. For example, the N fertiliser is too costly; there is also inherently poor soil fertility and N levels have also been depleted through soil N mining, and use has been made of unimproved varieties that do not tolerate low soil N conditions. Thus, breeding for maize cultivars that utilise N efficiently, could be effective in minimising N input and loss of N fertilisers in the field (Wang et al., 2005), resulting in a positive impact being made on both yield and the environment. Improved maize varieties with tolerance to low soil N would be a pertinent solution in SSA, where maize grain yields are still very low and erratic (Elings, 1997; Worku et al., 2007). Although most maize grain yields in Tanzania should come from intermediate altitudes by virtue of extended land area suitable for maize production, the high altitudes contribute more maize than the intermediate and/or low altitudes combined, because hybrid breeding is well established there (Lyimo, 2006). Nevertheless, studies on heterosis and the adaptation of hybrids in intermediate altitudes, especially under low soil N have been barely conducted. Knowledge of heterosis of hybrids under these conditions may help to extract breeding populations with genes of interest that are embedded in heterotic orientations when heterosis is expressed in crosses.

The anomaly of intermediate altitudes producing a poorer grain yield than the high altitudes of the Southern Agro-ecological Zone (SHZ) could suggest that it would be advantageous to identify potential hybrids and adapt them to intermediate altitudes. Kang (1994) asserted that in the hybrid breeding programme, any foreign germplasm must be evaluated against whether it:

i) introduces new genes for a particular breeding objective, and/or
ii) is heterotic with the local germplasm under pervasive production environment.

Improved varieties have been reported to utilize available inputs efficiently compared with unimproved varieties (Echarte et al., 2008). Bänziger and Lafitte (1997) reported that maize could be improved under low N inputs by targeting secondary traits rather than grain yield per se. Therefore, varieties that are improved and adapted to local environments may be stable and could justify the profitable use of inputs by farmers.

Factors responsible for causing heterosis have not been established in literature. The paucity of the causes of heterosis may suggest taking all available opportunity to utilise and evaluate heterosis under farmers' production conditions. Heterosis is specific to cross, trait, and environment, but superior heterotic groups have been widely copied across breeding programmes to improve maize (Hallauer and Miranda, 1988; Halluer et al., 1996). Despite the importance of heterosis in maize breeding, the phenomenon is mired in uncertainty. Firstly, the primary causes of heterosis are not clear to date (Hallauer et al., 1996), and Jinks and Jones (1958) raised this concern decades ago. Secondly, the heterotic patterns that have been established to utilise heterosis in breeding programmes are arbitrary. Heterosis fades with selection and sampling, thus regular mechanisms to ensure that heterosis is not compromised should be put in place. Such mechanisms may include regular, in-house checks on heterosis (Hallauer and Miranda, 1988; Falconer, 1989) and if any foreign germplasm is required to enrich the local germplasm, it should be tested and adapted to local environments (Kang, 1994). Therefore, heterosis should have tangible benefits to any maize breeding programme. A question that has not been attempted is whether heterosis will be compromised under low-N stress because the majority of maize in SSA is produced under stressed conditions, particularly low soil N. And among abiotic stresses, the effects of low N are at least systematic, such that the trends and conditions of the effects of low soil N may be determined.

In practical terms, standard heterosis refers to the relative superiority of mean performance of F_1 hybrids over commercial genotypes or the trial mean for traits of interest (Falconer and Mackay, 1996; Hill et al., 1998) such as grain yield in maize production. Improved mean performances for characters under study may imply the presence of heterosis and if the inbred parents were a point of reference, it may suggest that the two parents are in opposite heterotic groups and, vice versa (Vasal et al., 1992; Warbuton et al., 2002). Notwithstanding, the challenge to the breeder has been to maintain the stability of heterotic patterns (Hallauer and Miranda, 1988). Therefore, studying and evaluating heterosis under any particular prevailing maize production environment in SSA may not be negotiable but rather a crucial breeding goal.

Grain yield is an ultimate character resultant from the interaction of genotype and environment. It has not yet been established in literature on what determines final yield in maize. However, final yield has been used as a measure of adaptation to local stresses (Kang, 1994; Hageman and Lambert, 1996). Maize cultivars that produce well under both high and low N conditions have been considered to use N efficiently (Kikafunda et al., 2001). Investigations on heterosis for yield would therefore improve maize production particularly under or near farmers' production conditions. This has been at least conducted elsewhere. For instance, heterosis has been established for crosses B73 x Mo17 in the USA (Hallauer et al., 1996) and elsewhere other studies have been used to establish heterosis based on genetic distances and geographical differences. Lamkey et al. (1995) associated the observed heterosis in that cross to be due to positive epistasis. It has been established that the wider the genetic and geographical diversity, the higher the heterosis of the cross. However, heterosis has not been investigated under low N conditions in tropical maize. A yield penalty may be incurred if hybrids that perform well under both conditions are not identified. Therefore, the germplasm of inbred lines adapted to tropical Africa may be crossed and evaluated for their heterosis under low N, since African maize is mainly produced under these conditions (Bänziger et al., 2000; Worku et al., 2007). Heterosis can therefore be enhanced by crossing exotic lines adapted to tropical environments with local lines from the breeding programme in Tanzania.

While the effect of low soil N in maize grain yield has been established in literature, the same effect has not been studied on calendar physiological maturity and related characters. Yet another challenge would be to incorporate high dry matter into relatively earlier maturing cultivars of maize, particularly under low N conditions. Escalating changes in socioeconomic factors and decreasing growing seasons prompt breeding for improved maize varieties that have high grain yield, are earlier maturing and which are adapted to local tropical stresses. Although farmers in the SHZ produce maize under low soil N, they require early maturing cultivars with high grain yield potential. The key question stands as what could be the effect of low soil N on grain yield potential and maturity in maize. Therefore, breeding and evaluating cultivars under low soil N is a breeding goal that must be attempted. Provided that planting seasons are followed, the propagation of earlier-maturing and high-yielding maize cultivars that are adapted to low soil N conditions may mitigate food insecurity and the risk of losing a crop when the weather is unpredictable.

The present study was conducted to determine the level of tolerance to low soil N stress in hybrids, standard heterosis of hybrids under low and high N conditions, and to determine the effect of reducing the N application on the secondary traits, such as physiological maturity dates in hybrids among local inbreds from Tanzania and exotic lines from IITA-Nigeria (West Africa) and CIMMYT-Zimbabwe (Southern Africa). The research hypotheses were that:

i) grain yield and calendar physiological maturity and component traits related to yield and maturity are not dependent on soil N levels,

ii) standard heterosis is not significantly affected by the soil N conditions, and

iii) It was also expected that heterosis of hybrids could be maximised under both conditions by crossing exotic inbreds with local inbreds.

5.2 Materials and methods

5.2.1 Experimental design and management

Random biparental crosses were made among 28 inbred lines (Table 5.1) and only 48 F_1 hybrids with enough seed, plus 6 commercial hybrid checks were evaluated in a 9 x 6 alpha lattice design in two replications under 120 and 60 kg N ha^{-1} fertiliser application regimes. The N regimes were conducted side by side at low and high N sites for three consecutive seasons of dry (DS) in 2008, wet (WS) in 2009 and DS in 2009 at the Inyala Training Institute (S08°51.011' and E033°38.227') in Mbeya Rural district in Tanzania. The trial site is a flat slope (i.e. <1% and it is elevated at ~1520 m.a.s.l which is a typical intermediate altitude in tropical maize. The SHZ Maize Improvement Programme uses Inyala for inbred line-hybrid development and population improvement trials. The rainfall pattern is unimodal which begins early December to mid-May. Day temperatures are hotter than night-time temperatures. Two row plots were over-planted in 5.1 m plot length at the spacing of 75 between- and 30 cm within rows, then thinned to one plant per hill to make 44400 plants ha^{-1}. At planting, phosphorus (P) was applied at full dose at a rate of 30 kg P_2O_5 ha^{-1} and a first split of calcium ammonium nitrate (CAN), as source of N. The other two splits of N levels were then applied at knee height and near tasseling stages. The soil was tested prior to each planting to monitor and assess the trends of soil for N, also irrigation water was tested to check its quality and effect on maize nutrition. All standard cultural practices for maize production were followed including estimation of the need for irrigating the crop using the soil moisture meter (Hadeco Model). The seasonal soil analysis data also enabled to confirm the appropriateness of trial sites for high- and low N which were consistent over the seasons. Due to seasonal variation and the effect of low N on seed yield, only 40 experimental hybrids out of 48, plus six checks, made a total of 46 entries across three seasons. Standard heterosis was therefore estimated from only 46 hybrids. These were divided into seven sets as follows: local by local, southern Africa by southern Africa, West Africa by West Africa, southern Africa by local, West Africa by southern Africa, West Africa by local and popular commercial hybrid checks.

Table 5.1: Characteristics of maize inbred lines screened and involved in the production of hybrids

Inbred	Name	Pedigree	Origin	AD	SD	Pht (cm)	Eht (cm)
T3		UYL 4-1-6-11	Tanzania	82	81	215.1	104.7
T5		UYL 10-3-21-1-2-1-1	Tanzania	80	77	159.9	65
T6		UYL145 BC3-3-1-2-2	Tanzania	84	82	215.9	98.3
T7		UYL EARLY -5-09	Tanzania	74	74	128.6	54.2
T10		UYL005 X A104/P103 BC2 S4 4-1-1	Tanzania	82	83	167.2	78.5
T12		UYL36-1 B [MOD]	Tanzania	79	77	177.3	76.2
T13		UYL36-1 C [MOD]	Tanzania	80	81	191.2	100.8
T15		UYL3758 S8 12-2-3	Tanzania	77	81	174.1	79.9
T17		UYL 141	Tanzania	76	77	146.9	51.7
T20		UYL 15-11-1-8-5-15	Tanzania	75	75	134.9	68.7
C3	VL05617	[EarlyMid1/KatumaniSR]-#-169-2-4-B-1-#-BB	CIMMYT	68	75	141.8	54.5
C4	VL054799	[[[K64R/G16SR]-39-1/[K64R/G16SR]-20-2]-5-1-2-B*4/CML390]-B-38-1-B-7-#-B*6	CIMMYT	75	76	150	60.5
C14	VL05200	[CML144/[CML144/CML395]F2-8sx]-1-2-3-2-B*4-1-B	CIMMYT	82	81	117.6	44.9
C38	VL058014	ZEWAc1F2-254-2-1-B-1-BB-1-B	CIMMYT	69	71	74	17.2
C49	VH051847	[Ent52:92SEW1-2/[DMRESR-W]EarlySel-#L-2-1-B/CML386]-B-22-1-B-4-#-1-BB/[Ent320:92SEW2-77/[DMRESR-W]EarlySel-#I-2-4-B/CML386]-B-11-3-B-2-#-BB	CIMMYT	68	68	160	65.9
C56	VH053241	ZEWBc1F2-216-2-2-B-1/[SC/CML204//FR812]-X-30-2-3-2-1-BB	CIMMYT	67	69	179.9	76.2
C58	VH0710	MAS[MSR/312]-117-2-2-1-B*5/MAS[202/312]-86-1-3-1-B*4	CIMMYT	76	75	174.4	82.4
C60	VH051969	CML395-B/[MSRXG9]C1F2-205-1(OSU23i)-5-3-X-X-1-BBB-1-BBB	CIMMYT	78	79	215.1	107
C68		[CML395/CML440//[LPSC3H144-1-2-2-2-4-#-BB/SC/ZM605#b-19-2-X]-1-2-X-1-1-BB]-1-2-1-1-B]-3-2-1-1-BBB-B-B	CIMMYT	81	82	131.9	63.8
N_G1	TZEI 1	TZE-W Pop Co S6 Inb 1-2-4	IITA	71	71	128.1	51.8
N_G2	TZEI 2	TZE-W Pop X 1368 STR S6 Inb 2	IITA	73	73	138.9	51.4
N_G3	TZEI 3	TZE-W Pop X 1368 STR S6 Inb 4	IITA	72	72	149.8	67.4
N_G5	TZEI 5	TZE-W Pop X 1368 STR S6 Inb 9	IITA	77	75	98.9	34.4
N_G6	TZEI 6	TZE-W Pop X 1368 STR S6 Inb 13	IITA	78	78	104.6	47
N_G7	TZEI 7	WEC STR S7 Inb 12	IITA	75	75	121.4	44.8
N_G8	TZEI 8	TZE-Y Pop Co S6 Inb 62-3-3	IITA	69	67	111.5	41.7
N_G15	TZEI 15	TZE Comp5-Y C6 S6 Inb 25	IITA	78	81	121.2	46.5
N_G18	TZEI 18	TZE-W Pop Co S6 Inb 136-3-3	IITA	73	72	138.9	60.8

T = Tanzania, C = CIMMYT Zimbabwe, N_G = IITA-Nigeria inbred lines, AD = 50% anthesis date, SD = 50% silking date, Pht = plant height, Eht = ear height

5.2.2 Traits measurement

The traits were measured under both regimes of N.

Calendar flowering dates i.e. 50% anthesis date (AD) and 50% silking date (SD) were recorded when half of total plants in a plot from the date of planting reached that stage.

Leaf chlorophyll concentration (LCC) in SPAD values was measured as stated in section 3.2.3 of chapter 3 of this study. Some exceptions were that the LCC was an average from 7 - 8 plants per plot, whereas under GMA, the SPAD values were recorded on individual plants. Whereas the LCC_1 was an individual plot figure, the data from LCC_2 onwards were obtained by grouping genotypes at four-day intervals based on 50% SD, thus pooling the same genotypes in different replications, due to micro-environmental differences. The LCC of each hybrid was therefore an average of SPAD value per plot from the two replications.

The kernel dry-down (KDD) index was determined according to Afuakwa and Crookston (1984), Cross and Kabir (1989), and Ma and Dwyer (2001) but with some modifications. The index was determined at the 7th week from 50% silking (i.e. a week before harvesting) by randomly sampling 100 kernels i.e. 20 kernels from 5 plants per plot. Semi-destructive sampling was done where the husks were partially opened and kernels knifed off the rachis from the middle sections of each intact cob. The husks of partially dehusked ears were then tightened with rubber bands and then covered with water proof (pollination) bags to minimise excessive wetting from precipitation (i.e. rain, dew), and moisture loss from the ears via evapotranspiration, which would affect the kernel moisture contents at harvest. Only primary ears were considered when a prolific plant was encountered. Fresh weights of these kernels were recorded and then the kernels oven dried at a constant temperature of 70^0C, until more or less constant weights were attained. Each time kernels were removed from the oven in order to record the lost kernel weight, they were kept in the desiccators in order to stabilise kernel moisture contents.

Kernel dry-down index was calculated as (fresh weight ÷ constant weight)-1.

Since the tropical maize reaches physiological maturity (marked by 50% black layer i.e. 50% BL) at 35% kernel moisture content (Lafitte, 1994), the calendar physiological maturity for the present study was therefore computed against this standard developed by CIMMYT. To demonstrate, the calculation for calendar physiological maturity, say kernel moisture content at harvest, was 20.1% (79.9% dry matter) at 166 days from planting to day of harvest. Based on CIMMYT's standard of 35% kernel moisture content (65% dry matter), physiological maturity for the present study would be:

166 days------------------------------79.9% kernel dry matter

? days ---------------------------------65% kernel dry matter

(166 days x 65% dry matter)/79.9% kernel dry matter = 135 calendar days to physiological maturity

Effective duration of grain filling period (EFPD) was computed according to Cavalieri and Smith (1985) as:

days at 50% black layer (BL) - days at 50% silking.

Grain yield (t ha^{-1}) was estimated as:

[field weight * 10000 ÷ net area*((100 - %moisture content at harvest)/85) * 0.8)] ÷ 1000, assuming grain moisture content at 15 g kg^{-1} or 15% and 80% shelling percent.

5.2.3 Data analysis

The data were analysed in SAS over three seasons using the PROC GLM procedure with blocks, entry and replications as classifying variables. Each response variable was modelled against grain yield with entry means and least significant differences calculated at 5% level of probability. The PROC means procedure was employed to calculate entry means.

Relative performance loss due to application of a low N dose was calculated as:

[(mean of trait under high N conditions-mean of same trait under low N conditions)/mean of trait under high N conditions]*100

Taking, for example, grain yield depression due to application of low N, the estimation was:

[(mean grain yield in high N conditions-mean grain yield in low N conditions)/mean grain yield in high N conditions)]*100

Standard heterosis or relative grain yield under both low N (LN) and high (HN) conditions was estimated as follows:

Trial mean heterosis = [(F_1 hybrid - trial mean)/trial mean]*100
Local check heterosis = [(F_1 hybrid - local check)/local check]*100
Best check heterosis = [(F_1 hybrid - best check)/best check]*100
Mean check heterosis = [(F_1 hybrid - mean of checks)/mean of checks]*100

Relative performance across N regimes and standard heterosis could also be a measure of N-use efficiency (NUE) of a genotype.

5.3. Results

5.3.1 Relative grain yield performance of maize hybrids under high and low nitrogen conditions

Table 5.2 indicates that the two N regimes across three seasons were highly significantly different ($P \leq 0.001$) except for the HN dry season 2008, which was statistically significant at $P \leq 0.05$. The R^2 values for modelling for grain yield were about 90%. High N in the dry season of 2008 had the highest grain yield, followed by a high N regime for the dry season in 2009. The low N conditions had the lowest yields, which further appeared to be associated with wet seasons than in dry seasons. Table 5.3 indicates that all characteristics related to maturity had R^2 values of over 80% and these features were all statistically significant, except the high N regime of dry season year 2009 for EFPD, which was non-statistically significant and had R^2 of 65.5%. Grain yield loss due to reduced N dosage ranged between 20 and 31% for three seasons (Table 5.2).

Table 5.2: Summary statistics for grain yield (tons ha^{-1}) of 54 maize hybrids across environments

Environment	R^2 (%)	CV (%)	Grain yield (ton ha^{-1})				Error mean square	LSD	Genotypes (Pr>F)
			Mean	Min	Max	Difference			
HNDS08	80.9	17.2	8.74	3.66	13.58	9.92	2.258	3.05	*
LNDS08	85.2	13.7	5.99	2.91	8.72	5.81	0.673	1.66	**
% Mean diff.			31.46						
HN WS09	86.1	13.7	5.66	2.10	9.18	7.08	0.602	1.57	**
LN WS09	93.7	13.5	4.10	1.27	8.77	7.50	0.308	1.12	**
% Mean diff.			27.56						
HN DS09	91.8	13.0	7.60	2.90	12.00	9.10	0.970	2.00	**
LN DS09	90.5	14.9	6.02	1.97	10.60	8.63	0.802	1.82	**
% Mean diff.			20.79						

**, * = statistically significant at $p \leq 0.0001$, and $p \leq 0.05$. HNDS08 = high nitrogen fertiliser application rate at 120kg N ha^{-1} for dry season year 2008, HNDS09 = high nitrogen fertiliser application rate at 120kg N ha^{-1} during dry season year 2009, HNWS09 = high nitrogen fertiliser application rate at 120kg N ha^{-1} during wet season year 2009, LNDS09 = low nitrogen fertiliser application rate at 60 kg N ha^{-1} during dry season year 2008, LNDS09 = low nitrogen fertiliser application rate at 60kg N ha^{-1} , LNWS09 = low nitrogen fertiliser application rate at 60kg N ha^{-1} , R^2 = coefficient of determination, CV = coefficient of variation, LSD ((α=0.05, 37df,critical value of t = 0.02619)

Table 5.3: Summary statistics for maturity related characters of 54 maize hybrids across environments

Environment	R^2 (%)	CV (%)	Error mean square	LSD (α=0.05)	Pr>F
			50 % anthesis date		Genotype
HNDS08	96.2	1.71	2.25	3.11	**
HNDS09	94.9	1.76	2.75	3.36	**
HNWS09	96.2	1.96	2.33	2.96	**
LNDS08	96.4	1.74	2.49	3.20	**
LNDS09	88.5	2.53	6.36	5.11	**
LNWS09	94.6	2.53	3.80	3.95	**
			50% silking date		
HNDS08	96.5	1.56	2.02	2.88	**
HNDS09	94.6	1.85	3.11	3.57	**
HNWS09	96.1	2.03	2.33	3.09	**
LNDS08	96.1	1.81	2.83	3.41	**
LNDS09	93.7	2.16	4.78	4.43	**
LNWS09	85.4	4.56	12.80	7.25	**
			Leaf chlorophyll concentration		
HNDS08	95.9	2.88	1.97	2.85	**
HNDS09	88.2	6.28	7.52	5.56	**
HNWS09	94.1	5.34	5.04	4.55	**
LNDS08	96.0	4.31	3-00	3.51	**
LNDS09	89.7	7.26	6.23	5.06	**
LNWS09	92.1	9.35	9.25	6.62	**
			Kernel dry-down index		
HNDS08	91.6	8.77	0.004	0.13	**
HNDS09	79.7	19.5	0.010	0.20	*
HNWS09	87.7	12.01	0.005	0.15	**
LNDS08	91.2	9.75	0.005	0.14	**
LNDS09	85.2	10.21	0.003	0.11	**
LNWS09	91.0	12.64	0.006	0.16	**
			Kernel moisture content		
HNDS08	87.2	3.63	0.66	1.65	**
HNDS09	84.0	4.74	0.97	2.00	*
HNWS09	92.7	5.07	0.97	2.00	**
LNDS08	91.7	3.23	0.51	1.45	**
LNDS09	91.1	2.92	0.42	1.32	**
LNWS09	92.2	5.92	1.26	2.28	**
			Effective grain filling duration		
HNDS08	88.4	4.22	3.94	4.02	**
HNDS09	65.6	1.75	84.58	18.63	ns
HNWS09	85.3	5.18	4.68	4.38	**
LNDS08	88.1	4.53	4.54	4.32	**
LNDS09	90.7	5.05	5.53	4.76	**
LNWS09	82.8	9.08	12.37	7.13	*
			Calendar physiological maturity		
HNDS08	85.6	1.04	2.07	2.91	**
HNDS09	84.0	1.24	3.29	3.68	*
HNWS09	92.4	1.36	2.53	3.23	**
LNDS08	92.1	0.92	1.64	2.60	**
LNDS09	90.9	0.84	1.56	2.53	**
LNWS09	92.4	1.36	2.53	3.23	**

**, * = statistically significant at $p \leq 0.0001$, and $p \leq 0.05$; ns = statistically non-significant. HN and LN = high and low N; DS and WS = dry and wet seasons; 08 and 09 = 2008 and 2009

Figure 5.1 indicates the seasonal average of yield loss for hybrids ranging from 30-40% in DS08, 25-35% in WS09, and 25-40% in DS09. Relative yield loss of about -80% was observed at a frequency of one in dry season year 2009. Frequency distributions of hybrids for relative yield loss tended to normal curves in DS08 and WS09. However, DS09 had bimodal distribution, although the trend of the normal frequency curve was clear on the right-hand side of the bimodal curve. Generally, all the three frequency curves had the mean centred around 30% yield loss due to application of low N.

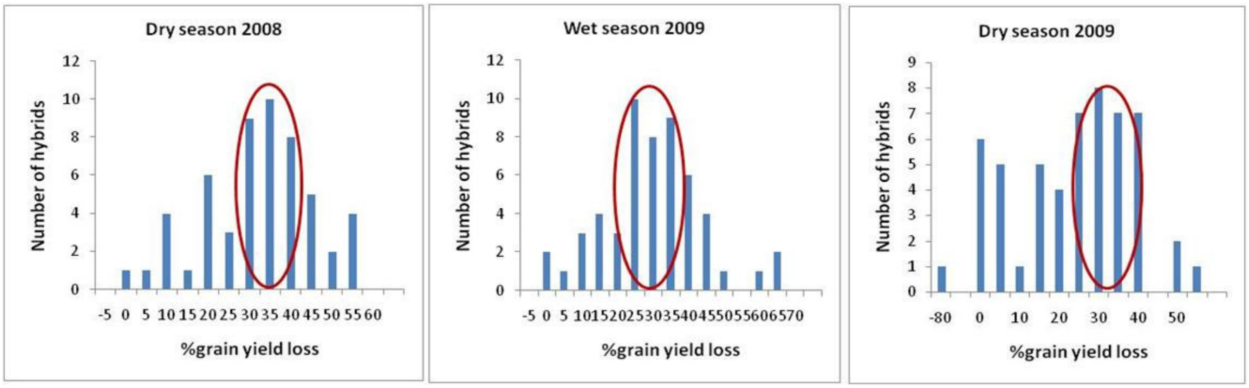

Figure 5.1: Frequency distributions of relative grain yield loss indices (%) for 54 hybrids due to application of low rate of N in different seasons

Table 5.4 shows mean and relative yield loss due to the application of low N. High N had a site mean of 7.46, with ranges from 3.88 to 9.45 t ha^{-1}. The range for low N was from 2.69 to 7.56, with a site mean of 5.44 t ha^{-1}. An average of 26.7% yield loss, due to low N application, was evident with a range from 6.1 to 43.2%. The results regarding the best and worst ten hybrids, due the response of high and low N, are also demonstrated in Table 5.4. Six and eight out of the best 10 hybrids involved local inbred lines under high and low N, respectively. Hybrids N_G2 x T3, T20 x T15, T5xT17, T6 x T13 and commercial check SC513 were consistent for both regimes of N. For the relative yield loss, hybrids C60 x T5, N_G18 x C4, N_G3 x T5, and T17 x C4 were consistent with low N with five out of 10 hybrids involving local inbred lines. There was no consistency between yield loss and high N.

For poor performance of hybrids in high N, except T3 x T5, all hybrids involved exotic crosses. Under low N, two hybrids involving local inbreds, including local check, were also observed for poor performance. Six out of 10 hybrids involving inbreds from IITA Nigeria were consistently poor yielders under both conditions of N. Considering the relative yield loss on the poor end, checks PAN77 and PAN67, and four out of 10 hybrids involving local inbreds performed poorly. Whereas only N_G2 x N_G1 was consistently poor yielder under both N regimes, hybrids N_G18 x N_G7, T15 x T12, T13 x T15 and N_G18 x T12 matched relative yield loss and low N.

Hybrids N_G18 x T12 and C58 x C4, which were among the best 10 yielders under high N, were poor in the relative yield loss under low N. Hybrids C60 x C68, C49 x C3, and T3 x T5, grown in high N on the poor side, were consistent with high relative yield loss on the list of the best end. Under low N the best 10 hybrids were not consistent for both yield performance and relative yield loss.

Entries T5 x T17, T20 x T15, T17 x T7, C58 x C38, C60 x T5, T17 x C4, N_G2 x T3, N_G3 x T5, and T15 x N_G7 were above the grand mean for both and individual regimes of N. In terms of individual sets, the local x local and West Africa x local sets were above average for both, and individual N regimes, but they were only slightly above the mean for tolerance to low N. However, southern Africa x local set and check SC513 were above the mean for both and individual regimes of N. Southern Africa x southern Africa, West Africa x West Africa sets were below the grand average for HN and LN, however, southern Africa x southern Africa had low tolerance to low N, which is advantageous in breeding for a wide adaptation across N regimes. By looking at means of sets, west Africa x local (8.23), check (7.96), local x local (7.89), and southern Africa x local (7.85) were above average for HN; southern Africa x local (6.26), west Africa x local (5.98), local x local (5.74), West Africa x southern Africa (5.46) were above average for LN; West Africa x local (7.13), southern Africa x local (7.05), local x local (6.82) and check (6.68 t ha^{-1}) were above grand mean across N regimes; for tolerance to LN, southern Africa x local (20.03%), West Africa x southern Africa (25.73%) and southern Africa x southern Africa (26.31%) were superior. Overall, best hybrid combinations were found between local and exotic inbred lines. Low N conditions gave lower yields than high N.

Table 5.4: Mean grain yield and relative yield loss of maize hybrids under high and low N conditions evaluated for three seasons

			High N		Low N		Across N regimes		Low N tolerance	
Entry#	Pedigree	Hybrid Set	t ha^{-1}	rank	t ha^{-1}	rank	t ha^{-1}	Rank	% yield loss	rank
1	T3 x T5	L x L	5.52	44.00	4.92	36.00	5.22	40.00	10.90	2.00
2	T15 x T12	L x L	6.79	33.00	4.26	42.00	5.53	37.50	37.20	43.00
3	T5 x T17	L x L	9.06	5.00	6.76	5.00	7.91	5.00	25.30	17.00
4	T7 x T20	L x L	8.17	16.00	5.37	25.00	6.77	20.50	34.20	38.00
5	T20 x T15	L x L	9.13	4.00	7.32	2.00	8.23	3.00	19.80	12.00
6	T17 x T7	L x L	8.01	18.00	5.90	15.00	6.96	16.50	26.30	20.00
7	T13 x T15	L x L	7.43	27.00	4.60	38.00	6.02	32.50	38.10	44.00
8	T6 x T13	L x L	8.99	6.00	6.82	3.00	7.91	4.50	24.20	15.00
	Set mean (n=8)		7.89	19.13	5.74	20.75	6.82	19.94	27.00	23.88
9	C56 x C58	S x S	7.54	24.00	5.13	31.00	6.34	27.50	32.00	32.00
10	C58 x C38	S x S	7.48	26.00	5.55	19.00	6.52	22.50	25.80	19.00
11	C4 x C56	S x S	7.96	20.00	5.15	30.00	6.56	25.00	35.30	40.00
12	C49 x C3	S x S	6.13	39.00	4.95	35.00	5.54	37.00	19.40	9.00
13	C14 x C58	S x S	6.74	34.00	5.45	22.00	6.10	28.00	19.10	8.00
14	C58 x C4	S x S	9.14	3.00	5.35	27.00	7.25	15.00	41.50	45.00
15	C60 x C68	S x S	6.16	38.00	5.48	21.00	5.82	29.50	11.10	3.00
	Set mean (n=7)		7.31	26.29	5.29	26.43	6.30	26.36	26.31	22.29
16	N_G18 x N_G7	W x W	7.20	28.00	4.69	37.00	5.95	32.50	34.90	39.00
17	N_G2 x N_G1	W x W	5.74	42.00	3.71	45.00	4.73	43.50	35.40	41.00
18	N_G2 x N_G18	W x W	6.00	40.00	4.51	40.00	5.26	40.00	24.90	16.00
19	N_G18 N_G3	W x W	5.64	43.00	4.52	39.00	5.08	41.00	19.80	11.00
20	N_G2 x N_G3	W x W	3.88	46.00	2.69	46.00	3.29	46.00	30.80	30.00
21	N_G15 x N_G8	W x W	5.35	45.00	3.71	44.00	4.53	44.50	30.60	28.00

			High N		Low N		Across N regimes		Low N tolerance	
Entry#	Pedigree	Hybrid Set	t ha^{-1}	rank	t ha^{-1}	rank	t ha^{-1}	Rank	% yield loss	rank
22	N_G7 x N_G15	W x W	6.34	37.00	5.02	34.00	5.68	35.50	20.90	13.00
	Set mean (n = 7)		5.74	40.14	4.12	40.71	4.93	40.43	28.19	25.43
23	C49 x T7	S x L	7.10	30.00	5.13	32.00	6.12	31.00	27.70	23.00
24	C60 x T5	S x L	8.06	17.00	7.56	1.00	7.81	9.00	6.10	1.00
25	T12 x C38	S x L	6.71	35.00	5.95	14.00	6.33	24.50	11.30	4.00
26	C3 x T3	S x L	8.59	8.00	6.03	13.00	7.31	10.50	29.80	27.00
27	T17 x C4	S x L	8.40	11.00	6.74	6.00	7.57	8.50	19.70	10.00
28	T15 x C14	S x L	8.23	15.00	6.12	11.00	7.18	13.00	25.60	18.00
	Set mean (n = 6)		7.85	19.33	6.26	12.83	7.05	16.08	20.03	13.83
29	C49 x N_G18	W x S	6.90	32.00	5.74	17.00	6.32	24.50	16.80	7.00
30	N_G7 x C56	W x S	7.54	25.00	5.42	24.00	6.48	24.50	28.20	25.00
31	N_G18 x C4	W x S	7.15	29.00	6.22	9.00	6.69	19.00	12.90	5.00
32	C3 x N_G3	W x S	5.80	41.00	3.90	43.00	4.85	42.00	32.70	36.00
33	C68 x N_G2	W x S	8.93	7.00	6.04	12.00	7.49	9.50	32.40	35.00
34	N_G1 x C58	W x S	7.93	21.00	5.44	23.00	6.69	22.00	31.40	31.00
	Set mean (n =6)		7.38	25.83	5.46	21.33	6.42	23.58	25.73	23.17
35	N_G2 x T3	W x L	9.20	2.00	6.77	4.00	7.99	3.00	26.40	21.00
36	N_G3 x T5	W x L	7.61	23.00	6.49	8.00	7.05	15.50	14.70	6.00
37	T7 x N_G1	W x L	7.07	31.00	5.08	33.00	6.08	32.00	28.10	24.00
38	N_G18 x T12	W x L	9.45	1.00	5.37	26.00	7.41	13.50	43.20	46.00
39	N_G15 x T10	W x L	7.98	19.00	5.65	18.00	6.82	18.50	29.30	26.00
40	T15 x N_G7	W x L	8.37	12.00	6.52	7.00	7.45	9.50	22.10	14.00

			High N		Low N		Across N regimes		Low N tolerance	
Entry#	Pedigree	Hybrid Set	t ha^{-1}	rank	t ha^{-1}	rank	t ha^{-1}	Rank	% yield loss	rank
	Set mean (n = 6)		8.28	14.67	5.98	16.00	7.13	15.33	27.30	22.83
41	SC513	Check	8.43	10.00	6.18	10.00	7.31	10.00	26.60	22.00
42	PAN77	Check	8.37	13.00	5.52	20.00	6.95	16.50	34.00	37.00
43	PAN67	Check	8.26	14.00	5.21	29.00	6.74	21.50	36.90	42.00
44	DKC8033	Check	8.51	9.00	5.79	16.00	7.15	12.50	32.00	33.00
45	SC407	Check	7.77	22.00	5.28	28.00	6.53	25.00	32.10	34.00
46	LOCAL	Check	6.40	36.00	4.43	41.00	5.42	38.50	30.80	29.00
	Set mean (n =6)		7.96	17.33	5.40	24.00	6.68	20.67	32.07	32.83
	Minimum		3.88		2.69		3.29		6.13	
	Maximum		9.45		7.56		8.23		43.18	
	Mean		7.46		5.44		6.46		26.70	

Key: T = Tanzania, C = CIMMYT Zimbabwe, N_G = IITA-Nigeria inbred lines, N = nitrogen, high N = 120 kg N ha^{-1} fertiliser application rate, Low N= 60 kg N ha^{-1} fertiliser application rate. L x L = local by local set, S x S = southern Africa by southern Africa set, W x W = West Africa by West Africa set, S x L = southern Africa by local set, W x S = West Africa by southern Africa, W x L = West Africa by local, check = commercial hybrid check

5.3.2 Relative effects of low nitrogen on maturity characters in maize hybrids

Table 5.5 indicates the relative effects of application of low N to maize hybrids over seven sets of categories, based on the origin of crosses and commercial checks. Generally, the effects of low N were not significant for kernel moisture content at harvest (KMC) and calendar physiological maturity. The overall minimum and maximum values for KMC were -1.2 and -0.2% and the means of all the sets were negative. Within sets, all checks and the southern Africa x local set were negative, while the other sets had mixed signs of positive and negative. The overall calendar physiological maturity had 1 and -3 maximum and minimum days. The overall mean was -1 day. Means of sets were all negative.

The calendar flowering dates (i.e. 50% AD and SD), EFPD, KDD and LCC were affected by regimes of N. Both 50% AD and 50% SD had an overall mean of -4 days and all the values were negative. The range was -1 to -9 and -1 to -10 for 50% AD and 50% SD, respectively. The maximum 50% AD across sets ranged from -4 days (local x local set) to -9 days (southern Africa x west Africa), whereas for 50% flowering, SD ranged from -6 (local x local, West Africa x West Africa, southern Africa x local) to -10 days (West Africa x local). The EFPD had all positive values. The days were longest for hybrids N_G12 x T12 (10), PAN67 and C58 x C4 (7 days each), N_G18 x C4 (6), T13 x T15, N_G15 x N_G8, C3 x N_G3 (5), the southern Africa x local set had <5 days. Leaf chlorophyll concentration had an overall mean of 21% and all the values were positive. The minimum LCC was 12% for hybrids C49 x C3 (southern Africa x southern Africa), and C60 x T5 (southern Africa x local). The maximum value was 33% for the check hybrid PAN67. The (southern Africa x southern Africa), and (southern Africa x local) sets had a LCC loss below the overall mean. The index of KDD had the overall mean of -4. All sets had a loss of 10% as maximum values, except the West Africa x West Africa set. High negative magnitudes were for hybrids C3 x N_G3 (39), N_G18 x T12 (16), C60 x C68, check DKC8033, (15), T15 x T12, C60 x T5 (12) and check PAN67 (10). However, hybrid T17 x T7 had a positive index of KDD of 16%. The set means were higher for southern Africa x western Africa (-9%) and check (-8%). Generally it was evident that among the studied features for maturity, the flowering dates (50% AD and SD), LCC, KDD and EFPD were only affected by N regimes, whereas low N had low effects on percent kernel moisture content at harvest and the calendar physiological maturity.

Table 5.5: Mean performance of maturity-related features under high and low nitrogen and relative performance loss due to application of low nitrogen for 46 hybrids over three seasons

a) Calendar days

			50% AD			50% SD			EFPD			P.maturity		
			Calendar days											
S/no.	Hybrid	Set	HN	LN	difference	HN	LN	difference	HN	LN	difference	HN	LN	difference
1	T3 x T5	L x L	94.3	96.0	-2	94.0	96.3	-2	43.0	41.9	1	137.0	138.3	-1
2	T15 x T12	L x L	91.5	94.8	-3	94.0	99.2	-5	42.7	38.3	4	136.7	137.4	-1
3	T5 x T17	L x L	86.5	88.8	-2	87.2	89.2	-2	49.5	49.0	1	136.7	138.1	-1
4	T7 x T20	L x L	87.3	88.3	-1	87.7	92.2	-5	44.6	42.2	2	132.3	134.4	-2
5	T20 x T15	L x L	88.8	90.0	-1	90.2	93.2	-3	44.9	43.8	1	135.1	137.0	-2
6	T17 x T7	L x L	84.5	87.8	-3	85.0	88.8	-4	47.2	45.2	2	132.2	134.1	-2
7	T13 x T15	L x L	94.3	98.2	-4	96.2	101.7	-6	41.3	36.6	5	137.5	138.3	-1
8	T6 x T13	L x L	93.2	95.5	-2	93.7	97.3	-4	42.9	39.4	4	136.5	136.8	0
	Set mean		90.1	92.4	-2	91.0	94.7	-4	44.5	42.1	2	135.5	136.8	-1
9	C56 x C58	S x S	84.5	85.8	-1	85.0	89.2	-4	48.9	46.6	2	133.9	135.7	-2
10	C58 x C38	S x S	81.2	83.0	-2	83.3	85.7	-2	47.7	47.3	0	131.1	133.0	-2
11	C4 x C56	S x S	81.3	85.3	-4	82.3	85.5	-3	49.7	47.1	3	132.0	132.6	-1
12	C49 x C3	S x S	79.3	80.2	-1	81.8	83.5	-2	49.7	48.9	1	131.5	132.4	-1
13	C14 x C58	S x S	90.3	93.8	-4	91.0	95.5	-5	45.5	43.0	3	136.5	138.5	-2
14	C58 x C4	S x S	82.8	87.3	-5	83.3	91.3	-8	48.6	41.8	7	131.9	133.1	-1
15	C60 x C68	S x S	89.8	93.8	-4	91.5	97.5	-6	42.9	39.9	3	136.3	137.9	-2
	Set mean		84.2	87.0	-3	85.5	89.7	-4	47.6	44.9	3	133.3	134.7	-1
16	N_G18 x N_G7	W x W	81.0	87.0	-6	84.3	89.5	-5	47.8	44.4	3	132.1	133.9	-2
17	N_G2 x N_G1	W x W	81.3	88.2	-7	84.5	90.5	-6	45.7	41.5	4	130.2	132.0	-2
18	N_G2 x N_G18	W x W	84.3	88.0	-4	85.7	89.0	-3	44.3	43.9	0	130.0	132.9	-3

			50% AD			50% SD			EFPD			P.maturity		
								Calendar days						
S/no.	Hybrid	Set	HN	LN	difference	HN	LN	difference	HN	LN	difference	HN	LN	difference
19	N_G18 N_G3	W x W	82.5	87,0	-5	85.5	88.8	-3	46.6	44.6	2	132.1	133.4	-1
20	N_G2 x N_G3	W x W	83.3	87.2	-4	86.2	88.8	-3	47.4	46.8	1	133.6	135.6	-2
21	N_G15 X N_G8	W x W	81.0	85.0	-4	82.3	86.8	-5	48.4	43.4	5	130.7	130.1	1
22	N_G7 X N_G15	W x W	83.8	88.5	-5	88.5	91.2	-3	44.2	42.4	2	132.7	133.6	-1
	Set mean		82.4	87.3	-5	85.3	89.2	-4	46.3	43.9	2	131.6	133.1	-2
23	C49 x T7	S x L	82.0	85.3	-3	81.5	86.0	-5	48.6	44.7	4	130.1	130.7	-1
24	C60 x T5	S x L	87.8	92.3	-5	88.3	92.5	-4	45.8	43.2	3	134.0	135.7	-2
25	T12 x C38	S x L	80.5	83.5	-3	81.3	84.3	-3	45.3	43.2	2	126.5	127.5	-1
26	C3 x T3	S x L	83.5	86.3	-3	85.8	90.3	-5	46.6	43.1	4	132.3	133.4	-1
27	T17 x C4	S x L	80.3	86.0	-6	80.3	85.8	-6	50.8	48.5	2	134.6	136.6	-2
28	T15 x C14	S x L	89.0	93.5	-5	90.3	96.5	-6	44.1	40.3	4	137.1	138.6	-2
	Set mean		83.8	87.8	-4	84.5	89.2	-5	46.8	43.8	3	132.4	133.8	-1
29	C49 x N_G18	W x S	81.5	86.0	-5	82.0	86.8	-5	48.6	45.8	3	133.0	134.3	-1
30	N_G7 x C56	W x S	80.0	82.8	-3	80.5	83.5	-3	48.7	46.1	3	129.2	129.6	0
31	N_G18 x C4	W x S	84.3	88.3	-4	85.5	93.0	-8	44.7	38.9	6	130.2	131.9	-2
32	C3 x N_G3	W x S	80.7	83.3	-3	80.3	86.0	-6	49.1	43.7	5	129.4	129.7	0
33	C68 x N_G2	W x S	83.5	92.5	-9	85.5	90.8	-5	47.1	43.6	4	132.6	134.3	-2
34	N_G1 x C58	W x S	83.3	88.3	-5	86.2	88.5	-2	47.0	45.9	1	133.2	135.2	-2
	Set mean		82.2	86.8	-5	83.3	88.1	-5	47.5	44.0	4	131.3	132.5	-1
35	N_G2 x T3	W x L	86.5	89.5	-3	86.5	90.3	-4	46.5	44.2	2	133.0	134.4	-1
36	N_G3 x T5	W x L	87.5	88.8	-1	88.0	88.8	-1	47.2	46.7	1	135.2	135.5	0
37	T7 x N_G1	W x L	82.0	84.5	-3	82.5	84.8	-2	46.7	46.0	1	129.2	130.8	-2

			50% AD			50% SD			EFPD			P.maturity		
								Calendar days						
S/no.	Hybrid	Set	HN	LN	difference	HN	LN	difference	HN	LN	difference	HN	LN	difference
38	N_G18 x T12	W x L	86.5	89.5	-3	86.3	96.5	-10	44.4	34.6	10	130.7	131.1	0
49	N_G15 x T10	W x L	85.5	89.5	-4	88.3	92.5	-4	44.3	40.6	4	132.6	133.1	-1
40	T15 x N_G7	W x L	86.8	91.5	-5	89.5	93.8	-4	44.7	40.4	4	134.2	134.2	0
	Set mean		85.8	88.9	-3	86.8	91.1	-4	45.6	42.1	4	132.5	133.2	-1
41	SC513	Check	85.0	88.8	-4	85.8	90.3	-5	47.5	44.4	3	133.3	134.7	-1
42	PAN77	Check	88.3	91.8	-4	89.0	93.5	-5	44.1	41.1	3	133.1	134.6	-2
43	PAN67	Check	86.5	91.8	-5	85.0	93.3	-8	48.7	41.8	7	133.7	135.0	-1
44	DKC8033	Check	86.3	91.7	-5	87.2	92.2	-5	45.9	43.5	2	133.1	135.7	-3
45	SC407	Check	83.3	88.0	-5	86.2	90.8	-5	47.6	44.6	3	133.8	135.5	-2
46	LOCAL	Check	94.3	100.0	-6	97.3	98.3	-1	40.7	40.7	0	138.0	139.1	-1
	Set mean		87.3	92.0	-5	88.4	93.1	-5	45.8	42.7	3	134.2	135.7	-2
	Minimum		79.3	80.2	-9	80.3	83.5	-10	40.7	34.6	0	126.5	127.5	-3
	Maximum		94.3	100.0	-1	97.3	101.7	-1	50.8	49.0	10	138.0	139.1	1
	Range		15	19.8	8	17.0	18.2	9	10.1	14.4	10	11.5	11.6	4
	Grand mean		85.3	89..0	-4	86.6	90.9	-4	46.3	43.3	3	133.1	134.4	-1

Key: AD = calendar 50% silking date, SD = calendar 50% silking date, EFPD = calendar effective grain fill period, P.mat = calendar physiological maturity, T = Tanzania, C = CIMMYT Zimbabwe, N_G = IITA-Nigeria inbred lines, N = nitrogen, HN (high nitrogen) = 120 kg N ha^{-1} fertiliser application rate, LN (low nitrogen) = 60 kg N ha^{-1} fertiliser application rate. L x L = local by local set, S x S = southern Africa by southern Africa set, W x W = West Africa by West Africa set, S x L = southern Africa by local set, W x S = West Africa by southern Africa, W x L = West Africa by local, check = commercial hybrid check

b) Percent loss

			LCC			KDD			KMC at harvest	
		SPAD units			Index			Percent		
Hybrid	Set	HN	LN	% loss	HN	LN	% loss	HN	LN	difference
T3 x T5	L x L	31.8	25.8	19	0.83	0.79	4	22.8	23.1	-0.3
T15 x T12	L x L	39.8	30.5	23	0.70	0.78	-12	22.6	22.6	0.0
T5 x T17	L x L	43.1	37.1	14	0.68	0.70	-2	22.6	23.0	-0.4
T7 x T20	L x L	45.3	36.8	19	0.59	0.57	3	19.9	20.8	-0.9
T20 x T15	L x L	44.7	33.3	26	0.64	0.66	-4	21.7	22.3	-0.6
T17 x T7	L x L	48.0	40.7	15	0.59	0.50	16	19.8	20.6	-0.8
T13 x T15	L x L	37.1	28.6	23	0.82	0.90	-9	23.0	23.1	-0.1
T6 x T13	L x L	39.1	28.7	27	0.77	0.77	0	22.5	22.2	0.3
Set mean		41.1	32.7	21	0.70	0.71	-1	21.9	22.2	-0.3
C56 x C58	S x S	45.3	35.6	21	0.56	0.59	-5	21.0	21.5	-0.5
C58 x C38	S x S	49.4	41.7	16	0.55	0.56	-2	19.2	19.9	-0.7
C4 x C56	S x S	47.5	38.3	19	0.56	0.56	0	19.9	19.6	0.3
C49 x C3	S x S	51.3	45.1	12	0.50	0.48	5	19.4	19.5	-0.1
C14 x C58	S x S	42.6	33.5	21	0.69	0.75	-8	22.5	23.2	-0.7
C58 x C4	S x S	44.3	35.2	21	0.56	0.61	-9	20.4	20.9	-0.5
C60 x C68	S x S	40.6	30.3	25	0.69	0.79	-15	22.4	22.8	-0.4
Set mean		45.9	37.1	19	0.59	0.62	-5	20.7	21.0	-0.3
N_G18 x N_G7	W x W	47.0	33.5	29	0.53	0.54	-2	19.8	20.4	-0.6
N_G2 x N_G1	W x W	43.5	31.6	27	0.50	0.51	-2	18.6	19.2	-0.6
N_G2 x N_G18	W x W	46.6	38.2	18	0.54	0.50	8	18.6	19.8	-1.2
N_G18 N_G3	W x W	46.8	37.3	20	0.52	0.51	2	19.8	20.1	-0.3
N_G2 x N_G3	W x W	40.8	32.4	21	0.58	0.58	0	20.8	21.4	-0.6
N_G15 X N_G8	W x W	41.2	31.8	23	0.45	0.47	-4	19.7	19.1	0.6
N_G7 X N_G15	W x W	43.5	37.0	15	0.63	0.61	2	20.2	20.2	0.0
Set mean		44.2	34.5	22	0.54	0.53	1	19.6	20.0	-0.4
C49 x T7	S x L	49.3	37.5	24	0.50	0.52	-4	19.3	19.6	-0.3
C60 x T5	S x L	38.8	34.2	12	0.63	0.70	-12	21.6	22.5	-0.9
T12 x C38	S x L	45.8	37.8	18	0.46	0.46	-1	16.9	17.3	-0.4
C3 x T3	S x L	40.3	34.0	16	0.55	0.54	1	20.6	21.2	-0.6
T17 x C4	S x L	49.5	41.0	17	0.64	0.66	-4	21.4	22.0	-0.6
T15 x C14	S x L	40.4	31.1	23	0.73	0.73	-1	22.8	23.3	-0.5
Set mean		44.0	35.9	18	0.58	0.60	-3	20.4	21.0	-0.6

		LCC			KDD			KMC at harvest		
		SPAD units			Index			Percent		
Hybrid	Set	HN	LN	% loss	HN	LN	% loss	HN	LN	difference
C49 x N_G18	W x S	51.0	41.6	18	0.52	0.54	-5	20.4	20.8	-0.4
N_G7 x C56	W x S	45.5	37.7	17	0.44	0.48	-8	18.7	18.8	-0.1
N_G18 x C4	W x S	45.3	34.4	24	0.49	0.53	-9	19.3	20.3	-1.0
C3 x N_G3	W x S	45.1	35.7	21	0.39	0.55	-39	18.6	18.5	0.1
C68 x N_G2	W x S	41.2	29.9	27	0.62	0.59	5	20.7	21.6	-0.9
N_G1 x C58	W x S	47.3	38.1	19	0.58	0.58	0	20.5	21.2	-0.7
Set mean		45.9	36.2	21	0.51	0.54	-9	19.7	20.2	-0.5
N_G2 x T3	W x L	37.2	28.6	23	0.65	0.67	-2	21.0	21.8	-0.8
N_G3 x T5	W x L	38.3	33.2	13	0.65	0.64	1	22.4	22.4	0.0
T7 x N_G1	W x L	46.6	39.4	15	0.47	0.48	-2	18.7	19.5	-0.8
N_G18 x T12	W x L	45.4	33.0	27	0.50	0.58	-16	19.6	19.7	-0.1
N_G15 x T10	W x L	37.6	27.2	28	0.53	0.55	-5	20.8	20.9	-0.1
T15 x N_G7	W x L	39.1	30.4	22	0.58	0.61	-4	21.9	21.6	0.3
Set mean		40.7	32.0	22	0.56	0.59	-5	20.7	21.0	-0.3
SC513	Check	44.3	33.9	24	0.66	0.71	-8	21.2	22.0	-0.8
PAN77	Check	43.7	31.1	29	0.62	0.66	-8	21.1	21.8	-0.7
PAN67	Check	45.2	30.4	33	0.57	0.63	-10	21.5	22.1	-0.6
DKC8033	Check	50.5	42.2	17	0.65	0.74	-15	20.4	21.5	-1.1
SC407	Check	44.9	34.5	23	0.62	0.64	-2	20.9	21.4	-0.5
LOCAL	Check	36.6	28.0	23	0.83	0.90	-8	23.4	23.6	-0.2
Set mean		44.2	33.3	25	0.66	0.71	-8	21.4	22.1	-0.7
Minimum		31.8	25.8	12	0.39	0.46	-39	16.9	17.3	-1.2
Maximum		51.3	45.1	33	0.83	0.90	16	23.4	23.6	-0.2
Range		19.5	19.3	21	0.44	0.44	55	6.5	6.3	1.0
Grand mean		43.7	34.5	21	0.59	0.62	-4	20.7	21.1	-0.4

Key: SG = mean leaf chlorophyll concentration in SPAD units, KDD = kernel dry down, KMC = percent kernel moisture content at harvest, T = Tanzania, C = CIMMYT Zimbabwe, N_G = IITA-Nigeria inbred lines, N = nitrogen, HN (high nitrogen) = 120 kg N ha^{-1} fertiliser application rate, LN (low nitrogen) = 60 kg N ha^{-1} fertiliser application rate. L x L = local by local set, S x S = southern Africa by southern Africa set, W x W = West Africa by West Africa set, S x L = southern Africa by local set, W x S = West Africa by southern Africa, W x L = West Africa by local, check = commercial hybrid check

5.3.3 Standard heterosis for grain yield under high and low nitrogen conditions

Hybrids T5 x T17, T20 x T15, T6 x T13, and N_G2 x T3 had positive standard heterosis across N regimes (Table 5.6). Entries C60 x T5, T17 x C4, N_G3 x T5, and T15 x N_G7 had positive heterosis under low N. Hybrids C58 x C4 and N_G18 x T12 mostly performed better under high N.

The West Africa x West Africa set had negative values for all types of heterosis that were studied (Table 5.6). Local check heterosis was higher in magnitudes, especially for LN than HN across sets, except for the check set. The best check heterosis was negative across hybrid sets and of relatively equal magnitude for sets in both N conditions, except for southern Africa x local, where HN had negative heterosis of 7.8% and insignificant positive heterosis under LN. The check set heterosis for LN doubled heterosis under HN. Heterosis for trial mean tripled under LN, compared to HN for the southern Africa x local set. Also, mean check heterosis was positive and high for this set under LN conditions, compared with HN, which was insignificant. The general trend was that the between-sets crosses had higher values of heterosis under LN, compared with HN conditions.

Table 5.6: Standard heterosis for relative grain yield loss due to application of low rate of nitrogen

Sno.	Hybrid	Hybrid set	Heterosis (%)							
			Trial mean		Local check		Best check		Mean check	
			HN	LN	HN	LN	HN	LN	HN	LN
1	T3 x T5	L x L	-26.1	-9.6	-13.8	11.0	-35.2	-20.4	-30.7	-9.0
2	T15 x T12	L x L	-9.0	-21.6	6.1	-3.8	-20.2	-31.0	-14.7	-21.1
3	T5 x T17	L x L	21.4	24.3	41.5	52.6	6.4	9.4	13.8	25.2
4	T7 x T20	L x L	9.5	-1.2	27.6	21.3	-4.0	-13.0	2.6	-0.5
5	T20 x T15	L x L	22.5	34.6	42.7	65.3	7.3	18.5	14.8	35.6
6	T17 x T7	L x L	7.4	8.5	25.2	33.3	-5.9	-4.5	0.7	9.3
7	T13 x T15	L x L	-0.4	-15.4	16.1	3.9	-12.7	-25.5	-6.7	-14.8
8	T6 x T13	L x L	20.5	25.4	40.5	54.0	5.7	10.4	13.0	26.3
	Set mean		5.7	5.6	23.2	29.7	-7.3	-7.0	-0.9	6.4
9	C56 x C58	S x S	1.1	-5.7	17.9	15.9	-11.3	-17.0	-5.2	-5.0
10	C58 x C38	S x S	0.2	2.0	16.9	25.2	-12.1	-10.2	-6.0	2.7
11	C4 x C56	S x S	6.6	-5.4	24.3	16.2	-6.5	-16.7	-0.1	-4.7
12	C49 x C3	S x S	-17.8	-9.1	-4.1	11.6	-27.9	-20.0	-22.9	-8.4
13	C14 x C58	S x S	-9.7	0.2	5.3	23.1	-20.8	-11.8	-15.3	1.0
14	C58 x C4	S x S	22.5	-1.7	42.8	20.8	7.4	-13.4	14.8	-0.9
15	C60 x C68	S x S	-17.4	0.7	-3.7	23.7	-27.6	-11.4	-22.6	1.4
	Set mean		-2.1	-2.7	14.2	19.5	-14.1	-14.4	-8.2	-2.0
16	N_G18 x N_G7	W x W	-3.5	-13.9	12.5	5.8	-15.4	-24.2	-9.5	-13.2
17	N_G2 x N_G1	W x W	-23.1	-31.8	-10.3	-16.3	-32.6	-40.0	-27.9	-31.3
18	N_G2 x N_G18	W x W	-19.6	-17.2	-6.3	1.7	-29.5	-27.1	-24.7	-16.6
19	N_G18 N_G3	W x W	-24.4	-16.9	-11.9	2.1	-33.7	-26.8	-29.2	-16.3
20	N_G2 x N_G3	W x W	-47.9	-50.6	-39.3	-39.4	-54.4	-56.5	-51.2	-50.3

Sno.			Heterosis (%)							
			Trial mean		Local check		Best check		Mean check	
	Hybrid	Hybrid set	HN	LN	HN	LN	HN	LN	HN	LN
21	N_G15 X N_G8	W x W	-28.3	-31.8	-16.4	-16.2	-37.1	-39.9	-32.8	-31.3
22	N_G7 X N_G15	W x W	-15.0	-7.8	-0.9	13.3	-25.5	-18.8	-20.3	-7.1
	Set mean		-23.1	-24.3	-10.4	-7.0	-32.6	-33.3	-27.9	-23.7
23	C49 x T7	S x L	-4.9	-5.7	10.9	15.8	-16.6	-17.0	-10.8	-5.0
24	C60 x T5	S x L	8.0	39.0	25.9	70.7	-5.3	22.4	1.2	40.0
25	T12 x C38	S x L	-10.1	9.3	4.8	34.3	-21.2	-3.7	-15.7	10.2
26	C3 x T3	S x L	15.2	10.9	34.3	36.2	1.0	-2.4	8.0	11.7
27	T17 x C4	S x L	12.6	24.0	31.3	52.2	-1.3	9.1	5.6	24.9
28	T15 x C14	S x L	10.3	12.4	28.5	38.1	-3.3	-1.0	3.3	13.3
	Set mean		5.2	15.0	22.6	41.2	-7.8	1.2	-1.4	15.9
29	C49 x N_G18	W x S	-7.5	5.6	7.8	29.7	-18.9	-7.1	-13.3	6.4
30	N_G7 x C56	W x S	1.1	-0.4	17.8	22.3	-11.4	-12.4	-5.3	0.3
31	N_G18 x C4	W x S	-4.2	14.4	11.7	40.4	-16.0	0.7	-10.2	15.2
32	C3 x N_G3	W x S	-22.3	-28.3	-9.4	-11.9	-31.9	-36.8	-27.2	-27.7
33	C68 x N_G2	W x S	19.6	10.9	39.5	36.2	4.9	-2.3	12.1	11.8
34	N_G1 x C58	W x S	6.3	0.0	23.9	22.8	-6.8	-12.0	-0.4	0.7
	Set mean		-1.2	0.4	15.2	23.3	-13.4	-11.7	-7.4	1.1
35	N_G2 x T3	W x L	23.3	24.5	43.7	52.8	8.1	9.6	15.5	25.4
36	N_G3 x T5	W x L	2.0	19.3	18.9	46.5	-10.6	5.0	-4.4	20.2
37	T7 x N_G1	W x L	-5.3	-6.6	10.4	14.7	-17.0	-17.8	-11.2	-5.9
38	N_G18 x T12	W x L	26.6	-1.3	47.6	21.2	11.0	-13.1	18.7	-0.6
39	N_G15 x T10	W x L	7.0	3.8	24.7	27.5	-6.2	-8.6	0.3	4.6

Sno.	Hybrid	Hybrid set	Heterosis (%)							
			Trial mean		Local check		Best check		Mean check	
			HN	LN	HN	LN	HN	LN	HN	LN
40	T15 x N_G7	W x L	12.2	19.9	30.8	47.2	-1.6	5.5	5.2	20.8
	Set mean		11.0	9.9	29.4	35.0	-2.7	-3.2	4.0	10.8
41	SC513	Check	13.0	13.7	31.7	39.6	-1.0	0.1	5.9	14.5
42	PAN77	Check	12.2	1.5	30.7	24.7	-1.7	-10.6	5.1	2.3
43	PAN67	Check	10.7	-4.2	29.0	17.6	-3.0	-15.7	3.8	-3.5
44	DKC8033	Check	14.1	6.4	33.0	30.7	0.0	-6.3	6.9	7.2
45	SC407	Check	4.2	-3.0	21.5	19.1	-8.6	-14.6	-2.3	-2.3
46	LOCAL	Check	-14.2	-18.6	0.0	-0.1	-24.8	-28.4	-19.6	-18.0
	Set mean		6.7	-0.7	24.3	21.9	-6.5	-12.6	0.0	0.0

Key: T = Tanzania, C = CIMMYT Zimbabwe, N_G = IITA-Nigeria inbred lines, N = nitrogen, high N = 120 kg N ha^{-1} fertiliser application rate, Low N= 60 kg N ha^{-1} fertiliser application rate. L x L = local by local set, S x S = southern Africa by southern Africa set, W x W = West Africa by West Africa set, S x L = southern Africa by local set, W x S = West Africa by southern Africa, W x L = West Africa by local, check = commercial hybrid check

5.4 Discussion

5.4.1 Relative performance and standard heterosis for N-stress tolerance

Nitrogen regimes in each season were significantly different. High N had higher mean yield compared to those under low N. Poor yield in low N was associated with the wet season. Overall, the R^2 values were about 90% (Table 5.2). Characters related to physiological maturity had high R^2 values of over 80%, except the grain fill duration in high N DS09, which was not statistically significant ($P<0.05$) (Table 5.2). These R^2 values were high enough to warrant breeding for maize varieties that are adapted under low N conditions. These statistics may also suggest that maize varieties that are both earlier maturing and high yielding could be designed under low N. It is evident from this study that early and high yielding varieties could be bred under both and specific high and low N conditions — a challenge that has been barely attempted previously. Therefore, in order to strike a balance between yield and early maturity, the genotypes must be comparable in terms of: their adaptation; being equally treated; their breeding history and place of origin; traits to be considered when estimating maturity of a cultivar; and scale of measurement of the trait under focus. For instance, a genotype aimed for short seasons would be source limited to perform well into long seasons that are sink limited, as Hageman and Lambert (1996) suggested. The challenge to breeders is to incorporate high dry matter into an earlier maturing variety, especially in SSA, where maize is typically produced under low-N conditions. Furthermore, this is irrespective of the intensity and severity of low N, as tangible estimates on relative loss due to application of low N in tropical maize is not established (Edmeades et al., 1997). Knowledge on quantitative loss due to application of low N may help to improve maize that uses N input efficiently.

A loss of about 30% due to application of low N is shown in Figure 5.1 and Table 5.4, and variances in the response of the cultivars for yield suggest the efficient breeding and stabilising of cultivars for N use, since N is not only a key nutrient to maize but is also highly volatile under farmers' conditions. Generally, the frequency distributions that tended to normal distribution confirmed the average figure of loss due to application of low N of about 30%. Although the literature indicates that farmers in Tanzania apply low rates of fertilisers (Nkonya, et al., 1997; Lyimo, 2006), and the trend can be applied to SSA as a whole (Worku et al., 2007), few systematic studies barely exist on what farmers lose by applying a low rate of N. The study, conducted between 1986 and 1995 in low-land tropical maize by Bänziger et al. (1997), under quantities of between 0 and 200 kg N ha^{-1} found generally equal and positive correlations for yield — but these decreased with increasing relative yield depression under low N. However, low and high N-specific genotypes were found, indicating that the two environments differed for yield. Since studies in intermediate altitude tropical maize under low N are not reported, the present study would be a benchmark for further research to improve maize productivity. Table 5.5 indicated that six out of 10 and eight out of 10 hybrids that involved local germplasm under high and low N conditions, respectively, were the best performers under such environments. For instance, hybrids N_G2 x T3, T20 x T15, and T6 x T13 were consistently in the top 10 under both regimes of N. However, the relative yield loss of hybrids C60 x T5, N_G18 x C4, N_G3 x T5, T17 x C4 were consistent with low N. Furthermore, 50% of the poor performers in low N conditions involved one or more local inbred lines. This could strongly suggest that searching and breeding for local hybrid adaptation of maize cultivars should continue.

The present study established that in the case of the intermediate maturing maize hybrids (i.e. ≤140 calendar physiological maturity days in Tanzania, according to Lyimo et al. [2006]), the chance was that some hybrids could perform consistently better under both high and low N environments. Since the majority of the experimental hybrids outperformed commercial hybrid checks in this study, it could be suggested that these hybrids would be proposed for release as single crosses or they may enter advanced evaluation trials for eventual release.

The local x local, West Africa x local, southern Africa x local and check SC513 sets performed above the grand mean. The southern Africa x southern Africa and West Africa x West Africa were inferior to the grand mean under both high and low N, however the low N tolerance index for the southern Africa x southern Africa set was the most desirable characteristic for breeding for wide adaptability to N conditions (Table 5.4). The IITA lines were the worst within and between sets for their hybrids' low values of low N tolerance indices. The results further suggest that the southern Africa germplasm could be suitable for breeding for tolerance to low N since the southern Africa x local, West Africa x southern Africa, and southern Africa x southern Africa sets were superior under low N conditions. And this may comply with Kang (1994) that any foreign germplasm must introduce new genes into the local breeding programme for the case of low-N tolerance indices from southern African materials. Furthermore, the southern Africa x local, West Africa x local and local x local sets had higher means for low N tolerance indices across N regimes. These results may suggest the possibility of improving maize cultivars for wide adaptation across regimes of N, firstly by banking on exploring local germplasm for their potentially better performance under LN, and secondly from foreign germplasm that should have been preceded by a screening exercise, as the present study had done. Interestingly, hybrids performing better for HN, LN and under both conditions of N regimes were identified in the present study, which suggested the possibility of improving yield under low N conditions.

5.4.2 Relative effects of low nitrogen on maturity characters in maize hybrids

There was mixed information among entry sets, with some hybrids indicating values for characters related to maturity above and below the grand average (Table 5.5). West Africa x West Africa, southern Africa x local and southern Africa x West Africa sets had mean performance for characters related to maturity of above the grand average, whereas local x local hybrid combinations for all characters were below grand average. This may suggest that local germplasm had no appreciable levels of genes for reduced calendar physiological maturity dates under both conditions of N. Local germplasm may have been bred and adapted to local stresses by fast DMA, which may require the foreign germplasm if breeding for early genotypes is desired. However, the challenge has been, particularly under low N stress, to balance fast dry matter accumulation (DMA) and maintain the accumulated dry matter in the kernels until the mark of calendar physiological maturity. Identification of the prolonged leaf chlorophyll concentration, kernel dry down index and grain fill duration in some hybrid combinations, where the relative effects of the application of low N was above 10% (Table 5.5), would improve the maize ideotype for both yield and early maturity under high and low N conditions. The non-senescence character (stay-green) and grain fill duration may be logically related and Hageman and Lambert (1996) asserted that the SG character was reported to be irrelevant to extra-early-maturing genotypes. As the maturity period of the genotypes decreases, the genotype tends to become source limited, but it becomes sink limited in late-maturing genotypes. Maize ideotypes with reduced vegetative growth stage but with extended grain fill duration have been reported and genetic variation for these traits was reported by Mock and Pearce (1975). Cross and Kabir (1989) reported that the high index of kernel dry-down was negatively associated with yield components and yield per se, while cob and kernel size were reported to be inversely related with the index of kernel dry-down. However, the challenge remains as to how to integrate the high index of kernel dry-down into a genotype aspired to increase DMA via extended leaf chlorophyll concentration and grain fill duration. To aggravate the challenge further, such traits must be considered under low-N conditions, where tropical maize in SSA is typically produced.

5.4.3 Standard heterosis for grain yield under high and low nitrogen conditions

The predominantly better performance of hybrids involving either both or one local inbred parent across individual regimes of N suggests the adaptation of local elite inbred lines (Table 5.6), thus demonstrating local adaptation under high and low N conditions, as Kang (1994) and Romay et al. (2010) reported. Positive standard heterosis across individual N regimes implied that the experimental crosses were better under high N and their performance decreased under low N. The present study may further demonstrate that lower values of standard heterosis are desired when it comes to developing NUE cultivars of intermediate maturing maize.

However, considering the fact that yield can be a measure of adaptation of a genotype (Lonquist, 1953; Moll et al., 1965), heterosis for yield would be an indicator of adaptation to local stresses. In the present study, some hybrids were identified for specific and combined N regimes. The possible reasons for local inbred lines yielding better to the majority of hybrids involving foreign inbreds could demonstrate local adaptation among hybrids involving local inbreds (Kang 1994; Romay et.al., 2010). The reason why a few local and exotic crosses had better performance under N regimes could be due to wide genetic distances between these inbred lines. Moll et al. (1965), Cheres et al. (2000), Menkir et al. (2004) and Dhliwayo et al. (2009) suggested that extremely diverse populations suffer low heterosis in their F_1 progeny due to non-harmonious gene combinations. And this could be the reason why most crosses performed poorly under high and low N conditions of the present study. Although these researchers did not study heterosis under high and low N conditions, as did the present study, it may be concluded that foreign germplasm may not have a direct application to the recipient local breeding programme without their heterotic values being tested. Meseka et al. (2006) studied drought tolerance in maize and reported that in order for hybrids to be drought tolerant and with improved yield, their parents must at least have one parent with genes for drought tolerance than both parents being susceptible to drought. Therefore, any foreign germplasm must be tested and adapted to the local environment or the inbred lines extracted from the hybrid combinations between the local and foreign germplasm for maize improvement programmes to benefit from breeding investments. In this case and to have heterotic cultivars, Giesbrecht (1960) suggested a cross between local inbred lines (high yield potential) x exotic inbred lines (with desired traits except high yield potential). Derera et al. (2006), after studying drought-tolerant genotypes, also recommended a similar approach. However, this approach could be the starting point to maize improvement, since the inbred line's yield per se may not be a good indicator of a subsequent hybrid's performance.

The West Africa x West Africa set had negative values for all types of heteroses that were studied. Best-check heterosis also had negative values, which suggested that yield for these hybrids may be improved, even under low N conditions. Wide genetic and geographical diversity among parents, especially from the West Africa germplasm set, would improve heterosis for yield under low N if they combined with other sources. The West Africa germplasm was specifically bred for striga resistance and earliness, which may justify relevance to NUE and suitability to low-N conditions. Generally, the between sets crosses expressed higher heterosis under LN, compared with HN (Table 5.6), which is in support of Mather and Jinks (1982) that heterosis improves as the environment becomes impoverished. In support of this, Meseka et al. (2006) reported average heterosis for yield of 129% under low N and 114% under HN. This is demonstrated by southern Africa x local and even trial mean of the present study, where heterosis under LN tripled that of HN. Local check heterosis had positive values of higher magnitudes, especially under LN, compared with HN across sets, except the set for checks. Interestingly, in addition to genetics and geographic distance, to make the heterosis phenomenon practicable, the present study estimated heterosis under similar environmental conditions under which farmers produce their maize.

5.4.4 Conclusions and implications to maize breeding

Overall results revealed significant variation among hybrids for yield and secondary traits under both N conditions, and indicated varying levels of N-stress tolerance and heterosis, with implications on hybrid designs:

1) The average relative yield loss of hybrids due to application of low-N fertilizer was estimated at about 20% to 30%. Generally, hybrids involving local inbred lines exhibited higher levels of tolerance to low soil N.
2) Four hybrids, T5 x T17, T20 x T15, T6 x T13, and N_G2 x T3 with positive heterosis under both soil N conditions were identified. Three of the four hybrids involved local x local inbreds, while one involved West Africa x local inbred, suggesting that selection of parents with local adaptation was crucial in designing hybrids that perform well under both N conditions.
3) Four hybrids, C60 x T5, T17 x C4, N_G3 x T5, and T15 x N_G7 displayed positive heterosis under low N conditions only, indicating that they were specifically adapted

to low-N conditions. All four hybrids had one local line as a parent, while the other parent was exotic.

4) Two hybrids, C58 x C4 and N_G18 x T12 exhibited positive heterosis under high N conditions. Only one of the hybrids was constituted between exotic lines from southern Africa, while the other involved a local x West African line. These hybrids are specifically adapted to high N conditions.
5) On average, a reduction of soil N application reduced the number of days for anthesis and silking of the hybrids by about four days. There were very few differences between the hybrid sets. Physiological maturity was also earlier by about one to three days when the level of N fertiliser was reduced.
6) The kernel dry-down index for hybrids was faster by about a one-to-nine proportion of increased dry-down but varied across the hybrid sets.
7) The reduction of N application resulted in an average loss of chlorophyll concentration of 18% to 25% across hybrid sets. Loss of chlorophyll concentration was lower in experimental hybrids than check hybrids and crosses between local and CIMMYT lines displayed minimum loss of leaf chlorophyll concentration.
8) Differences between hybrids for grain moisture content at harvest due to reduced N dosage were negligible.
9) The effective grain filling period increased by two to four calendar days due to reduced N dosage.
10) Heterosis of hybrids relative to the trial mean tripled under LN compared to HN for the southern Africa x local set and mean-check heterosis was positive and high for this set under LN conditions, compared with HN, which was insignificant. The general trend was that the hybrid sets displayed higher standard heterosis under LN, compared with HN conditions, implying that, in general, productivity of maize can be enhanced by promoting use of hybrids in low-N input environments.

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Chapter 6:
Relationships between secondary traits and grain yield in tropical maize hybrids among exotic and local inbred lines under low and high nitrogen conditions in Tanzania

Abstract

The effectiveness of direct or indirect selection via secondary traits to improve maize grain yield under low soil N in tropical maize has not been fully studied yet. This study was conducted to determine relationships between grain yield and secondary traits, which are crucial to improving yield by indirect selection, especially under LN conditions. Forty-eight F_1 experimental hybrids among inbreds from exotic materials (CIMMYT, IITA) and local (Tanzania) plus six commercial checks were separately evaluated in a 9 x 6 alpha lattice design in two replications under 120 (HN) and 60 kg N ha^{-1} (LN) over three seasons. Nitrogen treatments influenced relationships among traits and their contribution to final grain yield. The study confirmed that the number of ears per plant (prolificacy) and plant height were influential on grain yield, both directly and indirectly, under both N conditions. Regression coefficients (β) for the ear prolificacy were all positive and consistently higher under LN than under HN regimes, suggesting that the direct effects of prolificacy were more important in contributing to yield under stress than non-stress conditions. It was observed that mean-leaf chlorophyll concentration and an effective duration of the grain-filling period had strong positive correlation coefficients, but these traits exhibited negative associations with most other traits. Direct effects to grain yield were higher under LN, compared with HN for kernels per ear and plant height. It was revealed that indirect effects to grain yield were higher under HN than LN. Under HN, the indirect effects to grain yield from plant stand at harvest via the ear prolificacy were -0.418, while indirect effects to yield, due to mean-leaf chlorophyll concentration through the mid-anthesis date, were -0.376. Positive indirect effects to grain yield under HN were from plant height via mid-anthesis date (0.294) and from the number of kernels per ear via plant stand at harvest (0.229). The results suggested that correlated response to selection could be done for traits that correlated positively with grain yield under both N conditions, such as plant height, plant stand, and the number of kernels per ear.

Key words: grain yield, soil nitrogen, trait relationships, tropical maize.

6.1 Introduction

Very often, tropical maize is bred and produced under multiple stresses and a plethora of farmers' needs such that targeting one particular stress or need becomes difficult.

Relationships among genetic characters may help to identify the degree of prediction, association, and whether the characters are directly or indirectly related to improve a desired trait. Very strongly associated characters would imply that improving one character improves the other, although separate breeding programmes would be employed for weakly associated characters. Farmers in sub-Saharan Africa (SSA) and elsewhere in developing countries require maize varieties that are early maturing, locally adapted, and high yielding (Lyimo, 2006; Bisanda et al., 1998), yet maize is produced under low-nitrogen (N) conditions (FAO, 2006). Elsewhere, breeding for such objectives without compromising grain yield stability has been a time challenge (Witcombe and Virk, 2001). It could be much harder to achieve these objectives in SSA, where maize is produced under multi-stressed environments. Gradients related to low soil fertility are at least systematic, as compared with random stresses such as drought and heat, among others (Allard and Bradshaw, 1964), such that N regimes could be used as an evaluating environment for grain yield potential. Not only is relative performance loss due to use of low N in tropical maize not known but little is documented on interrelationships among traits under high and low N conditions.

No study has been conducted to connect regression, correlation and path coefficient analyses to a common abiotic stress in tropical maize, such as low N, irrespective of the fact that maize in SSA is produced under low N conditions. Yet another challenge has been discovering that breeding for final yield, particularly under multiple stresses, has not shown significant progress, thus requiring yield components that justify investigations and use of relationships among genetic characters to improve final yield. However, most of the studies have been conducted on path and correlation analyses at the expense of modelling and prediction (Willman et al., 1987b). Correlations and path analyses exploit relationships among secondary traits to improve final grain yield (Kang et al., 1986; Gravois and Helms, 1992; Kang, 1994; Wang et al., 1999) to generate potential information for breeding. Given the complex production environments in tropical maize (Lafitte, 1994), and lack of knowledge on what conditions final yield (Lee et al., 2005), an integrated approach on trait relationships is suggested. Regression could complement correlations and path coefficient analyses to identify a few key traits which improve final grain yield (GY) and physiological maturity under low and high N conditions.

Unpredictable and erratic production environments compel maize breeders to identify key adaptive traits to cope with the situation. Direct selection under low N conditions might not be effective, hence there is the need to clearly identify useful adaptive traits that are highly associated with final grain yield data under high and low N conditions. Among others, traits under low N include synchronised pollinations (Lafitte, 1994; Lafitte and Edmeades, 1995; Bänziger et al., 2000), prolonged leaf chlorophyll concentration (LCC) and effective duration of the grain fill period (EFPD), which maintains the sink capacity (i.e. number of kernels per ear or per plant), and stress escape mechanisms such as early calendar physiological maturity (Elings et al., 1997). Progress to breeding from secondary traits such as LCC, EFPD and kernels per ear and per plant, has been associated with a preponderance of additive genetic effects under stress. Such genetic effects govern the majority of complex stresses such as low N tolerance and they may increase as stresses intensify, implying the possibility of indirect selection (Betran et al., 2003). Since alleles work together to express a quantitative phenotype (Hallauer and Miranda, 1988; Tuberosa et al., 2002), various options on associations between traits would facilitate the approach of correlated response to selection, provided the genetic variation is sufficient. Furthermore, phenotypic correlations may approach genotypic correlations as the number of genotypes, traits and evaluating environments increase (Cheverud, 1988; Watt and Levin, 1998). This may be a key requirement for relationships among genetic characters targeted to improve a complex trait such as final yield under low N conditions.

The present study was conducted under low and high N conditions to:

- i) use multiple-trait linear regression to determine how yield components are influenced by N regimes, and
- ii) establish interrelationships among traits by Pearson's phenotypic correlation coefficients and path coefficient analyses.

The hypotheses tested were:

- i) some traits could predict final grain yield more than others under high and low N,
- ii) N does not affect association among traits and individual traits with final yield, and
- iii) the direct and/or indirect effects of secondary traits to final grain yield are not dependent on N.

6.2 Materials and methods

6.2.1 Experimental design and management

The design and management of the experiment is as indicated in section 5.2.1 of chapter five in this thesis.

6.2.2 Traits measurement

Plant and ear heights were recorded on 10 randomly selected plants per plot, when on average all genotypes had reached 50% silking date. The actual measurements of plant and ear heights were as stated in section 4.2.2 in chapter four of this study. The number of leaves above the ear was counted as an average of 8 plants per plot, and then the final count was averaged over two replications to obtain the hybrid's number of leaves above the ear. The number of kernels per ear (KPE) was calculated, as explained, in section 4.2.2 in chapter four of the present study. The exception was that the KPE was calculated on individual plants in chapter four, whereas in this chapter, KPE was an average of eight plants per plot per two replications.

The number of ears per plant was calculated as: total ears ÷ total plants at harvest.
The KDD index, calendar flowering dates and physiological maturity, EFPD, LCC, and grain yield (t ha^{-1}) were calculated as stated in section 5.2.2 of chapter five in this study.

6.2.3 Data analysis

The data were analysed in a SAS separately for N sites over three seasons using the PROC GLM procedure. Modelling dwelt on grain yield because the physiological maturity had small tolerance (low weight) in most models. PROC REG (with Stepwise and Backward regression procedures) was employed according to Neter et al. (1996) for multiple traits, modelling with grain yield as a dependent variable. With multiple regression, the fitting in and eliminating of some explanatory variables destabilises the regression system, therefore both stepwise and backward regression procedures were used to maintain the stability of the models. Both steps (stepwise and backward) tallied with the determination of final traits. All variables left in the models were significant at critical upper probability levels of 0.100 and 0.150 for backward and stepwise procedures, respectively. Taking the multiple-trait linear regression model for HNDS08, for example (Table 6.1):

$Y = -m + X_1\beta_1 + X_2\,\beta_2 + X_3\,\beta_3 + X_4\,\beta_4 + X_5\,\beta_5 + X_6\,\beta_6 +$ error mean square or residual

Where Y= dependent variable i.e. grain yield (ton ha^{-1})
m = Y intercept
$X_{1...6}$ = independent variables (1 = 50% calendar silking date, 2 = mean LCC in SPAD values, 3 = plant height in centimetres, 4 plant stand at harvest, 5 = ears $plant^{-1}$, 6 = kernels ear^{-1}),
$\beta_{1...6}$ = regression coefficients associated with independent variables 1 to 6.

PROC CORR procedure was used to compute Pearson's phenotypic correlation coefficients.

Pearson Correlation Coefficients, No. = 108; Prob > |r| under H_0: Rho = 0

High and low N trials for the three seasons were combined and analysed separately in SAS using PROC GLM, PROC REG and PROC CORR procedures, as stated above, in order to obtain key and significant traits for path coefficient analyses. Thirteen traits were used as independent variables against grain yield as a dependent variable. Stepwise and backward regressions under the PROC REG procedure reduced independent variables to 6 for each N regime. The analyses involved 54 hybrids and two replications for three seasons, totalling 324 (i.e. 54 entries x 2 replications x 3 seasons) genotypes x environmental combinations for each N regime, so corroborating Cheverud (1988) and Waitt and Levin (1998) that phenotypic correlations match genotypic correlations, as the sample size and evaluation environment of the genotypes increased. Path coefficient analyses for the two N regimes were analysed according to Kang (1994). Each N regime had six different independent variables and one common dependent variable (grain yield) that made a total of seven variables in the path coefficient analyses equations.

The equations for high N were as follows:

1. $r_{17} = P_{17} + r_{12} P_{27} + r_{13}P_{37} + r_{14}P_{47} + r_{15}P_{57} + r_{16}P_{67}$
2. $r_{27} = r_{12}P_{17} + P_{27} + r_{23}P_{37} + r_{24}P_{47} + r_{25}P_{57} + r_{26}P_{67}$
3. $r_{37} = r_{13}P_{17} + r_{23}P_{27} + P_{37} + r_{34}P_{47} + r_{35}P_{57} + r_{36}P_{67}$
4. $r_{47} = r_{14}P_{17} + r_{24}P_{27} + r_{34}P_{37} + P_{47} + r_{45}P_{57} + r_{46}P_{67}$
5. $r_{57} = r_{15}P_{17} + r_{25}P_{27} + r_{35}P_{37} + r_{45}P_{47} + P_{57} + r_{56}P_{67}$
6. $r_{67} = r_{16}P_{17} + r_{26}P_{27} + r_{36}P_{37} + r_{46}P_{47} + r_{56}P_{57} + P_{67}$

The residual effect was calculated as:

$$P_{X7} = [1 - (P_{17}^2 + P_{27}^2 + P_{37}^2 + P_{47}^2 + P_{57}^2 + P_{67}^2 + 2P_{17}r_{12}P_{27} + 2P_{17}r_{13}P_{37} + 2P_{17}r_{14}P_{47} + 2P_{17}r_{15}P_{57} + 2P_{17}r_{16}P_{67} + 2P_{27}r_{23}P_{37} + 2P_{27}r_{24}P_{47} + 2P_{27}r_{25}P_{57} + 2P_{27}r_{26}P_{67} + 2P_{37}r_{34}P_{47} + 2P_{37}r_{35}P_{57} + 2P_{37}r_{36}P_{67} + 2P_{47}r_{45}P_{57} + 2P_{47}r_{46}P_{67} + 2P_{57}r_{56}P_{67})]^{1/2}.$$

Where in the model:

r_{ij} = simple correlation coefficients for measuring the mutual association of two variables

P_{ij} = path coefficients for measuring direct influence between variables to grain yield

$r_{ij}P_{ij}$ = indirect effects of variables upon another through the other variable

Px = the residual effect in the path analysis model; computed as $1\text{-}P_{X7}^2$

i and j = (1…6)

Where 1 = plant height (cm), 2 = mean-leaf chlorophyll concentration (SPAD values), 3 = number of kernels ear^{-1}, 4 = calendar 50% anthesis date, 5 = plant stand at harvest, 6 = number of ears plant^{-1}, and 7 = the dependent variable i.e. grain yield (t ha^{-1}).

Taking equation 3 for example:

r_{37} = the correlation coefficient between 3 (number of kernels ear^{-1}) and 7 the dependent variable (grain yield)

$r_{13}P_1$ = the indirect path of the number of kernels ear^{-1} to grain yield through plant height

$r_{23}P_2$ = the indirect path of the number of kernels per ear^{-1} to grain yield through mean-leaf chlorophyll concentration

P_{37} = the direct path of the number of kernels ear^{-1} to grain yield

$r_{34}P_{47}$ = the indirect path of calendar 50% anthesis date to grain yield through the number of kernels ear^{-1}

$r_{35}P_{57}$ = the indirect path of plant stand at harvest to grain yield through the number of kernels ear^{-1}

$r_{36}P_{67}$ = the indirect path of the number of ears plant^{-1} to grain yield through the number of kernels ear^{-1}

The same interpretation and codes were applied for high and low N conditions, since the number of independent variables was the same and grain yield was the common dependent variable. Therefore, under low N, the trait codes were: 1 = mean-leaf chlorophyll concentration (SPAD values), 2 = plant height (cm), 3 = plant stand at harvest, 4 = number of ears plant^{-1}, 5 = number of kernels ear^{-1}, 6 = grain filling duration, and 7 = the dependent variable (grain yield in t ha^{-1}).

After substituting equations (1…6) above the values of r_{ij}s were written in the matrix form according to Kang (1994):

a. Matrix for high N (correlations for r and c are as indicated in Table 6.5)

$$\begin{array}{ccccc} r & & P & & c \\ \left|\begin{array}{rrrrrr} 1 & -0.415 & 0.294 & 0.566 & 0.241 & -0.254 \\ -0.415 & 1 & -0.2 & -0.723 & 0.109 & 0.366 \\ 0.294 & -0.2 & 1 & 0.096 & 0.286 & -0.196 \\ 0.566 & -0.723 & 0.096 & 1 & -0.193 & -0.141 \\ 0.241 & 0.109 & 0.286 & -0.93 & 1 & -0.326 \\ -0.254 & 0.366 & -0.196 & -0.141 & -0.326 & 1 \end{array}\right| & * & \left|\begin{array}{l} P_{17} \\ P_{27} \\ P_{37} \\ P_{47} \\ P_{57} \\ P_{67} \end{array}\right| & = & \left|\begin{array}{r} 0.542 \\ 0.158 \\ 0.328 \\ 0.162 \\ 0.357 \\ 0.096 \end{array}\right| \end{array}$$

b. Matrix for low N (correlations for r and c are as indicated in Table 6.5)

$$\begin{array}{ccccc} r & & P & & c \\ \left|\begin{array}{rrrrrr} 1 & -0.359 & 0.014 & 0.234 & 0.036 & 0.441 \\ -0.359 & 1 & 0.145 & -0.269 & 0.283 & -0.278 \\ 0.014 & 0.145 & 1 & -0.259 & 0.079 & 0.07 \\ 0.234 & -0.269 & -0.259 & 1 & -0.222 & 0.135 \\ 0.036 & 0.283 & 0.079 & -0.222 & 1 & 0.232 \\ 0.441 & -0.278 & 0.07 & 0.135 & 0.232 & 1 \end{array}\right| & * & \left|\begin{array}{l} P_{17} \\ P_{27} \\ P_{37} \\ P_{47} \\ P_{57} \\ P_{67} \end{array}\right| & = & \left|\begin{array}{r} 0.102 \\ 0.526 \\ 0.447 \\ 0.036 \\ 0.435 \\ 0.179 \end{array}\right| \end{array}$$

The matrices take the form of $rP = c$

Where:

r = correlation matrix of independent variables or traits (1…6)

P = path coefficients of traits 1 to 6 in association with trait 7

c = correlation vector of traits 1…6 with trait 7

The direct paths, Ps, were computed according to Payne et al. (2009) in GenStat (Version 12) as:

P = inverse $(r)^* + c$; which implies the inverse matrix $r^{-1}\ c$

The direct path coefficients for high N were: $P_{17} = 0.304$, $P_{27} = 0.466$, $P_{37} = 0.124$, $P_{47} = 0.520$, $P_{57} = 0.799$, $P_{67} = 0.361$.

The direct path coefficients for low N were: $P_{17} = 0.161$, $P_{27} = 0.564$, $P_{37} = 0.410$, $P_{47} = 0.299$, $P_{57} = 0.273$, $P_{67} = 0.133$.

The summation of direct path (effects) and indirect effects per respective association between independent variable and dependent variable (grain yield) in Table 6.6

approximated genotypic correlations (r_g) or Pearson's phenotypic correlation coefficients (Table 6.5) for respective N regime, as Cheverud (1988) and Waitt and Levin (1998) suggested. The index of reduction of direct path relative to indirect effects to final yield was therefore computed as follows:

Index reduction = [(direct path-indirect effects)/total of direct path and indirect effects]

6.3 Results

6.3.1 Multiple trait linear regression models

The explanatory variables had varying signs and magnitudes for the models over N regimes (Tables 6.1, 6.2, and 6.3). Ear plant^{-1} (EPP) and plant stand at harvest were consistent with positive regression coefficients (β) across sites and seasons. The number of kernels ear-1 also had positive β values and it was consistent across seasons and sites except in HN WS09. Ears per plant had high magnitude and positive regression coefficients (β) throughout regimes of N, which were consistently higher in LN than in HN. Plant height, mean-LCC, grain filling duration and kernels ear^{-1} had positive β values. Surprisingly, the rate of kernel dry down had positive and high β values in LNDS09. Characters related to maturity, such as 50% AD, the number of leaves above the ear, ear height, kernel dry-down index (with much effect), and kernel moisture content at harvest had negative β coefficients at LN for DS08 and WS09 environments. All the traits were statistically significant at $p \leq 0.05$, except 50% silking and kernels ear^{-1} in HNDS08, mean LCC in LNWS09, and EPP in HNDS09.

Table 6.1: Multiple trait linear regression models for grain yield (t ha^{-1}) of 54 maize hybrids in the dry season in 2008

Environment	Variable/trait	Parameter estimate	Pr > F	EMS (ANOVA)
HN DS08	Intercept	-23.244±5.945	0.0002	2.321
	50% silking date	0.087±0.046	0.0632	
	Mean-LCC	0.111±0.046	0.0182	
	Plant height	0.043±0.008	<.0001	
	Plant stand at harvest	0.167±0.053	0.0023	
	Ears plant^{-1}	2.458±0.806	0.0029	
	Kernel ear^{-1}	0.005±0.806	0.0984	
LN DS08	Intercept	-18.670±4.500	0.0003	0.694
	50% anthesis date	0.151±0.053	0.0058	
	Plant height	0.069±0.0112	<.0001	
	Ear height	-0.041±0.0156	0.0096	
	Leaf number above ear	-0.734±0.204	0.0005	
	Plant stand at harvest	0.077±0.035	0.0316	
	Ears plant^{-1}	3.694±1.018	0.0005	
	Kernel moisture content at harvest	-0.247±0.123	0.0472	
	Kernel ear^{-1}	0.007±0.002	<.0001	
	Kernel dry-down index	-2.280±1.093	0.0395	
	Grain filling duration	0.094±0.046	0.0459	

HN = high nitrogen fertiliser application regime (120 kg N ha^{-1}), LN = low nitrogen fertiliser application regime (60 kg N ha^{-1}), DS08 = dry season year 2008, LCC = leaf chlorophyll concentration (SPAD values), EMS = error mean square, ANOVA = analysis of variance

Table 6.2: Multiple trait linear regression models for grain yield (t ha^{-1}) of 54 maize hybrids in the wet season in 2009

Environment	Variable/trait	Parameter estimate	Pr > F	EMS (ANOVA)
HN WS09	Intercept	-23.177±4.574	<.0001	0.549
	50% silking date	0.082±0.040	0.0418	
	Mean-LCC	0.117±0.024	<.0001	
	Plant height	0.033±0.005	<.0001	
	Plant stand at harvest	0.281±0.049	<.0001	
	Ear $plant^{-1}$	1.773±0.618	0.0050	
	Grain filling duration	0.111±0.035	0.0018	
LN WS09	Intercept	-6.764±2.194	0.0027	0.459
	50% anthesis date	-0.038±0.019	0.0422	
	Mean-LCC	0.009±0.006	0.1344	
	Plant height	0.026±0.004	<.0001	
	Leaf number above ear	-0.285±0.144	0.0505	
	Plant stand at harvest	0.177±0.035	<.0001	
	Ear $plant^{-1}$	3.056±0.737	<.0001	
	Kernel ear^{-1}	0.005±0.001	0.0001	
	Grain filling duration	0.069±0.0153	<.0001	

N = high nitrogen fertiliser application regime (120 kg N ha^{-1}), LN = low nitrogen fertiliser application regime (60 kg N ha^{-1}), WS09 = wet season year 2009, LCC = leaf chlorophyll concentration (SPAD values), EMS = error mean square, ANOVA = analysis of variance

Table 6.3: Multiple trait linear regression models for grain yield (t ha^{-1}) of 54 maize hybrids in the dry season in 2009

Environment	Variable/trait	Parameter estimate	Pr > F	EMS (ANOVA)
HN DS09	Intercept	-25.986±6.522	0.0001	2.388
	50% anthesis date	0.149±0.053	0.0064	
	Mean-LCC	0.150±0.044	0.0010	
	Ear height	0.028±0.012	0.0248	
	Plant stand at harvest	0.288±0.075	0.0002	
	Ear $plant^{-1}$	1.662±0.875	0.0604	
	Kernel ear^{-1}	0.008±0.003	0.0040	
LN DS09	Intercept	-16.890±1.630	<.0001	0.958
	Mean-LCC	0.114±0.023	<.0001	
	Plant height	0.022±0.007	0.0016	
	Plant stand at harvest	0.385±0.044	<.0001	
	Ear $plant^{-1}$	2.902±0.478	<.0001	
	Kernel ear^{-1}	0.008±0.002	<.0001	
	Kernel dry-down index	3.557±1.395	0.0123	

HN = high nitrogen fertiliser application regime (120 kg N ha^{-1}), LN= low nitrogen fertiliser application regime (60 kg N ha^{-1}), DS09 = dry season year 2009, LCC = leaf chlorophyll concentration (SPAD values), EMS = error mean square, ANOVA = analysis of variance

6.3.2 Pearson's phenotypic correlations

The results on correlations (Table 6.4) complemented the results from the multiple trait linear regression models. Plant and ear heights, plant stand at harvest, and the kernels per ear^{-1} (KPE) had strong positive correlation coefficients with the final yield (Table 6.4a). Plant and ear heights and KPE had positive values and were significantly correlated with yield across sites and seasons. Grain yield and mean-LCC were significantly correlated only in HN WS09 and LN DS09. The characters that were related were affected similarly in their comparisons against others. These characters included plant and ear heights, kernel moisture contents at harvest and KDD, and physiological maturity and flowering dates, among others (Table 6.4b). Mean-LCC had a strong negatively consistent relationship with characters related to yield and maturity (i.e. flowering/reproductive characters, plant and ear heights, KDD, kernel moisture content at harvest, kernels ear^{-1}), except when it came to grain fill duration. Kernel moisture content at harvest and KDD, also physiological maturity and kernel moisture content at harvest, and maturity and KDD were strongly positively correlated.

Table 6.4a: Pearson's phenotypic correlation coefficients of traits with grain yield for 54 maize hybrids over 6 environments

Trait	Environment					
	DS08		WS09		DS09	
	HN	LN	HN	LN	HN	LN
50% anthesis	0.292**	0.304**	-0.103	-0.361***	0.119	0.102
50% silking	0.290**	0.274**	-0.105	-0.330***	0.100	-0.002
Mean LCC	-0.006	-0.130	0.264**	0.162	0.139	0.348***
Plant height	0.558****	0.585****	0.510****	0.534****	0.469****	0.388****
Ear height	0.485****	0.482****	0.324***	0.375****	0.469****	0.325***
Leaf above ear	0.163	0.055	0.032	0.132	-0.025	0.138
plant stand	0.291**	0.089	0.442***	0.535****	0.444****	0.472****
Ears per plant	0.167	0.192*	0.110	0.251*	0.021	0.036
Kernel moisture content	0.250**	0.328**	0.174	0.259*	0.112	0.108
Kernel per ear	0.224*	0.400****	0.252*	0.441****	0.356***	0.485****
Kernel dry down	0.261**	0.178	0.148	-0.103	0.076	0.293**
Grain filling duration	-0.212*	-0.130	0.304**	0.535****	-0.069	0.060
Physiological maturity	0.257**	0.335***	0.170	0.251*	0.109	0.109

*****, ***, **, *, statistically significant at $p \leq 0.0001$; 0.001, 0.01 and 0.05, respectively

Pearson's Correlation Coefficients, No. = 108. Prob > |r| under H_0: Rho=0

WS = wet season; DS = dry season; HN = high nitrogen regime (120 kg N ha^{-1}), LN = low nitrogen regime (60 kg N ha^{-1}); 08 and 09 refer to years 2008 and 2009

Table 6.4b: Statistically significant Pearson’s phenotypic correlation coefficients among components traits for grain yield and physiological maturity of 54 maize hybrids over six environments

Trait pair	Environment/N regime					
	DS 08		WS 09		DS 09	
	HN	LN	HN	LN	HN	LN
	Correlations of GY components with 50% SD					
LCC vs. SD	-0.599****	-0.660****	-0.823****	-0.301**		-0.606****
P ht vs. SD	0.511****	0.663****	0.510****	0.299**		0.493****
E ht vs. SD	0.556****	0.699****	0.576****	0.368****		0.479****
MC vs. SD	0.594****	0.702****	0.680****	0.448****		0.468****
KDD vs. SD	0.763****	0.747****	0.708****	0.628****		0.604****
Fill vs. SD	-0.874****	-0.854****	-0.732****	-0.795****	-0.959****	-0.880****
Pmat vs. SD	0.665****	0.708****	0.685****	0.452****		0.465****
	Correlations of GY components with mean-LCC					
P ht vs. LCC	-0.306**	-0.468****	-0.390****	-0.2000*	-0.210*	-0.221*
E ht vs. LCC	-0.346***	-0.502****	-0.448****	-0.196*	-0.244*	-0.190*
Leaf vs. LCC	-0.427****	-0.377****				
MC vs. LCC	-0.485****	-0.542****	-0.606****	-0.241*	-0.403****	-0.260**
KDD vs. LCC	-0.592****	-0.503****	-0.599****	-0.297**		
Fill vs. LCC	0.424****	0.505****	0.556****			0.472****
Pmat. Vs. LCC	-0.550****	-0.547****	-0.613****	-0.255**	-0.406****	-0.396****
	Correlations of GY components with plant and ear heights					
E ht vs. p ht	0.931****	0.909****	0.797****		0.874****	
Leaf vs. p ht	0.310**	0.321***	0.265**			
MC vs. p ht	0.615****	0.657****	0.570****	0.703****	0.299**	0.316**
KDD vs. p ht	0.565****	0.608****	0.598****	0.483****		0.537****
Fill vs. p ht	-0.273**	-0.447****				-0.389****
Pmat vs. p ht	0.605****	0.632****	0.569****	0.701****	0.298**	0.312**
Stand vs. p ht				0.277**	0.479****	
MC vs. e ht	0.578****	0.635****	0.586****	0.704****	0.397****	0.258**
KDD vs. e ht	0.535****	0.565****	0.543****	0.517****		0.484****
Fill vs. e ht	-0.348***	-0.503****	-0.240*			-0.405****
Pmat vs. e ht	0.582****	0.624****	0.588****	0.704****	0.397****	0.254**
	Correlations of GY components with other traits					
EPP vs. stand		-0.19508*	-.0.35713***		-0.408****	-0.513****
Pmat vs. MC	0.935****	0.987****	1.000****	1.000****	1.000****	
MC vs. KDD	0.672****	0.709****	0.648****	0.639****	0.318**	0.402****
Fill vs. KDD	0.533****	-0.524****	-0.362****	-0.251**		-0.467****

Trait pair	Environment/N regime					
	DS 08		WS 09		DS 09	
	HN	LN	HN	LN	HN	LN
Pmat vs. KDD	0.712****	0.684****	0.651****	0.647****	0.317**	0.401****

*****, ***, **, *, statistically significant at p≤0.0001; 0.001, 0.01 and 0.05, respectively.

Pearson Correlation Coefficients, No. = 108, Prob > |r| under H_0: Rho=0

Key: AD = 50% anthesis date; SD = 50% silking date; p ht = plant height (cm); e ht = ear height (cm); stand = number of plants at harvest per plot; MC = kernel moisture content at harvest (%); KDD = kernel dry down index; Fill = effective grain filling duration; Pmat = physiological maturity (calendar days); EPP = ears plant$^{-1,}$ LCC = mean-leaf chlorophyll concentration (SPAD values). WS = wet season; DS = dry season; HN = high nitrogen regime (120 kg N ha^{-1}), LN = low nitrogen regime (60 kg N ha^{-1}); 08 and 09 refer to years 2008 and 2009

6.3.3 Linking multiple trait linear regression, correlations and path coefficient analyses

Only plant height, plant stand at harvest, and the number of kernels per ear (KPE) were positively significantly correlated with yield under high and low N, although plant stand and KPE increased under low-N conditions (Tables 6.5). All the traits were the same for all N conditions, except 50% AD under high N and EFPD under low N. For convenience, significant Pearson's correlation coefficients were considered at ≥30% under both N conditions and specifically for high and low N. Under both N conditions, plant height correlated negatively with mean-LCC, KPE correlated positively with yield, plant height had a positive correlation with yield, and plant stand at harvest correlated positively with yield. Under high N conditions, plant height correlated positively with 50% AD and yield. Under the same conditions, mean-LCC correlated negatively with 50% AD and positively with EPP, whereas plant stand at harvest correlated negatively with EPP. Under low N conditions, only mean-LCC correlated positively with EFPD (Table 6.5).

Table 6.5: Pearson's phenotypic correlation coefficients between grain yield and grain yield components in maize selected by multiple trait linear regression under high and low N for three seasons

High N

	P ht	LCC	KPE	50% AD	Stand	EPP	GY
Plant height (P ht)	1						
Mean LCC (LCC)	-0.415****	1					
Kernels ear^{-1} (KPE)	0.294**	-0.200*	1				
50% anthesis date (50% AD)	0.566****	-0.723****	0.096	1			
Plant stand (Stand)	0.241*	0.109	0.286**	-0.193*	1		
Ears plant^{-1} (EPP)	-0.254**	0.366****	-0.196*	-0.141	-0.326***	1	
Grain yield (GY)	0.542****	0.158	0.328***	0.162	0.357***	0.096	1

Low N

	LCC	P ht	Stand	EPP	KPE	G fill	GY
Mean LCC (LCC)	1						
Plant height (P ht)	-0.359***	1					
Plant stand (Stand)	0.014	0.145	1				
Ears plant^{-1} (EPP)	0.234*	-0.269**	-0.259**	1			
Kernels ear^{-1}(KPE)	0.036	0.283**	0.079	-0.222*	1		
Grain fill duration (G fill)	0.441***	-0.278**	0.07	0.135	0.232*	1	
Grain yield (GY)	0.102	0.526****	0.447****	0.036	0.435****	0.179	1

*****, ***, **, *, statistically significant at $p \leq 0.0001$; 0.001, 0.01 and 0.05, respectively. Pearson Correlation Coefficients, No. = 108. Prob > |r| under H_0: Rho = 0

6.3.4 Path coefficients under high and low nitrogen

Direct paths (effects) and indirect effects were considered following same criterion of statistical significance of Pearson's phenotypic correlation coefficients of ≥30%. Table 6.6 therefore indicates direct and indirect path coefficients to grain yield under high and low N conditions. Under high N conditions, the indirect effects of plant height via 50% AD were 0.294. The mean-LCC had indirect effects to yield via 50% AD and EPP of -0.376 and 0.132, respectively. Under the same conditions, KPE had indirect effects of 0.229 to yield via plant stand at harvest, whereas the indirect effects of plant stand at harvest to yield via EPP were -0.418. The only indirect effects at low N were 0.058 for mean-LCC to yield via EFPD.

Across N regimes, the direct paths to yield through plant stand were 0.779 at high N and 0.410 under low N, through KPE were 0.124 under high N and 0.273 under low N, and through plant height were 0.304 under high N and 0.564 at low N. The only indirect effects under both N were plant height to yield via mean-LCC were -0.193 under high N and -0.203 under low N.

The index of reduction of direct path to indirect effects relative to total effects was computed in terms of magnitudes and signs. Table 6.6 indicates that in terms of magnitude, the direct effects were higher than indirect effects relative to total effects, and vice versa. For signs, higher index values meant that indirect effects were negative relative to direct effects. For example, EPP had more negative indirect effects under LN, so the index of reduction was highest (15.6). Although EPP was reduced under both regimes of N, the effect was higher under LN than HN. Negative index reduction was observed for KPE under HN.

Table 6.6: Path and Pearson's phenotypic correlation coefficients of selected to grain yield (ton ha^{-1}) under contrasting N conditions pooled over three environments

Trait	Direct path coefficients	Indirect path values through:						Total indirect effects	Index reduced	r_p
	High N									
		PH	LCC	KPE	50%AD	Stand	EPP			
Plant height (PH)	0.304		-0.193	0.036	0.294	0.193	-0.092	0.238	0.122	0.542****
Mean LCC (LCC)	0.466	-0.126		-0.025	-0.376	0.087	0.132	-0.308	4.899	0.158
Kernels per ear (KPE)	0.124	0.089	-0.093		0.05	0.229	-0.071	0.204	-0.244	0.238***
50% anthesis (50%AD)	0.52	0.172	-0.337	0.012		-0.154	-0.151	-0.358	5.42	0.162
Plant stand (Stand)	0.799	0.073	0.051	0.035	-0.183		-0.418	-0.442	3.476	0.357***
Ears per plant (EPP)	0.361	-0.077	0.171	-0.024	-0.075	-0.26		-0.265	6.521	0.096
	Low N									
		LCC	PH	Stand	EPP	KPE	G fill			
Mean LCC (LCC)	0.161		-0.203	0.006	0.07	0.01	0.058	-0.059	2.157	0.102
Plant height (PH)	0.564	-0.058		0.06	-0.08	0.077	-0.037	-0.038	1.144	0.526****
Plant stand (Stand)	0.41	0.002	0.082		-0.077	0.021	0.009	-0.037	1.000	0.447****
Ears per plant (EPP)	0.299	0.038	-0.152	-0.106		-0.061	0.018	-0.263	15.611	0.036
Kernels per ear (KPE)	0.273	0.006	0.16	0.032	-0.066		0.03	0.162	0.255	0.435***
Grain fill duration (G fill)	0.133	0.071	-0.157	0.029	0.04	0.063		0.046	0.486	0.179

High N = 120kghaa^{-1}; Low N = 60kgha^{-1}; LCC = leaf chlorophyll concentration; Index reduced = (direct path-total indirect effects)/total effects. *****, ***, total direct and indirect effects to grain yield (t ha^{-1}) statistically significant at p≤0.0001and 0.001. Pearson Correlation Coefficients, No. = 108. Prob > |r| under H_0: Rho = 0

6.4 Discussion

6.4.1 Multiple trait linear regression models

Multiple trait linear regression models were conducted over three seasons under high and low N conditions (Tables 6.1-3). The number of ears per plant (EPP) and plant stand at harvest had consistently positive regression coefficients (β) across N regimes and seasons. The higher and consistent EPP with plant stand at harvest, especially under LN conditions, indicates the relevance of improving yield productivity on soils in SSA. Ears per plant and plant stand at harvest were closely associated in regression models and they had positive βs, probably because these characters are highly related, since the former trait is calculated from the latter. The EPP has been considered as both a key component of yield and as a measure of stress index, i.e. high EPP imply resilience to a variety to poor environments (Williman et al., 1987a, b; Bänziger et al., 2000; Varga et al., 2004). Furthermore, kernels per ear had positive β values to support the preponderance of EPP in the present study. Kernels per ear were reported to be even more of an important measure of stress tolerance than the EPP under stress (Monneveux et al., 2005). Plant stand at harvest could be a yardstick for yield because the productivity of the varieties is estimated as grain yield per plant per unit area, such that optimum stands may lead to high productivity. However, Lal et al. (2010) warned that benefits from N might not be realised where farmers apply low N to thick plant stands. The authors advised that applying N in splits would improve grain productivity in farming systems, where farmers practice high plant density; also the possibility increases to benefit from residual effects of N on soils.

Other traits that enhance yield may appear to contribute indirectly to yield via EPP, since EPP is the highest component under the present multiple-trait linear regression exercise. The relative relationship between EPP and other GY components and final yield will be established in the next section on path coefficient analysis. Whereas plant height enhanced yield, the ear height, which is a trait related to plant height, had a converse influence on final yield under LN conditions; this may render the use of correlated response very difficult under sub-optimal conditions, thus supporting Falconer (1989), who cited that indirect selection is not guaranteed in maize. The negative values of regression coefficients for traits that are related to calendar physiological maturity over N regimes and seasons would support the hypothesis that earlier maturing genotypes may have low-yield potential. The regression models (Tables 6.1-3) suggest that each farming system may have its specific problem to respond to, as portrayed by the inconsistency of traits over N regimes and seasons. This is consistent with Lory et al. (1995), who reported that crop responses to N fertiliser rates are case specific. However, positive regression coefficients for plant height, mean-LCC and EFPD that are earmarked in the present study may suggest that yield and physiological maturity may be improved via these traits.

6.4.2 Pearson's phenotypic correlations between traits

Generally, Table 6.4 could be summarised as the efficiency of the source: sink relationship. This explains the relative contribution of component traits, with respect to calendar physiological maturity and grain yield. Like multiple trait linear regression, Pearson's correlation coefficients were computed over three seasons under two N regimes. Correlations partly complemented regression models. However, against expectation, the mean-LCC correlated feebly but positively with yield per se; although the mean-LCC correlated strongly negatively with all other components of physiological maturity and yield, except the EFPD. However, the literature appears to support this finding of the mean-LCC vs. yield, although it is silent on the relationship between mean-LCC and EFPD, which was the only pair that had a positive and strong correlation. The literature supports the present findings for a weak relationship between mean-LCC and yield, as the mean-LCC offers tolerance to multiple stresses, which does not necessarily imply high yield (Tollenaar et al., 1997). However, Ahmadzadeh et al. (2004) reported that leaf carbon exchange rate (CER), rather than potential leaf CER, positively correlated with DMA during grain-filling period and yield. Thomas and Smart, (1993) reported that yield and extended LCC may not show a perfect positive correlation, whereas Borrell et al. (2001) asserted that extended LCC correlated negatively with yield under N stress. Azeez et al. (2006) found a significant correlation between yield and leaf N uptake ($r = 0.33$; $P<0.01$). Although high yield is achievable, the genetics and maintenance of the LCC character until physiological maturity are not easy objectives (Gentinatta et al., 1987). Dodd (1977, 1980) reported a perfect relationship between grain yield, grain filling and the number of kernels per ear with stalk rot. Whereas high kernels per ear would starve the stalk of the assimilates since the ear is a primary sink, the low grain fill has also been associated with high incidences of stalk rots, as the authors suggested. It therefore appears that stalk rots and high yield cannot be separated. The strategy, therefore, should be to breed for N tolerance, as N is the key nutrient to maintain photosynthetic capacity, and regulate other nutrients and the metabolism, which may balance the source: sink relationship.

Factors that reduce the LCC or increase physical leaf senescence (i.e. defoliation) may be genetic, developmental and environmental in nature. However, the genetic mechanism and the inheritance of defoliation is not clear (Tollenaar and Daynard, 1978a, b, c), which may suggest a correlated response of the LCC character with other traits under N regimes, since LCC is conditioned by N. Furthermore, tropical maize is overly produced under low-N conditions. Researchers have been considering the 50% silking stage as the reference point to leaf defoliation effects. Defoliation (loss of leaf N) near that stage affects kernel number, whereas after that stage may reduce kernel weight (Tollenaar and Daynard, 1978a). Early leaf senescence (early loss of LCC) hastens the drying of kernels, thus supporting a negative correlation between the kernel dry-down (KDD) index and mean-LCC, which was found in the present study. However, plant traits controlling KDD are not clear (Cavalieri and Smith, 1985), also kernel texture (whether flintier or dent) could not clarify KDD index (Hunter et al., 1979). Since the present study investigated traits and environments that predispose maize to defoliation, the findings from correlation analyses could provide insights on the inheritance of defoliation via loss of leaf N and its regulatory role to the source: sink ratio.

Traits that are logically related were similarly affected in correlation analysis. Plant and ear heights, KDD index and kernel MC at harvest, calendar physiological maturity and kernel MC at harvest, calendar physiological maturity and flowering dates, and calendar physiological maturity and KDD index were strongly positively correlated. However, other researchers have found conflicting relationships among traits. Furthermore, previous works have not studied the influence of low N in tropical maize for traits related to yield and calendar physiological maturity. Kernel dry-down correlates positively with kernel fill rate and duration (Johnson and Tanner, 1972; Daynard, 1972; Cross, 1985), although the present study found the traits to be negatively correlated except in HNDS08. However, Rajcan and Tollenaar (1999) established that maize hybrids that take high N at grain filling stage would yield higher than those taking less N and the character of extended LCC would follow similar trends. Nevertheless, under normal situations, KDD has been associated with a reduction in key yield components (Cross, 1985; Cross and Kabir, 1989), but the present study found some weak positive correlations between yield and KDD, and strong positive correlations with traits related to calendar physiological maturity and final calendar physiological maturity per se, except EFPD (Tables 6.4a and b). This may suggest that early maturers may have high rates of grain filling, which would not necessarily result in high yield due to a short period of effective grain filling duration (EFPD), which is affected by N regimes.

The source: sink relationships established by the present study under high and low N with seasons could help to classify the varieties by performance for calendar physiological maturity and yield. The shorter duration of the leaf area (or early loss of LCC) has been associated with early physiological maturity (Crafts-Brandner and Poneleit, 1987), due to efficiency in remobilising N and low EFPD, linked to increased rate of kernel DMA. The shorter-season varieties have been considered as source limited (Hunter, 1980; Crafts-Brandner and Poneleit, 1987), which suggests that a longer filling duration would translate into a high rate of DMA. Contrary to the long season varieties that are sink limited, as Hunter (1980) reported, the source strength can be increased in relatively earlier- to intermediate maturing cultivars by increasing the EFPD via an extended LCC character. However, the challenge would be to identify a few key traits that increase and maintain high kernel DM until physiological maturity, especially under limiting soil N conditions. One possible strategy would be to target and maintain a high floret fertility index, thus high kernels per ear, as Wolf and Hallauer (1977) and Andrade et al. (1999) suggested that the high kernel numbers translates into improved yield. Crafts-Brandner and Poneleit (1987) supported further that for plants with equal kernel number, an increase in 5% kernel weight is possible for each additional day of EFPD. These findings may comply with the concept of the constant system capacity (Yan and Wallace, 1995), which hypothesises that each agro-ecology has its own limits that the breeder cannot extend.

Correlation analysis partly supports multiple trait linear regression. Positively correlated traits may suggest that indirect selection is possible while, on the other hand, direct selection for the two traits may be conducted for negatively correlated traits. However, this relationship should not be taken for granted for regression. For instance, as the present study established, regression in ear heights had negative β values, whereas the plant height had positive β values, disregarding the fact that plant and ear heights are highly positively associated. Nevertheless, this should not be surprising because correlation and regression analyses mean different things. Stronger associations that exist between calendar physiological maturity and plant height confirm the results of Mahmud and Kramer (1951). Plant and ear heights are key grain components to final yield, as any reduction of these traits, especially under low N, may reduce final yield (Bänziger et al., 2000; Bertin and Gallais, 2000; Worku et al., 2007). It should therefore be concluded that any stress that reduces the morphological size of plants may also compromise final yield. The general trend was that regardless of N regimes and signs of correlations, the magnitudes of correlation coefficients among traits and final yield decrease under low N compared with high N.

6.4.3 Correlations and path coefficient analyses for high and low nitrogen

Positive correlation of plant height, plant stand at harvest and KPE with yield under both conditions of N would suggest that these relationships could be enhanced to improve final yield under both conditions of N (Tables 6.5 and 6.6). However, while plant height had correlations of similar magnitude under both N conditions, the magnitudes for plant stand at harvest and KPE improved under low N. This may suggest that KPE will increase with optimum plant stands, even under low N conditions. Bänziger et al. (2000), Monneveux (2005) and Ojo et al. (2006) reported the increase of KPE under stress as a measure of stress index. This is further supported by positive correlations between mean-LCC and EFPD, and KPE with plant height under low N, as indicated in Table 6.5. However, a positive association between KPE and EPP under high N and the opposite relationship under low N would confirm that low N reduces yield. Negative correlations under both N conditions between mean-LCC vs. plant height, EPP vs. plant stand at harvest would confirm that the traits are negatively affected, regardless of regimes of N. Bänziger and Lafitte (1997) reported genetic correlations with yield of 0.78 and 0.24 for EPP and extended LCC, respectively, under low N, which contradicted the present study. In this study, the two traits were opposite and non-significant under low N (Table 6.5). Furthermore, EPP and mean-LCC were similar under both N conditions, whereas mean LCC increased under high N (Tables 6.5). High mean-LCC in high N experiments, relative to low N, would further confirm that LCC is a function of N, whereas EPP could be increased under both conditions of N.

Both direct and indirect effects to yield under high and low N were considered following the same criterion of significance according to Pearson's correlation coefficients (r_p) of ≥30% (Table 6.5). However, some of the direct and indirect effects were lower than the stated cut-off r_p of 30%. Under high N conditions, the indirect effects of plant height via 50% AD were 0.294. This may suggest that plant height promotes about 30% of yield if it is associated with a mid-anthesis date under high N conditions. Bänziger et al. (2000) reported the mid-anthesis date as a bit stable over stresses such as low N, compared with the mid-silking date. However, for better yields, synchronised pollinations under stresses must be attained. The mean-LCC had indirect effects to yield via 50% AD and EPP of -0.376 and 0.132, respectively. This translates to a chance yield penalty of about 38% when mean-LCC was related to mid-anthesis dates, while only the association of mean LCC with EPP would help promote yield by about 10%. This agrees with Hageman and Lambert (1996) that extended LCC (or stay-green trait) would be relevant as the maturity period of a genotype increases. This may further confirm that earlier-maturing genotypes would be source limited and vice versa to longer varieties. In addition, it would be possible to reduce calendar flowering dates, while increasing the photosynthetic capacity of genotypes, thus incorporating high DM into a relatively earlier genotype under high N conditions. However, this may not necessarily reduce the gestation period of the cultivar. Only the yield would be improved in cultivars with the same maturity periods. Still under high N conditions, KPE had indirect effects of 0.229 to yield via plant stand at harvest, whereas the indirect effects of plant stand at harvest to yield via EPP were -0.418. The chance was about 23% to increase yield if the number of kernels per ear was associated with plant stand at harvest, whereas there was the possibility of more than a 40% yield penalty when an additional ear per plant (EPP) was associated with plant stand. This positive association between plant stand and KPE may further suggest that the two traits may decrease or increase together under high N. The negative association between plant stand and EPP suggests that one trait decreases as the other increases. Barrenness is expected as plant stand increases and vice versa (Bänziger et al., 2000: Sangoi, 2001; Lal et al., 2010). Thus, efforts to raise yield by targeting KPE would be more relevant than EPP under high N conditions, as the present study established.

The only indirect effects at low N were 0.058 for mean-LCC to yield via EFPD. This may demonstrate that mean-LCC, when associated with grain-fill duration under low N, had a negligible contribution to final yield. In other words, the mean-LCC would be relevant under high N, since LCC and N are highly associated, as this study has established, and as the supporting literature shows, that high N experiments had higher mean values than low N trials.

Across N regimes, the direct effects to yield through plant stand were 0.779 at high N and 0.410 under low N. Kernels per ear had a direct effect to yield of 0.124 under high N and 0.273 under low N, whereas the direct effects of plant height to yield were 0.304 under high N and 0.564 at low N. Plant stand had the highest direct effects of about 80% under high N, which reduced by half under low N. Plant height had the highest direct effects of about 56% under low N. Furthermore, direct effects to yield almost doubled for KPE and plant height, as soil N declined. This may suggest that boosting the number of kernels per ear on a taller genotype would improve yield under low N conditions. However, the challenge would be to maintain such an enhanced role to final yield under low-N conditions because KPE is more highly affected by management regimes than any other yield component under farmers' production conditions (Varga et al., 2004; Monneveux et al., 2005; Ojo et al. 2006).

The only indirect effects under both N regimes were plant height to yield via mean-LCC, which were -0.193 under high N and -0.203 under low N. This demonstrates that the association between plant height and mean-LCC had a possible yield penalty of about 20% under both N conditions.

6.4.4 Conclusions and implications

1) The number of ears per plant (EPP) had the highest positive regression coefficients under N regimes but was higher under low N, thus confirming that it contributes more to grain yield under low N. Therefore grain yield can be enhanced by emphasising ear prolificacy under low N conditions.
2) Plant and ear heights, plant stand at harvest and the number of kernels per ear had significant strong positive Pearson's correlations, with yield across all N regimes and seasons. Thus, taller genotypes with increased ear placement under optimum plant stand under both N regimes may have many kernels per ear. There could be, if one utilises this relationship, the possibility of increasing the number of kernels per plant in the case of an

ear-prolific genotype. Raising the ear height may allow below-the-ear internodes to bear an additional ear, consequently increasing the number of kernels per plant.

3) Leaf chlorophyll concentration (LCC) and the duration of effective grain filling (EFPD) had significant strong Pearson's positive correlations but they exhibited significantly strong negative correlations with most of the other traits.
4) Under high N conditions, plant height had positive correlations with a 50% anthesis date (AD) and yield, whereas negative associations were evident between mean--CC and 50% AD and between plant stand and EPP. Under the same conditions, mean-LCC associated positively with EPP.
5) Under low N, the mean-LCC correlated positively with EFPD
6) Low N exhibited higher direct effects to yield via KPE and plant height compared to high N, whereas plant stand at harvest had a converse relationship. Grain yield could therefore be directly improved by targeting KPE and plant height, whereas optimum plant stand until harvesting time would improve yield under high N conditions.
7) The only indirect effects to yield across N regimes were plant height via mean-LCC; however, these effects were negative and not significantly different under both high and low N conditions.
8) Regarding indirect effects under high N, plant stand at harvest reduced more yield via EPP (-0.418), followed by mean-LCC through 50% AD (-0.376), whereas yield was promoted by plant height via 50% AD (0.294), followed by KPE via plant stand at harvest (0.229).
9) Under low N, the indirect effects to yield were negative and they involved only mean-LCC via EFPD, although these effects were not significantly different.
10) The index of reduction for indirect effects relative to direct effects confirmed that EPP was reduced under low N, while KPE was reduced under high N. These findings implied that the indirect effects of EPP and KPE to grain yield were highest under low N and high N, respectively. Therefore, yield could be improved under low N by increasing ear prolificacy, while raising the KPE would promote yield under high N conditions.

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7 Overview of research findings

7.1 Introduction

Nitrogen (N) is a key nutrient in maize production in the intermediate altitudes of Tanzania yet farmers produce maize under low soil N conditions, consequently obtaining low grain yields. This has a negative impact on grain production for the country and the low grain productivity has negative implications for national food security. Most pressing is the fact that the intermediate-altitude zone, ranging from 900-1600 m.a.s.l., is the most suitable one in Tanzania for maize production, although average farmers' yields have stagnated at <2 t ha^{-1} compared to a yield potential of about 8 t ha^{-1}. Among other factors, yield can be improved by developing varieties with high nitrogen use efficiency or tolerance to low N conditions in the smallholder sector. The other factors that can be pursued include improved access of farmers to fertiliser and improved seeds. However, in this study, possible plant breeding interventions were investigated in order to develop a viable breeding strategy. Genetic control of secondary traits, heterosis for yield in hybrids and their adaptation to low soil N in the intermediate zones of Tanzania have not been established, yet this knowledge would be crucial in designing appropriate hybrids for deployment in the smallholder sector, where the crop is produced under low soil N conditions.

The present study therefore focused on determining genetic effects and heterosis for grain yield and secondary effects, respectively, and also investigated the interrelationships among secondary traits such as leaf chlorophyll and calendar physiological maturity that are important for improving the grain yield of maize varieties among local and exotic inbred lines under low and high soil N conditions in Tanzania. This overview summarises the major findings of a study representing the intermediate altitudes of Tanzania. The study objectives are highlighted, followed by a summary of the major findings and their implications for improving grain yield of maize cultivars under low soil N conditions. Possible challenges that are encountered in breeding maize hybrids for low N conditions and directions for the future studies are also discussed.

The specific objectives of the study were to:

1) determine farmers' perceptions, production constraints and preferences for new maize cultivars in the Southern Highlands zone in Tanzania,
2) determine the genetic effects controlling leaf chlorophyll concentration from mid-silking to physiological maturity under low and high N conditions,
3) determine the genetic effects that control plant and ear heights, the number of leaves above the ear and the number of kernels per ear^{-} under low and high N conditions,
4) determine heterosis, relative grain yield loss and an earliness to mature when low N dosage is applied, and,
5) establish relationships among secondary traits and their association with grain yield under low and high N regimes.

7.2 Summary of the major findings and their implications for maize breeding

7.2.1 Social survey conducted in the Southern Highlands Zone

Prior to genetic studies a social survey was conducted and the results revealed the following:

a) Significant variation among the farmers in background training and experience, access to new technology and preferences for maize cultivars were identified. Over 70% of farmers in the study area had no formal agricultural skills, which partly explains their perceptions of and access to new technology. For example 35%, 53% and 12% of the farmers used local, improved, and mixtures of local and improved maize varieties, respectively. The use of unimproved varieties also partly explains low productivity in the small holder sector.

b) About 60% of farmers required extra-early maize cultivars that mature in two months. The trend of requiring cultivars decreased as the time to maturity of the cultivar increased. A trend for the need for earlier cultivars stood at 83%. Nonetheless, the duration of two months' physiological maturity appeared not feasible in the study area. However, according to tropical maize, such a maturity period would be relevant in low altitudes.

c) About 60% of farmers needed cultivars with fast kernel dry-down index. Sixty-six percent of farmers needed cultivars with the stay-green character. Farmers used home-saved seed, whereas 33% of them selected big cobs before shelling and 31% of farmers considered high productivity per ear and per plant when selecting for grain yield components in the case of the improved varieties that they preferred. Thus these traits should be included in the breeding objectives when targeting high yielding and early maturing varieties under low N and other abiotic stresses. Furthermore, varieties whose kernels dry down faster and retain high leaf chlorophyll concentration would provide dual purpose maize for food grain and feed forage for ruminant livestock. Cultivars that dry-down fast are easy to process and transfer, as they are less bulky and may have longer shelf lives due to low-kernel moisture contents to limit post-harvest pests and diseases.

d) Only 37% of farmers applied fertiliser; they commonly use N-based fertilisers — i.e. urea, di-ammonium phosphate, sulphate of ammonia, and calcium ammonium nitrate in that order. However, 87% of farmers reported that fertiliser was not needed. Despite all these limiting factors, 90% and about 70% of farmers did not have access to credit and extension services, respectively. This indicates that production could be improved by increased use of both improved varieties and high levels of fertiliser. Furthermore, the current study indicated that yield could be improved by 30% if recommended fertiliser rates were used with positive impact on national food security for the nation, because the SHZ is the breadbasket of Tanzania.

e) Maize productivity was influenced by the age and sex of farmers, the area under cultivation, type of variety grown, fertiliser use and fungicides. In fact, poverty and drought were among the major constraints to maize productivity in the study area. In addition to developing drought-tolerant varieties, improvements to the farmers' socio-economic situation might also impact positively on grain yield, with some multiplier effects for the rural economy.

f) Generally, farmers needed earlier maturing cultivars with high yield, which also incorporate most of the traits that resist multiple local stresses. This may suggest the need to carefully select and breed for adaptive traits that relate to productivity and not survival, because in tropical maize, grain yield is an objective. The other option would be a correlated response to selection, whereby, in the case of strongly positively correlated traits, one trait is chosen for the other and conversely for negatively associated traits.

g) For farmers, an "idea of a good variety" meant wide adaptation with relatively higher yields. This variety matured earlier than the current variety and was obtained either from the market or was the farmer's best landrace. High grain yield was based on productivity per plant and per unit area: such as many cobs per plant, many kernels per row, many kernels per ear and, overall, many kernels per plant. The earliness character was associated with the high rate of kernel dry-down, while maintaining its greenness upon physiological maturity, consequently there was a long grain fill.

7.2.2 Inheritance of leaf chlorophyll concentration under low and high nitrogen

Leaf chlorophyll concentration post mid-silking to the physiological maturity stage may reflect the rate and duration of grain filling in maize. Chlorophyll concentration at each grain-filling stage may therefore indicate the status of N with respect to dry matter accumulation and know the stage where N is needed the most in maize. Decrease in leaf N with time would imply that DMA is taking place. This may also indicate the maturity period of the genotype versus DMA. Fast decline of leaf N may indicate that the genotype is early (i.e. rate of DMA), whereas the slow rate of leaf N decline (i.e. duration of DMA) indicates the genotype is somewhat later maturing. Low N has been reported in literature to be severe at that window growth period in maize, thus negatively impacting on yield due to poor grain fill. However, the genetic control of leaf chlorophyll concentration across grain filling stages is scarcely reported in the literature. Breeding strategies would be devised if the genetic effects of leaf chlorophyll concentration at each stage were either fixable (additive and additive by additive) or non-fixable (dominance, additive by dominance, and dominance by dominance), or both. Recurrent selection (RS) is appropriate when fixable effects predominate, whereas non-fixable genetic effects allow the use of hybrid breeding and the possibility of the back-cross breeding approach. The equal proportion of fixable to non-fixable genetic effects suggests the use of reciprocal recurrent selection (RRS).

Generation mean analysis was conducted for two crosses under high- and low-N conditions, and the results were for two categories:

a) across the crosses and N regimes, and
b) specific to the cross and growth stage.

Across the crosses and N regimes the results were as follows:

a) The genetic effects were under high N only for both crosses — where the parents were significantly different, based on LSD at 5% level of probability, thus meeting the critical requirement of GMA. This finding suggested that the genetic effects and genetic potential for LCC character are relevant in optimal N conditions where the differences between parents are clear.

b) The relative contributions of genetic effects indicated that additive genetic effects decreased with the age of the growth stage, whereas the dominance effects increased with the age of the growth stage. It would imply that RS could be practiced for the LCC trait at early growth stages, while the hybridisation may be deferred for later growth stages.
c) Positive transgressive segregants were observed, indicating that LCC at each growth stage (i.e. traits) could be improved under both N regimes. Generally, the frequency distribution curves were normal and continuous, confirming that the traits of the leaf chlorophyll character were governed by many genes.
d) Leaf chlorophyll concentration began to decline at the milk stage; however, there were lower SPAD values under LN, compared to HN. Such a stage could be considered as a linear growth one, where N is needed the most in maize for sink formation. The overall consistency of the inferior parent for leaf chlorophyll concentration (P_1) with low SPAD values, irrespective of crosses and N regimes, may indicate the precision of the chlorophyll meter in quantifying leaf chlorophyll concentration.

Specific results for crosses and growth stages were:

Cross T20 x C58

The genetic effects were different across growth stages and N regimes. Overall, the ratio of fixable to non-fixable genetic effects was 74% to 26% under high N and 35% to 65% under low N, which implied that fixable effects were important under high N whereas non-fixable were relevant for low N conditions.

a) At mid-silking, the fixable genetic effects (additive and additive x additive) contributed about 84% and the additive x dominance effects contributed the bulk of non-fixable effects (dominance, additive x dominance and dominance x dominance) under high N; whereas the fixable effects contributed about 63% under low N and the remainder was non-fixable effects coming mainly from dominance x dominance effects.
b) About 90% of fixable genetic effects mainly came from the additive effects for the milk stage under high N. Under low N, the fixable effects of about 63% mainly came from

additive effects, whereas the non-fixable effects of almost equal proportions were observed.

c) At the dent stage, there were fixable genetic effects of about 64% and non-fixable effects of 36%, which came from the dominance effects under high N. Under low N, the non-fixable genetic effects were about 96%, which mainly came from dominance, dominance x dominance, and additive x dominance in that order.

d) At the maturity stage, both fixable and non-fixable genetic effects were in equal proportions under high N, notably the additive x additive effects were only the largest (11%), whereas only non-fixable components from epistasis were few. Under low N, the additive x additive effects contributed almost all of the fixable effects, whereas the non-fixable effects, which were about 78%, mainly came from dominance and dominance x dominance effects.

e) When averaged across growth stages, this indicated 81% of fixable effects, whereas the non-fixable effects came from dominance under high N. Non-fixable effects of about 76% were evident under low N, with a major contribution from dominance and dominance x dominance effects.

Cross T20 x N_G8

Genetic effects were also different across growth stages, as in cross T20 x C58. The ratio of fixable to non-fixable genetic effects was 37% to 63% under high N and 20% to 80% under low N, suggesting the opposite trends of genetic effects from cross T20 x C58 under N regimes.

a) There were 74% of non-fixable genetic effects at the mid-silking stage, where 69% came from additive x dominance effects under high N. About 62% of the fixable genetic effects were evident under low N, with much contribution from the additive effects.

b) Fixable effects predominated at the milk stage under high N, while it was the converse under low N. The additive x dominance effects contributed to total genetic effects more than the dominance x dominance effects under low N.

c) The non-fixable effects predominated at the dent stage, where the additive x dominance effects had a higher contribution than the dominance x dominance effects under high N. The non-fixable effects were 96%, with the additive x dominance effects contributing significantly higher than dominance x dominance effects under low N.

d) Non-fixable genetic effects predominated at maturity under both regimes of N.

e) When averaged across the growth stages, the dominance and additive genetic effects were about 40% each under high N, however, the additive x dominance effects inflated the non-fixable effects. The non-fixable effects predominated under low N, with the additive x dominance effects contributing more than dominance x dominance effects.

It could therefore be suggested from this study that where the respective fixable or the non-fixable genetic effects were larger than 60%, the recurrent selection (RS) for the former and selection with inbreeding, which is then followed by hybridisation for the latter effects, would be effective breeding strategies. Contradicting genetic effects from the two crosses and N regimes demonstrated that the inheritance of leaf chlorophyll concentration is not straight forward.

7.2.3 Inheritance of four secondary traits under low and high nitrogen

Plant and ear heights provide a mechanical support for cob attachment and so have a direct and/or indirect bearing on final grain yield. Leaves above the ear, as opposed to leaves below the ear, contribute directly to grain yield as they trap incident solar radiation, thus improving photosynthesis. The number of kernels per ear or the floret fertility index is a measure of the adaptation of a genotype, as many kernels indicate stress tolerance. The size of all of these traits are reduced under low-N conditions, however, the genetic control of these traits under low N is not well documented. The genetic study was therefore conducted to detect and estimate genetic effects for these traits under high and low N conditions. The results were:

a) With plant height in the crosses T20 x N_G8, non-fixable genetic effects (dominance, additive x dominance and dominance x dominance) predominated over fixable effects (additive and additive x additive) but with negligible contribution from epistasis under both N conditions.

b) For ear height in the cross T20 x N_G8, the dominance genetic effects contributed 90% of the non-fixable genetic effects under high N. The influence of both fixable and non-fixable genetic effects was almost equal under low N.

c) The number of leaves above the ear in cross N_G2 x C3 was typically governed by fixable genetic effects under both N conditions.

d) The number of kernels per ear was mainly controlled by non-fixable genetic effects under high N, whereas fixable genetic effects predominated under low N. The additive x additive effects of 18% and dominance x dominance effects of 32% predominated high and low N, respectively.

Genetic effects of ≥60% for either fixable or non-fixable genetic effects would imply that such effects for respective traits were predominant for the trait under N regime and an appropriate breeding strategy may therefore be devised. However, the N condition in which the trait and crosses were evaluated influenced genetic effects and this was the case for plant and ear heights and the number of kernels per ear. The number of leaves above the ear was typically governed by fixable genetic effects, implying that RS would be the most appropriate selection procedure to improve the trait under both N conditions. Nonetheless, this contradicted frequency distributions of progenies for this trait.

The frequency distributions for traits of parents against their segregating generations (F_2, BCP_1 and BCP_2) to illustrate, and infer presence of transgressive segregants and the number of genes controlling the referred traits indicated that:

a) Plant and ear heights in cross T20 x N_G8, and the number of kernels per ear in cross T20 x C58 exhibited normal and continuous frequency distributions, which confirmed observation of the influence of polygenes for these traits and transgressive segregants were observed for the respective N conditions.
b) However, the number of leaves above the ear in the cross N_G2 x C3 exhibited discrete frequency distributions under both N conditions, suggesting that a few genes were involved in governing the trait. Furthermore, the fixable genetic effects (additive and additive x additive) that predominated for this trait under both N regimes may imply that a few genes with possibly some modifiers governed the trait in an additive manner.

7.2.4 Adaptation and yield heterosis of hybrids under low and high nitrogen

In most cases, heterosis is expressed when inbred parents of different genetic backgrounds are crossed though background heterosis may not be ruled out. Standard heterosis is of practical relevancy to the breeder, as it measures the relative importance of experimental hybrids against the most popular commercial hybrid check. Nevertheless, the relative superiority of hybrids for characters required by farmers in elite maize cultivars has scarcely been reported under low N conditions, whereby tropical maize is produced in SSA. Standard heterosis from within and between crosses from elite inbred lines from Tanzania (local), West Africa and southern Africa were therefore conducted for grain yield, and components of yield and earliness under high and low N conditions. Results were:

a) Maize farmers lose about 20% to 30% of yield by applying less N. Generally, hybrids involving one or both local inbred parents exhibited higher tolerance to low N than hybrids involving exotic inbred parents only.
b) Hybrids with different adaptations emerged as follows: Four hybrids exhibited high performance across both N conditions, four were high yielding under low N only and two were outstanding under high-N conditions only. This may suggest that high-yielding hybrids that are adapted to low N conditions may be designed if local inbreds are used in hybrid combinations with exotic inbred lines. At the same time, hybrids that are adapted to both conditions can also be developed.
c) Local inbreds expressed the potential of adaptation to local environments, whereas the desired new genes — for instance, for early maturity — can be obtained by crossing local x West Africa inbreds. It is suggested that at least one inbred parent from West Africa should be crossed with an inbred with high grain yield potential if early maturity and high grain yield is a breeding objective.
d) On the average, the effects of application of low N were discernible on final yield and characters related to earliness, except for final physiological maturity and kernel moisture content at harvest. In the case of traits related to earliness, the effects of low N were evident on flowering days, mean-LCC, KDD index, and EFPD. For characters related to calendar dates, low values implied that low N extended the duration of calendar days for such traits, for example of flowering dates and physiological maturity. However, the positive loss of EFPDs demonstrated that low N reduced the grain-filling duration compared with high N. Low loss of kernel MC implied that hybrids under LN retained more moisture in their kernels, thus extending final physiological maturity. Low loss on the KDD index suggested that low N had a higher KDD index, which in other words implied that hybrids under low N held more water in their kernels and thus had low DM, which may explain why low N experiments had lower yield, compared with high N. Low-percent loss of mean-LCC indicated that low

N did not compromise mean-LCC and, in this case, low SPAD values were desired. The overall suggestion was that low values of relative loss, due to an application of low N for respective traits, were desired. Clearly, the secondary traits related to earliness suggested a logical link with kernel moisture-DM dynamics under low N, thus demonstrating that earlier-maturing maize cultivars with high yields under low N can be bred.

e) The general trend was that the hybrid sets displayed higher standard heterosis under LN, compared with HN conditions, implying that the productivity of maize can be enhanced by promoting the use of hybrids in low-N input environments.

f) These results disprove the time notion that open pollinated varieties (OPVs) are more suited to sub-optimal production conditions such as low N than hybrids. The results further disagree with farmers that landraces are better performing under similar conditions. The between-set heterosis (trial mean, best, local and mean of checks) that improved more under low N than under high N proved that heterosis could be maximised in crosses from different genetic backgrounds. However, the reasons why standard heterosis was expressed higher under low N than under high N could not be clearly established.

7.2.5 Relationships among adaptive traits and grain yield under low and high nitrogen

Due both to many environmental stresses and farmers' needs when it comes to elite maize varieties in SSA, it is suggested that targeting one particular stress or farmer's need in a breeding programme may not be feasible. A study that linked multiple trait linear regression and phenotypic correlation coefficients with cause-and-effect relation analyses was conducted under high and low N conditions to identify key traits to improve yield either under both or specific N regimes.

The results were as follows:

a) The number of ears per plant (EPP) and plant stand at harvest had higher positive regression coefficients (β) across N sites and seasons, indicating that yield could be enhanced by targeting these traits under either high and/or low N conditions. The EPP was the highest and had positive β values across N regimes but they were consistently higher in LN than in HN. The EPP is an important yield component under stress conditions and it complied with farmers' requirements in SSA, who produce maize under low N, as well as supported results from the social survey of the present study.

b) Plant and ear heights, plant stand at harvest, and the number of kernels per ear exhibited consistent significant positive correlation with yield across N regimes and seasons. The EFPD and mean-LCC were strongly positively correlated but, in most cases, they correlated negatively with other traits. Plant height, KPE, and plant stand at harvest correlated positively with yield, implying that yield could be enhanced through selection for these traits across N regimes. Mean-LCC was correlated negatively with plant height across N regimes and with 50% AD under HN and positively with EFPD under LN. This suggests that high DMA into earlier maturing cultivars across N regimes was possible. Cultivars that flower earlier and have high EPFD would be the most desired ideotypes (i.e. a cultivar with a defined and repeatable architecture and dimensions of the end-breeding trait or goal) to improve grain yield under low-N conditions. High KPE, tall cultivars, optimum plant stand at harvest and high EPPD would therefore enhance yield across N conditions.

c) Higher direct effects to grain yield via KPE and plant height under low N compared with high N, and the converse trend of plant stand at harvest may suggest the suitability of direct selection to improve yield via these traits under the respective N regimes. Indirectly, yield was significantly reduced by plant stand at harvest via EPP (-0.418), followed by mean-LCC through 50% AD (-0.376), whereas yield could be promoted by plant height via 50% AD (0.294), then by KPE via plant stand at harvest (0.229) under high N. Under low N, the indirect effects to yield were negative and involved mean-LCC via EFPD, although these effects were not significantly different under low- and high-N conditions. Generally, extension of leaf chlorophyll concentration and EFPD would lead to maize cultivars that are earlier and high-yielding under low N conditions. Occurrence of EFPD only under low N, as opposed to high N in path coefficient analysis, would suggest that this trait may be crucial in improving maize cultivars under low-N conditions. Furthermore, it would be easy to raise DMA, as the vegetative phase may be reduced and grain-filling duration increased, since the mean-LCC and EFPD were correlated negatively with flowering dates (i.e. 50% AD).

d) The number of ears per plant was reduced under low N, while the number of kernels per ear decreased under high N. These findings suggested that under low N, yield could be improved by breeding for prolificacy of ears, whereas yields could be increased under high N by raising the number of kernels per ear.

7.3 Challenges in breeding maize for low N conditions and directions for the future

a) Breeding earlier-maturing and high-yielding maize cultivars under low-N conditions is not an easy goal. An ideotype with such dimensions has never been attempted and could prove a time challenge with tropical maize production. Participatory maize breeding may at least help empirical breeders to design an ideotype of maize varieties that will be utilised by farmers, since such ideotypes would be socially acceptable, economically viable, resource-use efficient, and environmentally friendly. Incorporating the stay green (SG) and KDD traits into high grain-yielding and earlier-maturing maize cultivars under low soil N, as established by the social survey of this study, was attempted. The goal should be to increase DMA into an earlier-maturing cultivar by reducing the vegetative phase and extending the grain-filling duration via the SG and EFPD characters. However, the relevance of the SG character may decrease as the maturity of the cultivar increases. Low N at least extended calendar physiological maturity, retained more moisture in the kernels, reduced EFPD, had a higher KDD index, and a decreased mean-LCC which all may penalise yield potential. Thus, any breeding effort to improve maize for early maturity and increase yield under low-N conditions in tropical maize may target an ideotype that incorporates these traits. However, except for EFPD, the mean-LCC had compensatory effects with most of the other secondary traits, which may limit maize improvement for grain yield.

b) Estimates of genetic parameters such as heritability, number of genes controlling secondary traits and LCC under low N may not be reliable, since they did not comply with all the assumptions of GMA. Low heritability estimates under low N may limit the repeatability of experiments, consequently compromising the breeding progress of the traits. Many of the assumptions of the GMA were falsified and thus hindered the computations of such genetic parameters. Low N may have created high variation in hybrids, as was the case where all genetic effects were not significantly different based on the LSD at 50% level of probability. Biometrical tools such as frequency distributions of parents, versus their segregating generations (F_2, BCP_1 and BCP_2) may help to infer parameters such as number of genes governing a particular trait and thus their heritability and existence of transgressive segregants. Breeding approach and strategies may also be devised from such frequency distributions. Transgressive segregants that were observed under both N conditions and on both tails of the frequency distribution curves may provide the breeder with the choice to select and improve upon the respective traits. Diallel or biparental mating designs may be used directly, or selfing may first be done on the segregants then selection deferred to a few later cycles to improve the quantitatively inherited traits. Grain yield loss due to the application of low rates of N fertiliser in maize was real. However, it should be made clear that farmers produce maize under low N, because they have limited access to fertiliser due to high costs, or the fertilisers are not available in their locality; that there is inherent poor soil fertility or N volatility; drought compromises the benefits of applying N; there are alternative market benefits to using N fertilisers in maize versus other crops, which may force farmers to opt using fertiliser on other crops and so give little weight to maize; and that there is a lack of elite varieties with high NUE, such that farmers cannot differentiate the response of N to landraces or improved varieties. For example, in the Southern Highlands of Tanzania, there are maize subsidies for fertilisers and improved seed, although about 90% of farmers in the social survey of the present study reported no need for fertilisers when it came to growing maize. Yet maize grain yields are low and erratic, stagnating at 2 ton ha^{-1} and farmers request early cultivars with space and time constraints. Overwhelmingly, this may be compounded by a lack of NUE-improved maize cultivars for farmers to be able to discern differences between use and non-use of fertilisers in maize. Furthermore, inadequate access to credit and extension services of 90% and about 70%, respectively, identified in the social survey of this study, will perpetuate low maize productivity. Often, varieties designed for high fertiliser input may not yield well under low-fertiliser input conditions. This applies to tropical maize, which is produced under low and variable levels of soil N, either due to inherently low soil N status and/or deliberate application of low N by farmers. About two-thirds of world crop advancement has been attributed to genetic improvement. Thus, improved maize cultivars for NUE and making them available to farmers might be the most affordable and sustainable way of boosting maize productivity amidst pervasive stresses such as low N.

c) The prevalence of a plethora of stresses in tropical maize may suggest the identification and use of adaptive traits in improving maize productivity because targeting only a single trait may not be feasible in such areas. Trait relationships using multiple-trait linear regression, Pearson's correlation coefficients, path coefficient analysis, and simulations in combination with participatory, conventional and/or molecular breeding may help to focus maize breeding programmes. Investments in studying relationships among traits should be given attention as they may determine when, where and why to use direct or indirect selection procedures, thus pointing to efficiency of breeding programmes.