Genetic and Crop-Physiological Basis of Nitrogen Efficiency in Tropical Maize: Field Studies

von der Naturwissenschaftlichen Fakultät
der Universität Hannover
zur Erlangung des akademischen Grades eines

Doktors der Gartenbauwissenschaften
- Dr. rer. hort. -

genehmigte
Dissertation

von

Mosisa Worku (MSc)
geboren am 28.06.1962 in West Shoa, Oromia, Äthiopien

2005

Referent: Prof. Dr. Walter J. Horst, Uni. Hannover

Korreferent: Prof. Dr. Heiko C. Becker, Uni. Göttingen

Tag der Promotion: 11. May 2005

Dedicated to

the Children of the Horn of Africa who vanished because of famine

GENERAL ABSTRACT

Low nitrogen (N) stress is among the major abiotic stresses causing yield reductions in maize grown in the tropics. To alleviate the problem CIMMYT has identified maize cultivars with improved performance under low-N conditions. However, no information is available on the underlying mechanisms. The objectives of the present field studies were: (1) to estimate the magnitude of gene effects and combining ability under contrasting N environments, (2) to study the crop-physiological basis of N efficiency under field conditions, and (3) to study protein quality and quantity under a range of N levels. For genetic studies six hundred and thirty five experimental inbred lines were crossed in different crossing designs and evaluated under high-N and low-N conditions at CIMMYT-Zimbabwe while for physiological, and protein quantity and quality studies, sixteen maize cultivars (quality protein maize, QPM and non-QPM) differing in N efficiency were evaluated under three N levels each at Kiboko, Kenya in 2003 and Harare, Zimbabwe in 2003 and 2004. The relative contribution of non-additive gene effects for grain yield increased under low-N conditions as compared to high-N conditions. Better N-uptake and N-utilization efficiencies, greater leaf longevity, higher leaf chlorophyll concentration, higher root-length density in the subsoil (as measured for two contrasting N-efficient cultivars), and more dry matter production together with higher partitioning to the grains during and after flowering in the N-efficient cultivars contributed to improved performance under low-N conditions. However, total root-system size (as measured by root capacitance) was not positively related to N efficiency. QPM cultivars maintained their superiority over non-QPM cultivars in lysine and tryptophan contents in all environments reflecting the stable effect of the opaque-2 gene for protein quality across N supply levels and sites. In general, the results of these field studies indicated that different interrelated mechanisms contributed to improved performance under low-N conditions in CIMMYT tropical maize cultivars and there may be the possibility of developing N-efficient QPM cultivars that combine high yield potential and good protein quality under low-N conditions.

Key words: Maize cultivars, Gene effects, N efficiency

KURZFASSUNG

Stickstoff(N)mangel ist einer der Hauptursachen abiotischen Stresses, der zu Ertragsreduktionen im tropischen Maisanbau führt. Um dem Problem zu begegnen, hat CIMMYT Maissorten mit einer verbesserten Leistung unter N-Mangelbedingungen identifiziert. Es sind jedoch keine Informationen über die zugrundeliegenden Mechanismen verfügbar. Die Zielsetzungen der vorliegenden Feldstudien waren: (1) das Ausmaß der Geneffekte und der Kombinationseignung unter gegensätzlichen N-Standortbedingungen abzuschätzen, (2) die ertragsphysiologische Basis der N-Effizienz unter Feldbedingungen zu untersuchen und (3) die Proteinqualität und -quantität unter einer Reihe von N-Stufen zu untersuchen. Für die genetischen Studien wurden 635 Experimental-Inzuchtlinien in verschiedenen Kreuzungsmustern gekreuzt und unter hoher und niedriger N-Versorgung bei CIMMYT-Zimbabwe beurteilt, während für die physiologischen sowie für die Proteinquantitäts- und -qualitätsstudien 16 Maissorten (Qualitätsproteinmais, QPM und nicht-QPM) mit unterschiedlicher N-Effizienz unter einer Reihe von N-Stufen 2003 in Kiboko, Kenia und 2003 und 2004 in Harare, Zimbabwe, untersucht wurden. Der Anteil nicht-additiver Geneffekte am Kornertrag stieg unter N-Mangel im Vergleich zur hohen N-Versorgung an. Eine bessere N-Aufnahme- und Verwertungseffizienz, eine längere Blattlebensdauer, höhere Chlorophyllkonzentrationen in den Blättern, höhere Wurzellängendichten im Unterboden (gemessen an zwei unterschiedlich N-effizienten Sorten) und eine höhere Trockenmasseproduktion verbunden mit einer stärkeren Verteilung in die Körner während und nach der Blüte N-effizienter Sorten trugen zur verbesserten Leistungsfähigkeit unter N-Mangel bei. Die Gesamtgröße des Wurzelsystems (gemessen mittels der Wurzelkapazität) war dagegen nicht positiv mit der N-Effizienz korreliert. Die QPM-Sorten behielten ihre Überlegenheit in den Lysin- und Tryptophangehalten gegenüber den nicht-QPM Sorten auch unter N-Mangel bei, was den stabilen Effekt des Opaque-2 Gens auf die Proteinqualität über eine Reihe von Bodenfruchtbarkeitsstufen und Standorten widerspiegelt. Allgemein zeigen die Ergebnisse dieser Feldstudien, dass verschiedene miteinander verbundene Mechanismen zur verbesserten Leistung der tropischen CIMMYT-Maissorten unter N-Mangel beitrugen und dass vermutlich die Möglichkeit besteht, N-effiziente QPM-Sorten zu entwickeln, die unter N-Mangel ein hohes Ertragspotential mit einer guten Proteinqualität verbinden.

Schlagworte: Maissorten, Geneffekte, N-Effizienz

TABLE OF CONTENTS

ABBREVIATIONS

AD	anthesis date
AMMI	Additive Main effect and Multiplicative Interaction
ASI	anthesis-silking interval
b	regression coefficient
BIOF	aboveground biomass at anthesis
BIOFH	dry matter accumulation after anthesis
BIOH	aboveground biomass at physiological maturity
BIONF	aboveground biomass nitrogen at anthesis
BIONH-F	nitrogen accumulation in the biomass after anthesis
C	cultivar/genotype
°C	degree Celsius
C/N	carbon/nitrogen ratio
$CaCl_2$	calcium chloride
CHL	leaf chlorophyll
CHL1	leaf chlorophyll content at anthesis
CHL2	leaf chlorophyll content 14 days after anthesis
CHL3	leaf chlorophyll content 28 days after anthesis
CIMMYT	International Maize and Wheat Improvement Centre
CIRC	stem circumference
cm	centimeter
CML	CIMMYT line
CNS	carbon, nitrogen, sulfur
CV%	coefficient of variation
E	environment
EH	ear height
EI	environmental index
EPP	ears per plant
ER	ear rot
G	genotype/cultivar
g	gram
GCA	general combining ability
GL	green leaves
GL1	green leaf number at anthesis
GL2	green leaf number 14 days after anthesis
GL3	green leaf number 28 days after anthesis
GLS	gray leaf spot
GP	grain protein
Grain rot%	grain rot percent
GY	grain yield
ha	hectare
HHN	Harare high-N
HI	harvest index
HLN	Harare low-N
HN	high-N
ICRAF	International Crop Research and Agro-Forestry
IPCA1	Interaction Principal Component Analysis axis 1
IPCA2	Interaction Principal Component Analysis axis 2
K	potassium

K03N1	Kiboko 2003 low-N
K03N2	Kiboko 2003 medium-N
K03N3	Kiboko 2003 high-N
KCl	potassium chloride
kg	kilogram
KN	kernel number per ear
KPR	kernels per row
KRN	kernel row number
KW	kernel weight
L	line
l	liter
LAI	leaf area index
LN	total leaf number
LS	leaf senescence
LSD	least significant difference
M	molarity
m	meter
ml	milliliter
mm	millimeter
µm	micrometer
MOI%	grain moisture percent
N	nitrogen
nF	nano farad
$NH4^+$	ammonium
NHI	nitrogen harvest index
NO_3^-	nitrate
ns	not significant
NUP	nitrogen uptake in the aboveground biomass
NUT	nitrogen utilization
P_2O_5	phosphorus pentoxide
PH	plant height
pH	negative logarithm of proton concentration
QI	quality index
QPM	quality protein maize
r	linear correlation
RAHN	Rattray Arnold high-N
RALN	Rattray Arnold low-N
RC1	root capacitance reading at flowering
RC2	root capacitance reading 14 days after flowering
SCA	specific combining ability
SD	silking date
SE	standard error
SEN1	leaf senescence score at anthesis
SEN2	leaf senescence score 14 days after anthesis
SEN3	leaf senescence score 28 days after anthesis
SEN4	leaf senescence score 42 days after anthesis
SPAD	Single Photon Avalande Diode
SS	sum of square
STONH	stover nitrogen at harvest
T	tester

t	ton
Z03N1	Harare (Zimbabwe) 2003 low-N
Z03N2	Harare (Zimbabwe) 2003 medium-N
Z03N3	Harare (Zimbabwe) 2003 high-N
Z04N1	Harare (Zimbabwe) 2004 low-N
Z04N2	Harare (Zimbabwe) 2004 medium-N
Z04N3	Harare (Zimbabwe) 2004 high-N

GENERAL INTRODUCTION

Food security is a great challenge in the twenty first century in the developing world, particularly in sub-Saharan Africa (Cakmak, 2001; Mugo and Hoisington, 2002). Maize is one of the most important food crops in that region and is the staple food in east and southern Africa (Byerlee and Heisey, 1996; Diallo et al., 2003). It is estimated that maize demand in sub-Saharan Africa is expected to increase from 27 million tons in 1995 to 52 million tons in 2020 (Pingali and Pandey, 2001). To fulfill this projected demand maize production in the future has to be realized predominantly on the existing cultivated land, since an expansion of cultivated land is severely limited because of population increase, environmental concerns, urbanization and diminishing water resources (Cakmak, 2001).

The maize area in eastern and southern Africa averaged, from 1997 – 1999, 15.4 million hectares while the mean yield obtained on farmers field is very low, 1.5 t ha^{-1} (Aquino et al., 2001). Abiotic stresses are as important as biotic stresses in limiting maize production in the region. Moisture stress (i.e., drought) and low soil fertility are the most important abiotic stresses (Friesen et al., 2002). Nutrient deficiency, N in particular, is a wide-spread problem in the region due to the low use of purchased inputs and the lack of soil fertility enriching rotations or fallows in maize-producing areas (Ransom et al., 1993; Asfaw et al., 1997; Edmeades et al., 2003). Nitrogen is the most limiting nutrient as it is the most mobile in the soil and the nutrient needed in the largest quantities by the crop (Ransom et al., 1993; Laegreid et al., 1999). It affects photosynthetic rate, leaf area, size of the sink and thus yield (Dass et al., 1997).

Fertilizer use in sub-Saharan Africa averages 10 kg nutrient (NPK) per hectare while it is 83 kg ha^{-1} and 120 kg ha^{-1} in the developing world as a whole and in Europe, respectively (Heisey and Mwangi, 1997; Cakmak, 2001). As a result maize fields of small holder farmers are poor in soil fertility as compared to well fertilized research-station soils (Zambezi and Mwambula, 1997). This is reflected in the wide gap between research-station yields (8 – 10 t ha^{-1}) and maize yields of small holder farmers (1.5 t ha^{-1}) in eastern and southern Africa and in the wide gap between average maize yield in industrialized countries (8 t ha^{-1}) and sub-Saharan Africa (2.5 t ha^{-1}) (Aquino et al., 2001; Pingali and Pandey, 2001).

To date, two major problems exist with mineral-N: (1) low-N stress to maize plants due to unavailability of mineral fertilizers, particularly in sub-Saharan Africa and (2) environmental

pollution of both air and water where high N fertilizer doses are applied to achieve maximum yields (Lynch, 1998; Horst et al., 2003). In low-input agriculture N-efficient cultivars, cultivars with better grain yield under low-N conditions (Graham, 1984; Sattelmacher et al., 1994), are recommended as one of the key elements for sustainable agriculture (Horst et al., 2003). Zaidi et al. (2003) also suggested that utilization of drought-tolerant and N-efficient cultivars in maize production of the tropics could lead to better stability of grain yield across the environments.

Traditional maize breeding programs have generally focused on increasing grain yield under conditions where N supply is large and does not significantly constrain grain yield (Lafitte and Edmeades, 1994c; Bänziger et al., 2000). However, under poor soil-fertility conditions of the fields of small holder farmers the result may be different, i.e., the highest yielding genotype under well-fertilized condition may not be the highest yielding under low-N conditions (Ceccarelli, 1989; Bänziger et al., 1997; Bänziger et al., 2000).

Lynch (1998) presented three approaches of germplasm improvement for grain yield: (1) improving yield response to high nutrient fertilization, (2) improving yield response to low nutrient availability (efficiency) and (3) improving yield response to both low and high nutrient inputs (efficiency and response). The authour also stated that in the first case the varieties may be less efficient at low level of nutrient input while in the second case the varieties may be less responsive under high input level. Thus, for maximum gains under both low input and high input agriculture, in addition to a high-N environment, inclusion of a low-N environment in the breeding program has been recommended (Bänziger et al., 1997). Evaluation under both conditions gives an opportunity to select genotypes which adapt to both conditions (Dass et al., 1997; Sallah et al., 1997; Santos et al., 1997).

In recent years, CIMMYT (International Maize and Wheat Improvement Center) maize breeders and physiologists have attempted to increase grain yield under low-N by exploiting the genetic variability in adaptation to low-N stress in maize germplasm (Bänziger et al., 1997; Friesen et al., 2002). Progress has been made in developing efficient maize cultivars which perform better than the checks under all fertility conditions in east and southern Africa (Friesen et al., 2002; Bänziger et al., 2005). However, detailed information on physiological mechanisms and the gene effects underlying the N efficiency is largely missing.

Limited information is available from the studies conducted by the physiology program at CIMMYT-Mexico about N efficiency mechanisms that originate from selecting maize genotypes under low-N conditions. One open pollinated variety (OPV) selected for N efficiency was assessed (Lafitte and Edmeades, 1994a, b, c). Other OPVs assessed under low-N conditions included populations selected for drought tolerance (Bänziger et al., 1999, 2002). No detailed studies were conducted so far to assess the morpho-physiological changes associated with improved performance under low-N stress of maize germplasm selected within the CIMMYT N efficiency breeding program. No study has been also conducted to assess protein quality and quantity under low-N condition. Furthermore, the information on the gene effects under contrasting N environments is limited. In the present study five areas were assessed:

(i) Gene effects under contrasting N fertility environments (Chapter 1)

(ii) Nitrogen uptake and utilization efficiencies (Chapter 2)

(iii) Relationship between N efficiency and root-system size, N depletion in the soil and leaf traits (Chapter 3)

(iv) Dry matter partitioning and N efficiency (Chapter 4)

(v) N efficiency and protein quality (Chapter 5)

Thus, this project was initiated with the following main objectives: (1) to estimate the magnitude of gene effects and combining ability in CIMMYT tropical mid-altitude inbred lines under contrasting N environments, (2) to study the crop-physiological basis of N efficiency under field conditions, and (3) to study protein quality and quantity under a range of N levels.

CHAPTER 1

MODES OF GENE ACTION AND COMBINING ABILITY AMONG TROPICAL MAIZE (*ZEA MAYS* L.) INBRED LINES UNDER CONTRASTING NITROGEN ENVIRONMENTS

ABSTRACT

Low-N stress is among the major abiotic stresses causing yield reductions in maize grown in the mid-altitude tropical environments of Africa. This study estimates the magnitude of gene effects and combining ability in CIMMYT's tropical mid-altitude inbred lines under contrasting N environments. Six hundred and thirty five inbred lines ($S_2 - S_7$) were evaluated in different crossing designs (Diallels, North Carolina Design II and Line x Tester cross). Results of experiments conducted under low-N and high-N at the same site within the same year and season from 1999 – 2003 were compared. The contribution of general combining ability (GCA), indicative of additive gene effects, was higher than specific combining ability (SCA), non-additive gene effects, for most of the secondary traits under both high-N and low-N levels. However, significant crossover interactions were observed for GCA effects of the inbred lines for grain yield. Pair-wise t-test for diallels and Design IIs showed significant difference ($P<0.05$) between the proportion of specific combining ability (SCA) sum of squares for grain yield under high-N and low-N conditions. The average relative contribution of SCA, indicative of non-additive gene effects, on progeny performance to genetic components, for grain yield under low-N accounted for 51% (average across all trials) but only for 36% under high-N. Average narrow sense heritability for grain yield across the experiments was reduced from 0.48 under high-N to 0.32 under low-N conditions. This suggests the need to use different breeding strategies to increase grain yield on the fields of resource poor farmers who mainly produce maize under low-N conditions, particularly in sub-Saharan Africa.

Key words: Gene effects, Inbred lines, Nitrogen environments, *Zea mays* L.

INTRODUCTION

Crop performance is a function of the genotype and the nature of the production environment (Cooper and Byth, 1996). Genotypic differences for grain yield observed in the absence of stress are largely unrelated to differences observed in the presence of severe stress (Ceccarelli, 1989; Ceccarelli and Grando, 1991; Ceccarelli et al., 1992; Bänziger et al., 1997). This may indicate that different physiological mechanisms are associated with high yield in favourable conditions and high yield in unfavourable conditions (Ceccarelli, 1996; Blum, 1997). Variation for quantitative characters is under the control of many genes and the contribution of the genes can differ among environments (Delacy et al., 1996; Basford and Cooper, 1998). This conditional contribution of genes is the basis of genotype-by-environment (G x E) interactions.

Low-N stress is among the major abiotic stresses causing yield reductions in maize grown in the tropics (Lafitte and Edmeades, 1994c; Beck et al., 1996; Bänziger et al., 2000; Bänziger and Meyer, 2002). Understanding the genetic basis of hybrid performance under this stress is crucial to the design of appropriate breeding strategies (Hallauer and Miranda, 1988; Betran et al., 2003 a, b). Although improved N efficiency has been a desirable goal of maize breeders, the information available regarding gene action and combining ability for different traits related to N efficiency is limited (Below et al., 1997; Dass et al., 1997).

Below et al. (1997) evaluated single cross hybrids forming a diallel mating design under high-N and low-N availability (where inadequate N results in approximately 35% yield reduction) in a temperate environment and reported that the mean squares for general combining ability (GCA) and specific combining ability (SCA) were significant for all traits measured at both levels of N. They concluded that, based on the magnitude of the difference between GCA and SCA mean squares, the majority of the genetic effects were associated with GCA, indicative of additive gene effects. Kling et al. (1997) conducted a diallel experiment in the tropical lowlands of West Africa for one season under high-N and low-N conditions and reported that GCA for grain yield was significant under both N treatments while SCA was only significant under high-N. For ears per plant, GCA was significant only under low-N while SCA was significant under high-N.

Non-additive gene effects under low-N were common in other studies. Betran et al. (2003a) evaluated diallel crosses under high-N and low-N for one season and reported that under low-

N non-additive gene effects were more important for grain yield than the additive gene effects. A significant crossover interaction was observed between the GCA of lines under low-N and high-N conditions. Similar results were reported by Lafitte and Edmeades (1995). Bänziger et al. (1997) found that N stress severity influenced genotype-by-N stress interactions. The contradictory results of different researchers may, therefore, be due to differences in the N stress level (testing environment) under which the genotypes were evaluated and/or genotypic difference among sets of genotypes included in the studies.

A detailed study of combining ability and modes of gene action under contrasting N environments is crucial to generate precise information and design breeding strategies that serve the interests of resource-poor farmers in the tropics, particularly in sub-Saharan Africa (Bänziger et al., 2000). This study estimates the magnitude of gene effects and combining ability in several sets of CIMMYT tropical mid-altitude inbred lines under contrasting N environments.

MATERIALS AND METHODS

Germplasm, experimental site and trial management

Four different diallel cross (Griffing Method IV) trials (Griffing, 1956), two different North Carolina Design II cross (L x T) trials and eight Line x Tester cross (L x T) trials (Dhillon and Pollmer, 1978; Singh and Chaudhary, 1985; Hallauer and Miranda, 1988) were conducted at CIMMYT-Zimbabwe between 1999 and 2003 and used for this study. Experiments were conducted in Harare, Zimbabwe (17°49'S, 31°1'E and 1478 m above sea level) at high-N (HHN) and low-N (HLN), and in Rattray Arnold, Zimbabwe (17°40'S, 31°1'E and 1308 m above sea level) at high-N (RAHN) and low-N (RALN) (Table 1). Alpha (0,1) lattice experimental designs with two replications (Patterson and Williams, 1976) were used for most of the trials while two trials in 2001 were evaluated using augumented designs (Federer, 1977).

All parental lines were CIMMYT experimental inbred lines of tropical mid-altitude adaptation which had been selected for agronomic performance including resistance to streak virus, turcicum leaf blight (*Exserohilum turcicum*), common rust (*Puccinia sorghi*) and gray leaf spot (*Cercospora zeae-maydis*) and other agronomic traits at CIMMYT-Zimbabwe. In total, 1707 crosses of 635 different inbred lines were used for the study (Table 1).

Experiments under low-N conditions were planted in fields that had been depleted of N by growing unfertilized, non-leguminous crops for several seasons and removing the crop biomass after each season. No additional N fertilizer was applied for low-N experiments. Under high-N conditions, 168 kg N ha^{-1} was applied in splits at planting (28 kg N ha^{-1}), 30 and 50 days after planting (70 kg N ha^{-1} each).

Plot size was 4 x 0.75 m with one row for each entry. Plant spacings were 0.75 m and 0.25 m between rows and plants, respectively, corresponding to 53,333 plants per hectare. During harvesting one border plant on either side of the row was excluded to avoid border effects. Phosphorus (142 kg P_2O_5 ha^{-1}) and potassium (32 kg K ha^{-1}) fertilizers were applied uniformly prior to planting. The experiments were conducted under rainfed conditions and were kept clean of weeds using herbicides (Atrazine, 4.5 l ha^{-1}, and Dual, 1.8 l ha^{-1}, 96% Metalchlor) and hand weeding.

Table 1. Trials, mating designs and testing sites of the crosses used for the study (1999 – 2003), CIMMYT-Zimbabwe. Harare high-N (HHN), Harare low-N (HLN), Rattray Arnold high-N (RAHN) and Rattray Arnold low-N (RALN).

Mating	No. of	Trial	Trial	No. of	No.of	Field		Testing site			
design	parents	No.	code	entries	reps	design	Year	HHN	HLN	RAHN	RALN
Diallel	7	1	99ELITESC	144	2	Alpha lattice	1999	x	x		
	9	2	01ELISC	84	2	Alpha lattice	2001	x	x		
	8	3	VESB01	60	2	Alpha lattice	2001	x	x		
	11	4	VESA03	196	2	Alpha lattice	2003	x	x		
Design II	7 x 7	5	99ELITESC	144	2	Alpha lattice	1999	x	x		
	15 x11	6	00ELISC	192	2	Alpha lattice	2000	x	x		
	15 x11	7	00ELISC	192	2	Alpha lattice	2000			x	x
L x T	103x3	8	VETA01	316	1	Augumented	2001	x	x		
	96x3	9	VETB01	292	1	Augumented	2001	x	x		
	26x3	10	VETB02	180	2	Alpha lattice	2002	x	x		
	83x2	11	VETC02	170	2	Alpha lattice	2002	x	x		
	69x2	12	VETD02	165	2	Alpha lattice	2002	x	x		
	68x2	13	VETE02	156	2	Alpha lattice	2002	x	x		
	52x2	14	VETD03	108	2	Alpha lattice	2003	x	x		
	67x2	15	VETH03	140	2	Alpha lattice	2003	x	x		

Measurements

Anthesis (AD) and silking (SD) dates were measured as number of days after planting when 50% of the plants in the plot shed pollen and extruded silks, respectively. Anthesis-silking interval (ASI) was calculated as: ASI = SD – AD. Plant height (PH) was recorded from ground level to the insertion of the first tassel branch of the same plant. Ear height was measured from ground level to the insertion of the top ear of the same plant. The number of ears per plant (EPP) was equal to the number of ears with at least one fully developed grain divided by the number of harvested plants. An EPP of below 1.0 indicates partial barrenness while an EPP of above 1.0 indicates partial prolificacy. Leaf senescence was rated on a scale of 1 (10% dead leaf area) to 10 (100% dead leaf area) during several stages of grain-filling. Gray leaf spot (*Cercospora zeae-maydis)* was assessed on a scale of 1 (clean, no infection) to 5 (severely diseased). The percentage of rotten ears was also recorded. All ears from the harvestable area were shelled, and grain weight and grain moisture percentage recorded. Grain yield was adjusted to 12.5% grain moisture.

Statistical analyses

Lattice-adjusted genotype means were calculated for each experiment using PROC MIXED procedures of SAS (SAS, 2001) with genotypes as fixed effects and replicate and incomplete blocks within replicates as random factors. Adjusted means were used to estimate general combining ability (GCA) and specific combining ability (SCA) effects. The checks were excluded for genetic analysis. Griffing method IV (Fixed Model) for diallel analysis was used to estimate GCA and SCA effects of the lines and crosses, respectively in each environment and across environments for each diallel (Griffing 1956; Baker, 1978). Analyses of GCA and SCA effects in each environment and across environments in factorial crosses (Design II and L x T) were conducted using the procedures described in Dhillon and Pollmer (1978) and Singh and Chaudhary (1985). Genetic analyses were computed using the SAS program (SAS, 2001).

For the diallels, the significance of GCA (additive) and SCA (non-additive) sources of variation in each N environment was determined using the error mean square while in the across environment analysis, they were tested against their interaction with the environment. Interactions of GCA and SCA with the environment were tested in the across environment analysis using the pooled error to test their significance (Betran et al., 2003b). In the factorial

crosses, the significance of GCA of line (GCA (L)) and GCA of tester (GCA (T)) in each N environment was determined using the L x T interaction as error term. The significance of the L x T interaction (SCA) was determined using the error mean square. In the combined analysis across environments, the significance of GCA (L) and GCA (T) was determined using the L x T interaction as error term. The significance of the GCA (L) and GCA (T) interaction with the environment was determined using the SCA interaction with environment as error term. The significance of SCA (L x T interaction) and SCA x environment were determined using SCA x environment and the pooled error mean square, respectively, as error terms (Dhillon and Pollimer, 1978; Singh and Chaudhary, 1985). Combined analysis was carried out only for grain yield in the factorial crosses, and for grain yield and secondary traits in the first three diallel crosses.

The relative importance of GCA vs. SCA on progeny performance in the diallels was calculated as the ratio between sum of squares due to GCA or SCA and total genetic variance (GCA and SCA sum of squares) (Baker, 1978; Betran et al., 2003a). Similarly, proportional contribution of lines (GCA (L)), testers (GCA (T)) and their interactions (SCA (L x T)) to total genetic variance (total sum of squares among the crosses) in the factorial crosses (Design II and Line x Tester) were calculated as the ratio between sum of squares of each component and total sum of squares among the crosses (Singh and Chaudhary, 1985). Narrow sense heritability for grain yield (Hallauer and Miranda, 1988; Falconer, 1990) under contrasting N environments was calculated. Simple linear correlation coefficients were also calculated to determine relationships between traits.

RESULTS

Mean grain yield and low-N stress intensity

Mean trial yields under high-N (HN) varied from 5.40 t ha^{-1} (trial 10) to 8.91 t ha^{-1} (trial 4) at Harare in 2002 and 2003, respectively (Table 2). Under low-N, mean grain yields of the trials varied from 1.39 t ha^{-1} (trial 11) to 5.18 t ha^{-1} (trial 15), both at Harare (Table 2). Low-N stress reduced grain yield by 64% as compared to high-N conditions across all trials. The highest grain yield reduction was recorded in trial 11 in 2002 and the lowest reduction was recorded in trial 15 in 2003, both at Harare. The level of yield loss between low-N and high-N varied depending on the degree of N depletion in different fields. Simple linear correlation coefficients (r) between grain yields under high-N and low-N conditions at each site were not

significant in most of the trials (mean r = 0.17) (Table 2) indicating that ranking and/or response of the genotypes were different under low-N and high-N conditions. However, significant genotypic differences were observed under both N fertility environments at both sites in all trials included in this study (data not shown).

Table 2. Mean trial grain yields under high-N (HN) and low-N (LN), percentage of low-N yield (LNGY) as compared to high-N yield (HNGY) and simple correlations (r) between HN and LN grain yields.

Trial No.	Mean grain yield, t ha^{-1}		LNGY as % of HNGY	r
	HN	LN		
1	6.28	3.36	54	0.29*
2	7.59	2.20	29	0.12
3	6.64	2.06	31	0.45*
4	8.91	3.33	37	0.31*
5	6.28	3.36	54	0.29*
6	7.04	2.45	35	0.24
7	8.06	2.08	26	0.22
8	6.82	2.13	31	0.09
9	7.20	2.14	30	-0.01
10	5.40	1.68	31	0.04
11	8.08	1.39	17	-0.03
12	5.79	1.67	29	0.19
13	8.50	2.92	34	0.08
14	8.67	2.49	29	0.23
15	7.34	5.18	71	0.07
Mean	7.24	2.56	36	0.17

*significant at P<0.05.

Combining ability and GCA vs. SCA sum of squares for grain yield

In all diallels, the GCA effect was significant under both high-N and low-N conditions (Table 3). SCA was significant under high-N conditions in two out of four diallels. Estimates of GCA effects for males (testers, T) in the three Design II experiments also showed significant differences among the GCA of the males under both N environments. GCA effects for the females (lines, L) were significant under both N levels except for trial 6 in Harare at low-N in 2000 (Table 3). The mean square for L x T (SCA) was significant under high-N in all Design II experiments but it was significant under low-N only for trial 7 at Rattray Arnold in 2000.

Line x Tester (L x T) crosses differed significantly for GCA of lines for four experiments under high-N and five experiments under low-N conditions (Table 4). GCA effects for testers

were significant for six experiments under high-N and seven experiments under low-N. The L x T interaction (SCA) was significant for five experiments under low-N conditions and two experiments under high-N conditions.

Table 3. Mean square for general combining ability (GCA) and specific combining ability (SCA) and percent sum of squares of GCA and SCA under high-N (HN) and low-N (LN) for grain yields for different diallel (1 – 4) and Design II (5 – 7) trials.

Trial		Mean square		% Sum of squares	
No.	Gene effect	HN	LN	HN	LN
1	GCA	6.2**	0.9^{+}	79	35
	SCA	0.7*	0.7	21	65
2	GCA	12.4**	0.5*	80	33
	SCA	0.9	0.3*	20	67
3	GCA	7.2**	1.4**	58	76
	SCA	1.8**	0.2	42	24
4	GCA	3.3*	0.6^{+}	42	27
	SCA	1.0	0.4	58	73
Mean	GCA			65	43
	SCA			35	57
5	Line, L (GCA)	5.8**	2.3**	39	30
	Tester, T (GCA)	3.9**	2.0**	26	27
	L x T (SCA)	0.9**	0.5	35	43
6	Line, L (GCA)	8.3**	0.7	35	8
	Tester, T (GCA)	11.2**	3.4**	34	31
	L x T (SCA)	0.7*	0.5	31	61
7	Line, L (GCA)	14.7**	1.1**	30	24
	Tester, T (GCA)	30.1**	1.2**	44	18
	L x T (SCA)	1.3**	0.3**	26	58
Mean	Line, L (GCA)			35	21
	Tester, T (GCA)			34	25
	L x T (SCA)			31	54

$^{+}$, *, ** Significant at P<0.1, 0.05 and 0.01, respectively.

Combined analysis across N levels for the first three diallels showed significant GCA x environment and SCA x environment interactions indicating the changes in the magnitude of GCA and SCA effects among the genotypes at different N levels (Table 5). Combined analysis across N levels for each group of factorial crosses (Design II and Line x Tester crosses) also showed significant differences in GCA (L) and GCA (T) effects and their interactions with the environment in most of the trials. SCA (L x T) effects and their interactions with environment were also significant in some of the trials (Table 6).

General combining ability for grain yield varied considerably among the inbred lines in all groups of crosses (Diallels, Design II's and Line x Tester crosses). Both significant positive and negative GCA effects were observed for some inbred lines in each experiment under both N environments (data not shown). Some inbred lines had consistent GCA effects for grain yield under both N levels while others had opposing GCA effects under low-N and high-N (Figure 1A). Similarly, some combinations had consistent SCA effects under both N levels while the SCA of others changed between low-N and high-N (Figure 1B). Simple linear correlation coefficients (r) indicated that there was a very low association (mean r = 0.27) between GCA of inbred lines under high-N and low-N conditions. The relationship between GCA of inbred lines under high or low N conditions of two different sites was more strongly associated than the GCAs of inbred lines under high-N and low-N conditions at the same site (Table 7).

Comparison of GCA sum of squares to SCA sum of squares under high-N and low-N conditions revealed that GCA was more important than SCA for grain yield under high-N conditions. However, the proportion of SCA sum of squares increased dramatically under low-N conditions in all diallels except in trial 3 at Harare 2001 (Table 3). On average, GCA sum of squares explained 65% of variation among crosses (GCA and SCA) under high-N conditions whereas it explained only 43% under low N conditions.

Table 4. Mean squares and percent of general combining ability (GCA) and specific combining ability (SCA) sum of squares for grain yields for different Line x Tester (L x T) trials.

Source	Trial																	
	8		9		10		11		12		13		14		15		Mean	
	HN	LN	HN	LN	HN	LN	HN	LN	HN	LN	HN	LN	HN	LN	HN	LN	HN	LN
Line, L (GCA)	0.7*	0.6**	0.6*	0.4	1.3	0.3*	-	0.3**	1.8	0.3**	1.7	0.6	2.1^{+}	0.2	2.3**	0.9^{+}	-	-
Tester, T (GCA)	33.4**	3.7**	28.3**	2.6**	3.3*	2.6**	-	1.1*	5.7*	0.6^{+}	0.3	0.9	16.8**	2.6**	4.6*	8.3**	-	-
L x T (SCA)	0.5	0.4*	0.4	0.4**	1.0**	0.2	-	0.2**	1.4*	0.2	1.5	0.5*	1.4	0.2^{+}	1.1	0.6	-	-
%GCA SS (L)	30	43	29	35	37	37	-	61	55	63	54	57	55	48	67	54	47	50
%GCA SS (T)	29	5	29	5	7	22	-	3	3	2	0	1	9	10	2	8	11	7
%SCA SS (L x T)	41	52	42	60	56	41	-	36	42	35	46	42	36	42	31	38	42	43

$^{+}$, *, ** Significant at P < 0.1, 0.05 and 0.01, respectively.

Table 5. Mean squares and percent of general combining ability (GCA) and specific combining ability (SCA) sum of squares for grain yields (GY), anthesis-silking interval (ASI), ear per plant (EPP), anthesis date (AD), plant height (PH) and ear rot (ER) for the first three diallel trials across N environments.

Source	Trial														
	1				2						3				
	GY	AD	PH	ER	GY	ASI	EPP	AD	PH	ER	GY	ASI	AD	PH	ER
GCA	4.7	79.7**	1058^{+}	355	7.3	9.0	0.02	84.5**	3181**	281	6.1	8.4^{+}	38.8**	1214*	479
SCA	0.6	2.9	197*	18	1.0	1.9	0.01	2.3*	99	57	1.1	2.1	4.8*	172^{+}	109
GCA x Env	2.4**	2.3^{+}	305**	188**	4.69**	5.2**	0.02**	3.4**	72	269**	2.4**	2.4*	2.6*	226**	695**
SCA x Env	0.8*	1.7	71	17	0.9**	2.8**	0.01*	1.1	61^{+}	76	0.8*	1.8^{+}	2.1*	84	101^{+}
%GCA SS	76	92	70	89	69	58	35	92	90	59	65	58	74	71	61
%SCA SS	24	8	30	11	31	42	65	8	10	41	35	42	26	29	39

$^{+}$, *, ** Significant at P < 0.1, 0.05 and 0.01, respectively.

Table 6. Mean squares and percent of general combining ability (GCA) and specific combining ability (SCA) sum of squares for grain yield (GY) for different factorial cross trials across environments (E). Trial 6 and 7 are same crosses tested under high-N and low-N conditions of Harare and Rattray Arnold (four environments).

Source	Trial								
	5	6 and 7	8	9	10	12	13	14	15
ENV. (E)	135.2**	1578.0**	3394.5**	3615.6**	603.5**	1184.4**	2077.6**	1985.3**	296.6**
Line, L (GCA)	3.2**	11.7**	0.8**	0.6^{+}	1.1*	1.3*	1.2	1.3^{+}	1.7**
Tester, T (GCA)	4.5**	28.4**	10.9**	11.0**	0.6	1.3	1.2	16.3**	12.6**
E x L (GCA)	4.9**	4.4**	0.5^{+}	0.5^{+}	0.6	0.8	1.1^{+}	1.1^{+}	1.5*
E x T (GCA)	1.3	5.8**	26.3**	19.8**	5.2**	5.0*	0.1	3.1*	0.3
L x T (SCA)	0.6	0.9*	0.5*	0.4	0.6	0.8	1.1	0.9	0.8
E x L x T (SCA)	0.8**	0.6**	0.4	0.4	0.6*	0.8*	0.8	0.7	1.0
%GCA SS (L)	29	29	41	34	46	62	53	52	65
%GCA SS (T)	40	50	11	14	2	1	1	13	7
%SCA SS (LxT)	31	21	48	52	52	37	47	35	28

$^{+}$, *, ** Significant at P < 0.1, 0.05 and 0.01, respectively.

Similarly, the proportion of GCA sum of squares of females (L) and males (T) for grain yield was higher under high-N conditions than under low-N conditions in all Design II crosses (Table 3). On average, SCA (L x T interaction) explained 54% of the total sum of squares among crosses under low-N conditions but only 31% under high-N conditions. Similar trends were observed in all Line x Tester (L x T) crosses except in all L x T crosses of 2002 (Table 4). A pair-wise t-test for diallels and design II's showed a significant difference ($P<0.05$) between the proportion of SCA sum of squares for grain yield under high-N and low-N conditions. Average narrow sense heritability for grain yield across the trials was significantly reduced from 0.48 under high-N to 0.32 under low-N conditions.

The combined analysis across N environments for the first three diallels revealed that the proportion of GCA sum of squares was higher than SCA sum of squares for grain yield (Table 5). Combined analysis for grain yield in the factorial crosses also showed that the proportion of GCA sum of squares was higher than SCA sum of squares except in trial 9 and trial 10 (Table 6).

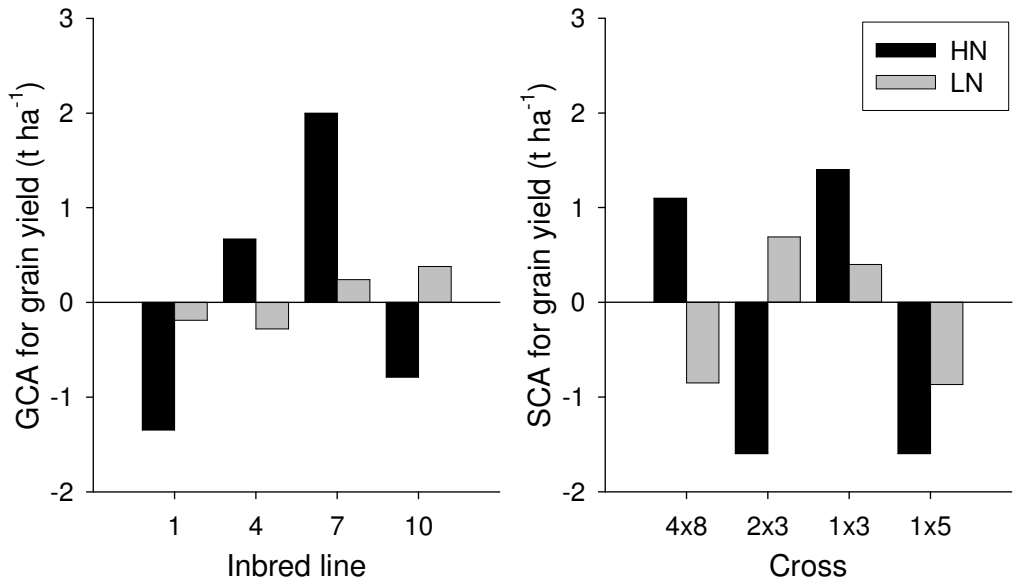

Figure 1. GCA for grain yield (t ha^{-1}) of selected inbred lines in trial 6 (A) and SCA for grain yield (t ha^{-1}) of selected crosses in trial 2 (B) under high-N and low-N.

Table 7. Simple correlation (r) between GCA of inbred lines under high-N (HN) vs. low-N (LN) at the same site (first three Diallels, 1, 2 and 3, and Design IIs, 5 and 6 at Harare; Design II, 7 at Rattray Arnold) and HN vs. HN and LN vs. LN at different sites (Harare vs. Rattray Arnold, 6 vs. 7).

Trial	GCA	r
1	HN vs. LN	0.48
2	HN vs. LN	-0.50
3	HN vs. LN	0.59
5	GCA (Line) HN vs. LN	-0.24
	GCA (Tester) HN vs. LN	0.58
6	GCA (Line) HHN vs. HLN	0.26
	GCA (Tester) HHN vs. HLN	0.43
7	GCA (Line) RHN vs. RLN	0.00
	GCA (Tester) RHN vs. RLN	0.83**
	Mean	0.27
6 vs. 7	GCA (Line) HHN vs. RHN	0.82**
	GCA (Line) HLN vs. RLN	0.61*
	GCA (Tester) HHN vs. RHN	0.96**
	GCA (Tester) HLN vs. RLN	0.57
	Mean	0.74

*, **, Significant at $P < 0.05$ and 0.01, respectively.

Combining ability and GCA vs. SCA sum of squares for secondary traits

Estimates of GCA mean squares for anthesis date (AD), ear height (EH), plant height (PH) and ear rot (ER) were significant in both N environments for all diallels (Table 8). SCA effects were only significant under both N conditions for plant height in trial 1 and for AD in trial 2. GCA and SCA effects were significant for gray leaf spot (GLS) under high-N in three diallels (where it was recorded) and GCA effect was significant for leaf senescence (LS) in all diallels under low-N (where it was recorded). Both GCA and SCA effects were significant for anthesis-silking interval (ASI) under both N levels in all diallels. GCA and SCA effects were also significant for ears per plant (EPP) in all diallels under high-N. Under low-N, GCA for EPP was generally non-significant whereas SCA for EPP was significant only for trial 2 (Table 8). Combined analysis also showed that GCA x environment interactions were significant for most of the secondary traits while SCA x environment interactions were mainly significant for ASI and EPP (Table 5).

In Design II crosses GCA mean squares for females (L) and males (T) were significant for PH, EH, ER and AD under both N levels. GCA effects for GLS and LS were also significant

(Table 8). The SCA effect for ASI was significant under low-N in trial 6 at Harare and for EPP in the same trial (trial 7) at Rattray Arnold under low-N conditions.

For most of the secondary traits (AD, EH, PH, ER) the proportion of GCA sum of squares accounted for a higher percentage of the total sum of squares among crosses than SCA sum of squares in all diallels under both N environments implying that additive gene effects were more important than non-additive gene effects for these traits across all N environments (Table 9). Similar results were observed in all Design II crosses. The relative importance of GCA was greater than SCA for AD, EH and PH under both N environments. The proportion of GCA was also higher than SCA for GLS under high-N and LS under low-N (where it was recorded) in all trials. However, SCA sum of squares explained the greater portion for ER than GCA sum of squares under low-N conditions in all Design II crosses (Table 9).

Comparison of GCA sum of squares with SCA sum of squares revealed that SCA (non-additive gene effects) was more important than additive gene effects for EPP under low-N in all Diallels and Design II crosses. The percentages of SCA sum of squares were also higher than GCA sum of squares for ASI under low-N condition than under high-N condition in all Diallels and Design II crosses (Table 9). However, the combined analysis across N environments for the first three diallels revealed that the proportion of GCA sum of squares was higher than SCA sum of squares for all traits studied except for EPP (Table 5).

Table 8. Mean square for general combining ability (GCA) and specific combining ability (SCA) for anthesis-silking interval (ASI), ear per plant (EPP), leaf senescence (LS), anthesis date (AD), ear (EH) and plant (PH) heights, ear rot (ER) and gray leaf spot (GLS) in different diallel (1 – 4) and Design II (5 – 7) trials under high-N (HN) and low-N (LN).

Trial	Gene effect	ASI		EPP		LS	AD		EH		PH		ER		GLS
		HN	LN	HN	LN	LN	HN	LN	HN	LN	HN	LN	HN	LN	HN
1	GCA	-	-	-	-	7.8**	38.2**	43.8**	934**	204**	1208**	155^{+}	473**	70**	1.7**
	SCA	-	-	-	-	0.10	1.2	3.3*	117*	56	146*	122^{+}	15	20	0.5**
2	GCA	3.1**	11.1**	0.04**	0.01	0.3**	28.6**	59.3**	1210**	977**	1945**	1308**	57**	762**	1.5**
	SCA	0.7**	3.9^{+}	0.01**	0.01**	0.2*	1.0**	2.3**	55	49	73**	87^{+}	13^{+}	196	0.6**
3	GCA	1.6**	9.2**	-	-	0.7**	17.7**	23.7**	510**	269**	995**	445**	72**	1102**	1.6**
	SCA	0.6**	3.3*	-	-	0.2*	2.2**	4.7**	120**	90**	160**	97	23	188^{+}	0.1*
4	GCA	2.2**	10.1**	0.04**	0.01	1.0**	15**	32**	591**	462**	-	-	46**	35	-
	SCA	0.7	5.5**	0.02*	0.00	0.2*	1.6	4.1**	97**	80+	-	-	15**	30	-
5	Line, L (GCA)	1.7*	1.3	0.02*	-	0.5**	44.4**	92.2**	1497**	210**	2231**	448**	372**	45	1.3*
	Tester, T (GCA)	3.3**	5.61*	0.04**	-	0.9**	26.7**	57.6**	764**	255**	663**	349**	112	23	1.0*
	L x T (SCA)	0.6	2.3	0.01	-	0.1	3.1**	2.6*	74	40	87	73	61**	25**	0.4**
6	Line, L (GCA)	4.1**	9.3	0.05**	0.04**	1.5**	30.4**	48.1**	1809**	644**	1868**	971**	2022**	202**	4.9**
	Tester, T (GCA)	10.2**	24.1**	0.05**	0.04**	1.4**	68.8**	76.8**	2327**	941**	1793**	684**	918**	157**	3.9**
	L x T (SCA)	0.7**	6.0**	0.01**	0.01	0.2	1.3**	5.7	89*	54*	124	98**	80**	43	0.3**
7	Line, L (GCA)	-	2.6*	0.04**	0.08**	-	-	22.2**	1628**	416**	1264**	740**	897**	667**	-
	Tester, T (GCA)	-	6.6**	0.07**	0.02	-	-	47.3**	3543**	570**	2014**	681**	389**	623**	-
	L x T (SCA)	-	1.5	0.01**	0.02**	-	-	2.1^{+}	96**	115**	87**	142	70**	163*	-

$^{+}$, *, ** Significant at P < 0.1, 0.05 and 0.01, respectively.

Table 9. Proportion (%) of sum o squares for general combining ability (GCA) and specific combining ability (SCA) for anthesis-silking interval (ASI), ear per plant (EPP), leaf senescence (LS), anthesis date (AD), ear (EH) and plant (PH) heights, ear rot (ER) and gray leaf spot (GLS) in different diallel (1 – 4) and Design II (5 – 7) trials under high-N (HN) and low-N (LN)

Trial	Gene	ASI		EPP		LS	AD		EH		PH		ER		GLS
	effect	HN	LN	HN	LN	LN	HN	LN	HN	LN	HN	LN	HN	LN	HN
1	GCA	-	-	-	-	69	93	85	77	61	78	35	93	60	58
	SCA	-	-	-	-	31	7	15	23	39	22	65	7	40	42
2	GCA	56	45	49	15	34	89	88	87	85	89	82	56	54	43
	SCA	44	55	51	85	66	11	12	13	15	11	18	44	46	57
3	GCA	49	49	-	-	57	74	64	60	51	69	62	53	67	84
	SCA	51	51	-	-	43	26	36	40	49	31	38	47	33	16
4	GCA	41	29	29	30	58	69	64	58	57	-	-	40	21	-
	SCA	59	71	71	70	42	31	36	42	43	-	-	60	79	-
Mean	GCA	49	41	39	23	55	81	75	71	64	79	60	61	51	62
	SCA	51	59	61	77	45	19	25	29	36	21	40	39	49	38
5	GCA (L)	20	6	18	-	28	50	55	56	30	65	37	44	21	27
	GCA (T)	37	27	44	-	52	29	35	28	36	20	28	13	10	22
	SCA (LxT)	44	67	38	-	20	21	10	16	34	15	35	43	69	51
6	GCA (L)	22	11	27	25	38	33	30	42	35	43	40	58	28	44
	GCA (T)	39	20	19	17	25	53	34	38	36	29	20	19	15	25
	SCA (LxT)	39	69	54	59	37	14	36	20	29	28	40	23	57	31
7	GCA (L)	-	12	21	28	-	-	29	32	21	36	28	48	24	-
	GCA (T)	-	21	26	5	-	-	44	49	21	40	18	15	16	-
	SCA (LxT)	-	68	53	67	-	-	27	19	58	24	54	37	59	-
Mean	GCA (L)	21	10	22	26	33	42	38	44	29	48	35	50	24	35
	GCA (T)	38	22	30	11	39	41	38	38	31	30	22	16	14	24
	SCA (LxT)	42	68	48	63	28	18	24	18	40	22	43	34	62	41

DISCUSSION

In this study, 635 CIMMYT tropical mid-altitude inbred lines, including experimental inbred lines (S_2 – S_7) and well tested CIMMYT Maize Lines (CMLs), were used in different groups of crosses. The inbred lines originated from different CIMMYT populations and pools. Although intensive selection was practiced during the development of these inbred lines, there is no doubt that a large sample of tropical maize germplasm was included in the study.

Cooper and Byth (1996) argued that the association between quantitative characters measured in two separate environments is a function of the degree to which the same genes influence genetic variation in both environments. They further explained that the greater the degree of genotype-by-environment interaction, the more dissimilar the genetic systems controlling the physiological processes conferring adaptation to both environments. The ranking of maize genotypes also differed between low-N and high-N conditions in this study, implying that alleles controlling high grain yield under low-N conditions are at least partially different from those controlling high grain yield under high-N conditions. This confirms results reported by Ceccarelli et al. (1992) for barley and Bänziger et al. (1997) for tropical maize.

When an organism is subjected to environmental stress, its function may depend on those genes that are stress responsive and stress adaptive (Blum, 1997). Betran et al. (2003a) and Diallo et al (2003) reported a greater importance of SCA effects for maize grain yield under low-N conditions as compared to high-N conditions. Our results confirm that, although additive gene effects constitutively contributed to grain yield across high-N and low-N conditions, the relative contribution of genes with non-additive effects was more important than additive gene effects under low-N conditions. The contributions of GCA sum of squares were higher than SCA sum of squares only in the 2002 Line x Tester crosses. Severe drought occurred in southern Africa in 2002 and this might have influenced the results. Betran et al. (2003a) reported that additive gene effects are more important than non-additive gene effects under drought conditions.

The association between GCA effects of inbred lines for grain yield under high-N at different sites within the same agro-ecology was stronger than the association between GCA effects of inbred lines under high-N and low-N conditions at the same site. This indicates that different genes are responsible for high yield under low-N and high-N. Significant crossover interactions were also observed for GCA effects of the inbred lines; even though, there were

some inbred lines and some specific hybrid combinations with consistent GCA and SCA effects across the N levels. This is in agreement with the results of Betran et al. (2003a) who reported a genotype-by-environment interaction for GCA effects of CIMMYT inbred lines. The evaluation of genotypes under optimum and stress conditions will enable the identification of genotypes with consistent performance across the environments (Dass et al., 1997; Menkir et al., 2003).

The contribution of additive gene effects (GCA sum of squares) was higher than non-additive gene effects (SCA sum of squares) for most of the secondary traits (Table 9). This indicates that selection for secondary traits could be carried out at an early stage of inbred line development. This is in agreement with the results of Lafitte and Edmeades (1995) and Betran et al. (2003b) who indicated that inbred line performance *per se* for secondary traits could be predictive of hybrid performance for secondary traits across N fertility gradients. Non-additive gene effects were more important than additive gene effects for EPP and ASI under low-N conditions, which is not consistent with the earlier report from Betran et al. (2003b).

Lamkey and Edwards (1999) reported that additive genetic variance for grain yield is usually two to four times larger than dominance variance for maize populations grown in temperate environments. The results of different experiments in this study indicate that additive gene effects for grain yield were more important than non-additive gene effects under high-N conditions. The relative contribution of additive gene effects on progeny performance was less as compared to the non-additive gene effects under low-N conditions. This shows the necessity of selecting test crosses or hybrid combinations under both managed low-N stress and optimum-N conditions (Laffite and Edmeades, 1995; Bänziger et al., 2000; Presterl et al., 2002, 2003) to target resource-poor farmers in the tropical mid-altitude ecology who produce maize mainly under low-N conditions.

CHAPTER 2

NITROGEN UPTAKE AND UTILIZATION EFFICIENCIES IN CONTRASTING NITROGEN-EFFICIENT TROPICAL MID-ALTITUDE MAIZE (*ZEA MAYS* L.) CULTIVARS

ABSTRACT

CIMMYT has identified maize cultivars (C) with improved performance under low-N conditions. So far, no information on nitrogen (N) uptake and utilization, or the underlying physiological mechanisms is available for these cultivars. This study assesses N-uptake and N-utilization efficiencies and genotype-by-environment interactions of maize (*Zea mays* L.) cultivars differing in N efficiency across a range of N levels. Sixteen cultivars were evaluated in an alpha (0,1) lattice design under three N levels (N1, N2, N3) at Harare, Zimbabwe, in 2003 (Z03) and 2004 (Z04) and Kiboko, Kenya, in 2003 (K03). At physiological maturity, mean N accumulation in the aboveground biomass ranged from 47 kg N ha^{-1} at Z03N1 (Harare 2003 N1) to 278 kg N ha^{-1} at Z03N3 (Harare 2003 N3). Grain yields ranged from 1.5 – 4.3 t ha^{-1} and 10.6 – 14.9 t ha^{-1} for the same experiments, respectively. Significant ($P<0.01$) genotype-by-environment (G x E) interactions were observed. The best commercial cultivar under high-N conditions was the most responsive to N supply, but it was inefficient under low-N conditions. The mean yield advantage of CIMMYT's N-efficient cultivars (10 cultivars) over the mean yields of the N-inefficient commercial cultivars (2 cultivars) averaged 0.6 t ha^{-1} (22%) under severe low-N stress at Harare. Some N-efficient cultivars also gave reasonable grain yield under high-N conditions indicating that selection for improved performance under low-N increased grain yield under both low-N and high-N conditions. The strong relationships between grain yield and N-uptake and N-utilization efficiencies under low-N conditions across the years and sites indicated that selection for improved performance under low-N improved both N-uptake and N-utilization efficiencies under low-N stress conditions.

Key words: Cultivar, G x E interaction, N efficiency, *Zea mays* L.

INTRODUCTION

Environment is a general term that covers conditions under which plants grow, and may vary between locations and years with respect to climatic variables (radiation, temperature and rainfall), soil fertility levels, other management practices, or combinations of these factors (Romagosa and Fox, 1993). Every non-genetic factor that is a part of the plant environment has the potential to cause differential genotypic performance resulting in a significant genotype-by-environment (G x E) interaction (Fehr, 1991). In mid-altitude maize growing areas of sub-Saharan Africa, low soil fertility, especially low nitrogen (N) is among the major abiotic stresses limiting maize production in fields of smallholder farmers and results in significant G x E interactions in crop performance (Lafitte and Edmeades, 1994a, b, c; Friesen et al., 2002; Bänziger et al., 2005).

Different researchers have reported the existence of genetic variability in N efficiency under low-N conditions (Wiesler and Horst, 1993; Lafitte and Edmeades, 1994c; Oikeh, 1996; Bänziger et al., 1997; van Beem and Smith, 1997; Below et al., 1997; Oikeh et al., 1999; Akintoye et al., 1999; Horst et al., 2003; Presterl et al., 2003; Paponov and Engels, 2003). According to Lynch (1998), efficiency is the ability of a system to convert inputs into desired outputs or to minimize the conversion of inputs into waste. The author further stated that the amount of mineral nutrients might be considered as an input whereas plant growth, physiological activity or yields are typical outputs. Nitrogen efficiency has been defined as the ability of a genotype to realize an above average grain yield under conditions of low N availability or suboptimal N supply (Graham, 1984; Sattelmacher et al., 1994).

Moll et al. (1982) evaluated eight unselected single cross hybrids under low-N and high-N levels and attributed genetic differences in N efficiency under low-N to differences in the utilization of accumulated N for grain yield production. Under high-N, they attributed genetic differences in grain yield largely to variation in N uptake. Kamprath et al. (1982) also compared three population hybrids under three N levels. They associated N efficiency under low and medium N with a higher N-uptake efficiency whereas higher grain yield under high-N was linked to the ability to utilize N accumulated in the plant. Other authors reported that high N efficiency was achieved by a combination of high N-uptake and N-utilization efficiencies in maize (Wiesler et al., 2001) and in wheat (Ortiz-Monasterio et al., 2001).

Variation in the number and the nature of the sample genotypes and testing environment or a combination of both might explain these contradictory results on the relative importance of N-uptake and N-utilization efficiencies. CIMMYT has identified cultivars with improved grain yield performance under low-N conditions (Beck, 1996; Friesen et al., 2002; Bänziger and Meyer, 2002; Bänziger et al., 2005). So far, no information on the underlying physiological mechanisms is available for these cultivars. This study assesses N-uptake and N-utilization efficiencies and the genotype-by-environment interaction of contrasting N-efficient tropical mid-altitude maize cultivars from CIMMYT across a range of N levels.

MATERIALS AND METHODS

Cultivars

Fourteen maize hybrids (cultivars) from CIMMYT-Zimbabwe, CIMMYT-Kenya and CIMMYT-Mexico, and two commercial hybrids (cultivars) from Seed-Co International were used. Based on existing data from CIMMYT, their performance differs under low-N, indicating potential differences in N efficiency characteristics (Table 1).

Table 1. Maize cultivars/hybrids used for the study.

No.	Cultivar (Hybrid)	Source	Performance under low-N
1	CML444/CML445//CML440	CIMMYT-Zimbabwe	Good
2	CML395/CML444//CML440	CIMMYT-Zimbabwe	Good
3	CML202/CML395//CML205	CIMMYT-Zimbabwe	Poor
4	SC515	Seed-CO-Zimbabwe	Poor
5	CML395/CML444//CML442	CIMMYT-Zimbabwe	Good
6	CML444/CML197//CML443	CIMMYT-Zimbabwe	Good
7	SC633	Seed-CO-Zimbabwe	Poor
8	CML181/CZL01005//CZL01006	CIMMYT-Zimbabwe	Best among QPM[1]
9	CML181/CML182//CML176	CIMMYT-Zimbabwe	Best among QPM[1]
10	CML144/(16304/6303Q)-B-6-1-3-3-B*6	CIMMYT-Mexico	Poor (QPM[1])
11	CML247//CML254	CIMMYT-Mexico	Good
12	CML78/CML373	CIMMYT-Mexico	Good
13	CML264/CML311//CML334	CIMMYT-Mexico	Poor
14	CML442/CML444//[MSRXPL9]C1F2-205-1(OSU23i)-1-1-X-1-X-B-B	CIMMYT-Kenya	Good
15	LPSC4F273-2-2-1-B-B-B/CML202//CML384	CIMMYT-Kenya	Good
16	CML312/CML247//CML78	CIMMYT-Kenya	Good

[1] Quality Protein Maize

Experimental sites and trial management

The experiments were conducted at the CIMMYT research station at Harare, Zimbabwe (2003 and 2004) and at the Kenya Agricultural Research Institute substation at Kiboko (2003), representing sub-humid and dry mid-altitude areas of sub-Saharan Africa, respectively (Corbett, 1998; Hornetz et al., 2000). The research site at Harare is located at 17°49'E, 31°1'S and 1478 m above sea level. The soil is a reddish brown clay soil (nitosol) with a bulk density of 1.4 g cm^{-3}, 1.5 g cm^{-3} and 1.5 g cm^{-3} for 0 – 30 cm, 30 – 60 cm and 60 – 90 cm depth, respectively. The pH ($CaCl_2$) of the topsoil (0 – 30 cm) and subsoil (30 – 60 cm) was 5.89 and 5.93, respectively. The annual mono-modal rainfall averaged 684 mm (2003 and 2004) and maximum and minimum temperature during the growing season (October to April 2004) averaged 28.3°C and 14.2°C, respectively. The site at Kiboko, Kenya, is located at 2°10'S, 37°40'E at an elevation of 975 m above sea level. Long-term annual rainfall is 561 mm in a bimodal distribution (328 mm from October to January; 233 mm from March to June). Average, maximum and minimum temperatures are 33°C and 14°C from March to August,

respectively. The soil is an eutric fluvisol with sandy-clay texture. The pH ($CaCl_2$) of the topsoil was 7.9, decreasing to 5.8 in the subsoil (70 – 80 cm). The high pH of the topsoil was attributed to the Kiboko river irrigation water (Hornetz et al., 2000). The bulk density of the soil was 1.5 g cm^{-3}, 1.6 g cm^{-3} and 1.6 g cm^{-3} for 0 – 30 cm, 30 – 60 cm and 60 – 90 cm depth, respectively.

The cultivars were tested under three N levels (low, medium and high N) at both sites, resulting in nine environments: Harare 2003 low-N (Z03N1), Harare 2003 medium-N (Z03N2), Harare 2003 high-N (Z03N3), Kiboko low-N (K03N1), Kiboko medium-N (K03N2), Kiboko high-N (K03N3), Harare 2004 low-N (Z04N1), Harare 2004 medium-N (Z04N2) and Harare 2004 high-N (Z04N3). Choice of N applications at each station was intended to create a range of relevant soil-N levels and depended on soil type, cropping history, fertilizer recommendation and experience of researchers at each station (Table 2). High N rates were split. Phosphorus and potassium were applied uniformly based on the recommendation of each center prior to planting.

Trials at both sites were hand-planted with two seeds per hill and thinned at the 3-leaf stage. Planting dates at Harare were on 3, 7 and 4 December 2002 for Z03N1, Z03N2 and Z03N3 and on 26, 28 and 27 November 2003 for Z04N1, Z04N2 and Z04N3, respectively. Harvest was between 31 April 2003 and 5 May 2003 in 2003 and between 16 April 2004 and 25 April 2004 in 2004. The trials at Kiboko were planted on 14 April 2003 and harvested between 27 August 2003 and 1 September 2003. Pre-emergence herbicides, Atrazine (4.5 l ha^{-1}) and Dual (1.8 l ha^{-1}, 96% Metalchlor), were applied at planting to control the weeds. Then, the weeds were controlled by hand weeding and application of Basagran (3 l ha^{-1}, 48% Bentazon). Furadan (20 kg ha^{-1}, carbofuran) was applied at planting. Fungual diseases (*Cercospora zea-maydis, Excerohilum turcicum* and *Puccinia sorghi*) were controlled using Tilt 250EC (0.5 l ha^{-1}). Thiodan 1G (4 kg ha^{-1}) and Thionex (230 g ha^{-1}, endosulfan) were used to control stalk borers (*Busseola fusca* and *Chilo partellus*). Cutworms were controlled with Karate (5 g ha^{-1}, Lambda-cyhalothrin) applied at emergence. The trials at Harare were irrigated to field capacity at planting using sprinkler irrigation. A second irrigation of 20 – 30 mm was applied 6 – 7 days after planting to facilitate germination. Thereafter, trials were irrigated to field capacity whenever soil moisture was less than 40% of field capacity. Similar procedures were followed for trials at Kiboko. A plot size of 4 m length by 4.5 m width with six rows per plot

was used. Spacing was 0.75 m and 0.25 m between rows and plants, respectively. A plant density of 53,333 plants per hectare was kept after thinning.

Table 2. Fertilizer application (N1, N2, N3) and cropping history of the testing environments in Harare 2003 and 2004 (Z03, Z04) and Kiboko 2003 (K03).

Environment	Applied N (kg ha^{-1})	Cropping history and residue management
Z03N1	0	Depleted, residue removed
Z03N2	0	Previously fertilized, residue incorporated
Z03N3	168	Previously fertilized, residue incorporated
K03N1	18	Depleted, residue removed
K03N2	18	Previously fertilized, residue removed
K03N3	90	Previously fertilized, residue incorporated
Z04N1	0	Depleted, residue removed
Z04N2	0	Previously not fertilized, residue partially incorporated
Z04N3	168	Previously fertilized, residue incorporated

Measurements

Anthesis date (AD) was recorded for each plot when 50% of the plants in the two central rows shed pollen. On the following day, twelve plants were harvested from an area of 2.25 m^2 in the central four rows for dry matter determination and plant N analysis at anthesis. An area of 5.65 m^2, corresponding to 32 plants in the central four rows, was harvested immediately after physiological maturity for dry matter determination and plant N analysis at harvest. During both harvests, two border plants at the ends of each row were excluded to avoid border effects.

Grain yield was recorded from all ears in the harvest area at physiological maturity. Ears were shelled, grain weight and grain moisture percentage were recorded and grain yield (t ha^{-1}) calculated at 12.5% moisture. Fresh weight of the stover harvested at anthesis and physiological maturity was recorded after cutting plants at ground level. One quarter of the plants were chopped using a VIKING 220 chopping machine. At harvest, a quarter of the shelled cobs were added before chopping. A homogenized stover sub-sample and grain sample were taken for each plot, weighed, oven-dried to constant weight at 80^{o}C for 72 hours, weighed again and total stover, grain and plant biomass was calculated. Grain and stover sub-samples were milled with an analytical mill and analyzed for N at the Institute of Plant

Nutrition Laboratory, University of Hannover, Germany, using a CNS analyzer (Vario EL, Elementar Analysis Systems, Hanau, Germany).

Nitrogen uptake, utilization and harvest index were calculated from data taken at physiological maturity. Nitrogen uptake was set equal to the total N in the aboveground biomass. Nitrogen utilization was calculated as the ratio of grain yield to total N in the aboveground biomass. Nitrogen harvest index was calculated as the ratio of N in the grain to total N in the aboveground biomass. In addition, N uptake at anthesis (BIONF), N uptake after anthesis (BIONH-F) and stover N at physiological maturity (STONH) were calculated. Plant height, PH (height from ground level to the base of the tassel), and stem circumference, CIRC (at 6 cm above the ground), were also measured and relationships with N uptake were calculated.

Experimental design and statistical analyses

The cultivars were planted using alpha (0,1) lattice designs (Patterson and Williams, 1976) with four replications. Within each experiment, lattice-adjusted cultivar means were calculated using the PROC MIXED procedure of SAS (SAS, 2001), with cultivars as a fixed factor, and replicate and incomplete blocks within replicates as random factors. Across-experiment analysis was conducted from lattice-adjusted cultivar means, using cultivars as fixed factor and experiment as random factor (Bänziger et al., 2002). Although three N levels were used at both sites, N availability in the soil differed across sites and seasons. Therefore, each experiment was considered as a different environment in the across-experiment analysis (Bänziger et al., 1999; Bänziger et al., 2002). The significance for genotype mean square was tested against the genotype-by-environment interaction (G x E) mean square while the significance of G x E mean square was tested against the pooled error. Environmental index (EI) for each environment was also calculated as the difference between mean grain yield in each environment and the grand mean across the environments.

In addition, genotype-by-environment interactions were analyzed using Additive Main effect and Multiplicative Interaction (AMMI) analysis (Crossa et al., 1990; Gauch, 1992; Ebdon and Gauch, 2002) to assess similarity and dissimilarity among testing environments and interaction patterns between cultivars and environments. Biplots of the first two Interaction Principal Component Analysis axes (IPCA1 and IPCA2) were used to indicate the patterns. Close points within environments and cultivars indicate similarity while distant points

indicate dissimilarity. Cultivars and environments that are close together also tend to have similar interaction patterns (Gauch, 1992; Fox et al., 1997; Betran et al., 2003a). AMMI analysis was computed using SAS (SAS, 2001). Simple linear regression coefficient was also calculated for each cultivar by regressing the yield of individual cultivar on environmental index and slope of the regression was tested for significant difference from unity using t-test (Eberhart and Russell, 1966). Finally, simple linear correlation coefficients were calculated to assess the association between traits.

RESULTS

Nitrogen stress intensity and grain yield

The nine environments significantly ($P<0.01$) varied in grain yield, N uptake, N utilization and N harvest index (Table 3 and 4). Estimates of the environmental index showed that Z03N1 was the lowest yielding environment while Z03N3 was the highest yielding environment. Severe stress under low-N at Harare reduced grain yield by 65% and 77% in 2003 and by 47% and 70% in 2004 as compared to medium-N and high-N treatments, respectively. At Kiboko, low-N stress in K03N1 reduced grain yield by 25% and 40% as compared to K03N2 and K03N3, respectively, indicating that the severity of low-N stress was less than at Harare. Grain yields of cultivars ranged from 1.5 – 4.3 t ha^{-1}, 6.1 – 9.8 t ha^{-1} and 10.6 – 14.9 t ha^{-1} in 2003, and 2.2 – 4.4 t ha^{-1}, 4.6 – 9.4 t ha^{-1} and 8.7 – 13.5 t ha^{-1} in 2004 for Harare low-N, medium-N and high-N experiments, respectively, while they ranged from 3.7 – 7.3 t ha^{-1}, 5.8 – 10.9 t ha^{-1} and 5.5 – 13.2 t ha^{-1} for Kiboko low-N, medium-N and high-N experiments, respectively. Differences between the cultivars and G x E interactions were significant ($P<0.01$) (Table 4).

Table 3. Environmental index (mean grain yield in each testing environment minus grand mean) and mean grain yield of the testing environments.

Environment	Environmental index	Mean grain yield (t ha^{-1})
Harare 2003 low-N (Z03N1)	-4.90	2.9
Harare 2003 medium-N (Z03N2)	0.56	8.4
Harare 2003 high-N (Z03N3)	4.91	12.7
Kiboko low-N (K03N1)	-1.47	6.3
Kiboko medium-N (K03N2)	0.60	8.4
Kiboko high-N (K03N3)	2.77	10.6
Harare 2004 low-N (Z04N1)	-4.42	3.4
Harare 2004 medium-N (Z04N2)	-1.39	6.4
Harare 2004 high-N (Z04N3)	3.31	11.1

Genotype-by-environment interaction

Generally the correlation between grain yields of high-N treatments were higher than the correlation between grain yields of high-N and severe low-N stress treatments (Table 5). The correlation (r) between grain yields of Harare severe low-N stress experiments, Z03N1 and Z04N1, was 0.46^{+} ($P<0.10$) and the relationship between grain yields of Harare high-N experiments, Z03N3 and Z04N3 was 0.77**. However, grain yields in N1 and N3 treatments within the same year were not correlated at Harare, implying that the performance of the cultivars differed between severe low-N stress and high-N. The relationship between grain yield of low-N and high-N experiments at Kiboko, on the other hand, was close. The grain yield performance of the cultivars under severe low-N conditions of Harare was in the main in agreement with the breeders' classification of the cultivars for grain yield performance under low-N (Table 1 and Figure 2B).

Table 4. Days to anthesis (AD), nitrogen uptake in the aboveground biomass at flowering (BIONF, kg N ha^{-1}), nitrogen uptake in aboveground biomass at physiological maturity (NUP, kg N ha^{-1}), nitrogen utilization (NUT, ratio between grain yield and NUP), nitrogen harvest index (NHI, %), grain yield (GY), and linear regression coefficient of yield of individual cultivars on the environmental index (b) of maize cultivars differing in N efficiency.

Cultivar	AD (d)	BIONF (kg N ha^{-1})	NUP (kg N ha^{-1})	NUT	NHI (%)	GY (t ha^{-1})	b
1	67	95	136	54	63	7.6	0.89
2	68	99	144	52	59	8.1	0.94
3	69	92	134	50	61	7.0	0.97
4	67	81	121	51	59	6.8	0.81
5	71	96	144	54	59	8.6	1.14
6	72	109	147	53	57	8.2	1.16
7	68	97	149	54	62	9.0	1.30*
8	69	97	139	49	57	7.8	1.08
9	68	96	149	50	60	7.8	0.91
10	76	109	128	38	51	5.5	0.81
11	76	105	150	42	54	7.0	0.88
12	68	99	158	53	61	9.0	0.93
13	72	98	158	42	56	7.3	0.97
14	72	107	156	50	61	8.6	1.15
15	75	103	148	51	60	8.4	1.14
16	70	85	146	51	61	8.1	0.88
Mean	70	98	144	50	59	7.8	
CV%	1.5	18	17	12	8	14	
$LSD_{0.05}$	1.0	11	16	4	3	0.9	
G	**	**	**	**	**	**	
E	**	**	**	**	**	**	
G x E	**	**	**	**	**	**	

** Significant at P<0.01.

Table 5. Linear correlation coefficients (r) between grain yields of 16 maize cultivars grown in the testing environments differing in levels of N stress. Harare 2003 (Z03N1, Z03N2, Z03N3), Kiboko, Kenya 2003 (K03N1, K03N2, K03N3) and Harare 2004 (Z04N1, Z04N2, Z04N3).

	Z03N1	Z03N2	Z03N3	K03N1	K03N2	K03N3	Z04N1	Z04N2	Z04N3
Z03N1		0.52*	0.36	0.52*	0.18	0.35	0.46^{+}	0.36	0.46^{+}
Z03N2			0.55*	0.46^{+}	0.30	0.49^{+}	0.37	0.69**	0.78**
Z03N3				0.48^{+}	0.36	0.54*	0.16	0.20	0.77**
K03N1					0.70**	0.86**	0.53*	0.52*	0.50*
K03N2						0.79**	0.16	0.38	0.41
K03N3							0.29	0.50*	0.61*
Z04N1								0.58*	0.19
Z04N2									0.57*
Z04N3									
GY (t ha^{-1})	2.89	8.35	12.66	6.32	8.39	10.57	3.37	6.40	11.13

$^{+}$, *, ** Significant at P<0.10, 0.05 and 0.01, respectively (each correlation with n = 16 points)

AMMI (Additive Main effect and Multiplicative Interaction) biplots for grain yield (Figure 1) showed distinct patterns for the testing environments. The high potential environments of Harare (Z03N3 and Z04N3) were in quadrant one while the low potential environments (Z03N1 and Z04N1) were in quadrant four. Z03N2 (Harare 2003 N2) was in quadrant one while Z04N2 (Harare 2004 N2) was in quadrant four indicating mainly the difference in the N level in the soil. Kiboko N treatments (N1, N2 and N3) were also in different quadrants from Harare N treatments (N1, N2 and N3) indicating the difference in N level and interference of other environmental factors. IPCA1 (Interaction Principal Component Analysis axis 1) was significant (P<0.01) and explained 37.3% of the G x E interaction. IPCA2 (Interaction Principal Component Analysis axis 2) was also significant (P<0.01) and explained 28.6% of the G x E interaction. Thus, the two principal components explained 65.9% of the G x E variation. AMMI's biplots positioned the cultivars according to their relative grain yield performance across the environments (Figure 1). Cultivar 7 was among the cultivars with large positive IPCA1 score indicating better performance of this cultivar under high-N conditions than under low-N conditions. Except for cultivar 7, none of the cultivars had a regression coefficient (linear regression of grain yield of individual cultivar on environmental index) that significantly differed from 1.0 (Table 4). Cultivar 7 had the largest regression coefficient indicating that it was responsive to favourable N fertility environments but relatively poor performing under low-N stress. Figure 2A shows grain yield performance of

the cultivars under high and medium N conditions of Harare 2003 while figure 2B shows the grain yield performance of the cultivars under high-N and low-N conditions (average of Harare 2003 and 2004).

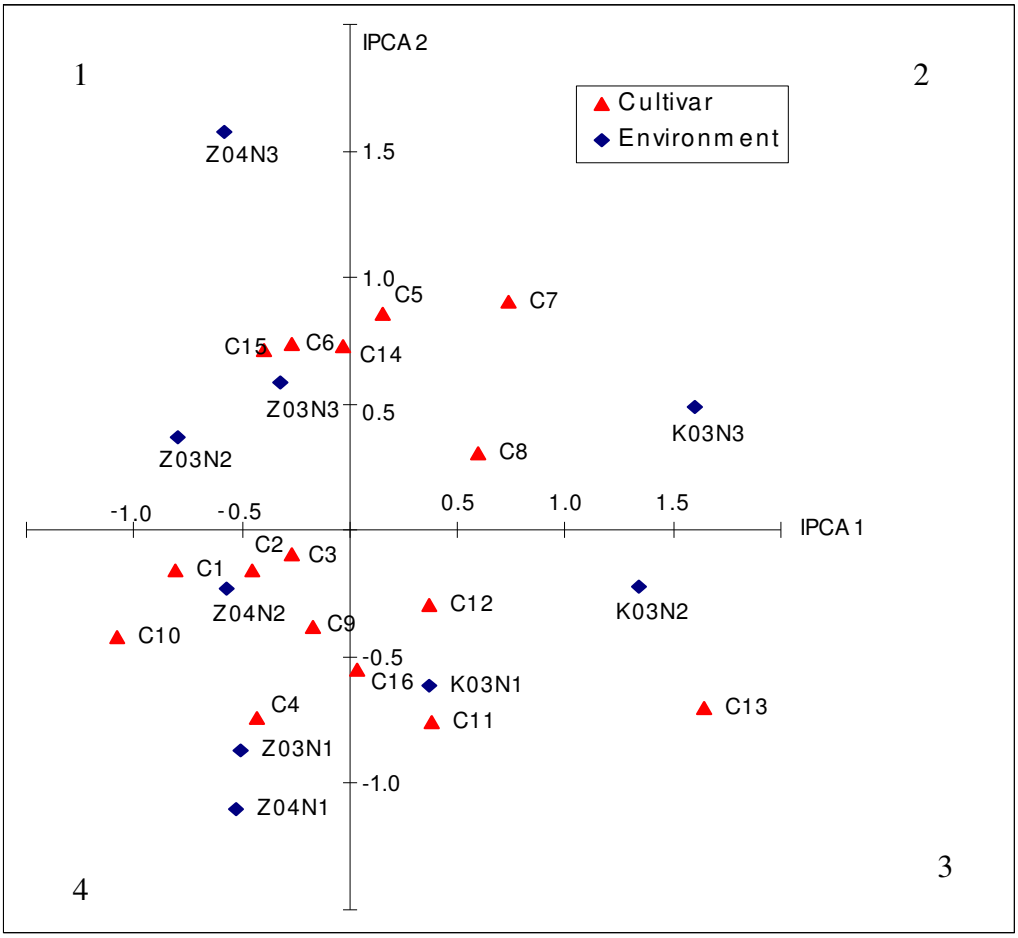

Figure 1. Additive Main effect and Multiplicative Interaction (AMMI) biplots for 16 cultivars (C) and nine environments (Harare 2003 (Z03N1, Z03N2, Z03N3), Kenya 2003 (K03N1, K03N2, K03N3) and Harare 2004 (Z04N1, Z04N2, Z04N3)) for grain yield. IPCA, Interaction Principal Component Analysis axis.

Focussing on the trials in Zimbabwe where the environmental indices differed most and AMMI biplots showed dissimilarity of high-N and low-N environments, cultivar 7 was among the highest yielding cultivars at Harare high-N (Z03N3 and Z04N3) while it was among the N-inefficient cultivars at Harare low-N (Z03N1 and Z04N1). However, the relatively N-efficient cultivars 6 and 15 had comparable grain yields to cultivar 7 at high-N conditions. Cultivars 16 and 12 were the most N-efficient cultivars (cultivars with above average grain yield under conditions of low-N) but they were less responsive as compared to cultivar 7. Cultivar 7 was also the best yielding at Harare 2003 medium-N (Z03N2), where AMMI biplots showed N2 similarity to N3 environments, indicating that the crossover

interactions occurred at low-N stress conditions (Figure 2A, B). The mean yield advantage of CIMMYT's N-efficient maize cultivars (10 cultivars) over the mean yields of the N-inefficient commercial cultivars (2 cultivars) averaged 0.62 t ha^{-1} (22%) under low yielding environments (Z03N1 and Z04N1). Although cultivars varied significantly ($P<0.01$) for days to anthesis, anthesis date was significantly related to grain yield only at Z03N2 and Z04N2 (Table 6).

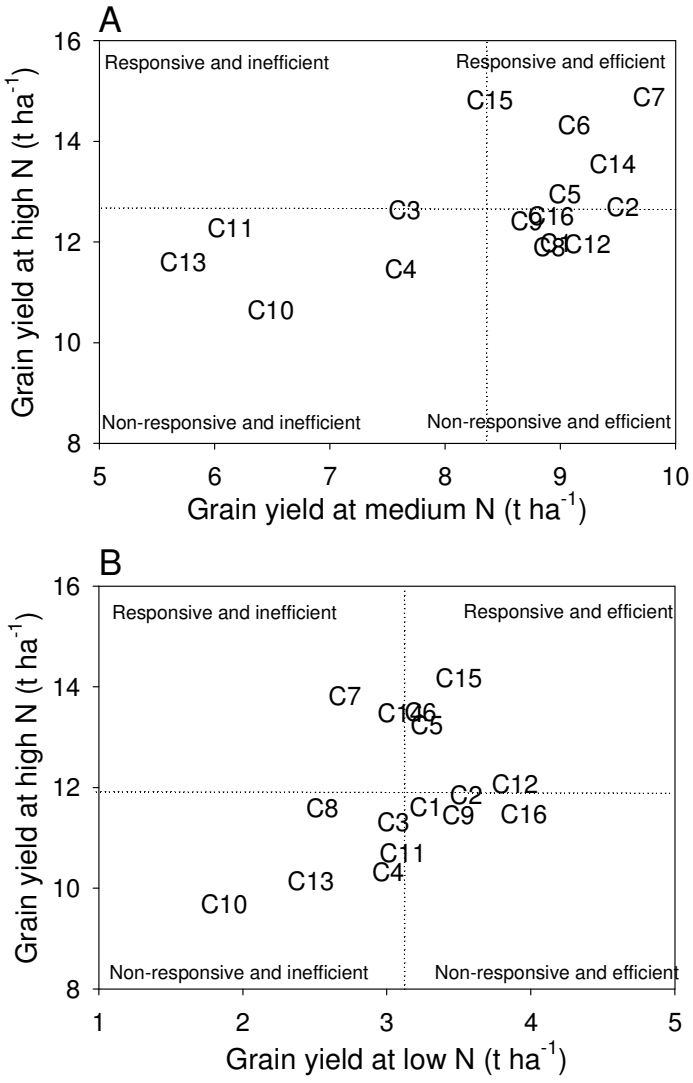

Figure 2. Relationships between grain yields of 16 maize cultivars at high-N and medium-N (A) (Harare 2003) and grain yields at Harare high-N and low-N (B) (means of Harare 2003 and 2004).

Nitrogen uptake and utilization efficiencies

Nitrogen uptake into the aboveground biomass increased as N level in the soil increased. At maturity, it varied from 47 kg N ha^{-1} at Z03N1 to 278 kg N ha^{-1} at Z03N3 (Figure 3). It was high at Harare high-N in both years as compared to Kiboko high-N, but N uptake was higher at Kiboko low-N as compared to Harare low-N in both years. More N was also accumulated

at Harare medium-N in 2003 than at Harare medium-N in 2004 and Kiboko medium-N in 2003, indicating that the N-stress level differed across years and sites. Significant differences ($P<0.01$) were observed among the cultivars for N uptake in all environments (data not shown). The correlation between grain yield and N uptake was always positive and close at low-N conditions at both sites (Table 6). However, the relation between N uptake at anthesis (BIONF) and grain yield was low except at K03N2 where there was a positive relationship. A significantly positive relationship was observed between N uptake after anthesis (BIONH-F) and grain yield at low-N conditions (Table 6).

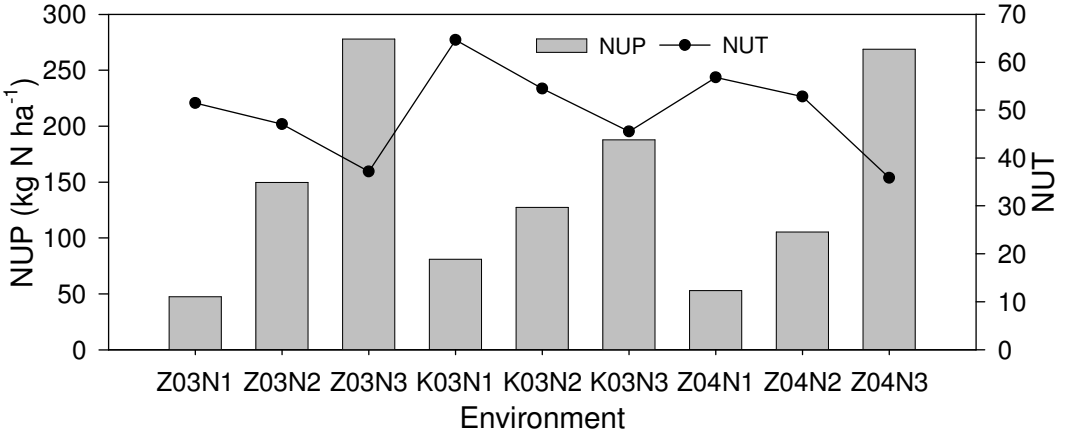

Figure 3. Mean N uptake (NUP) and utilization (NUT) across environments (Harare 2003 (Z03N1, Z03N2, Z03N3), Kenya 2003 (K03N1, K03N2, K03N3) and Harare 2004 (Z04N1, Z04N2, Z04N3)).

The N-efficient and relatively less responsive cultivars, cultivar 16 and cultivar 12 were among the cultivars with a high N uptake under low-N conditions, but their N uptake under high-N conditions was less as compared to the responsive cultivars. Some of the N-efficient and relatively responsive cultivars, for example cultivar 15 and cultivar 6, had a good N uptake under both high-N and low-N conditions (Figure 4A). On the other hand, the responsive cultivar, cultivar 7, had a high N uptake only under high-N conditions indicating crossover interaction for N uptake under different environments. Under severe low-N stress of Harare (Z03N1 and Z04N1), the difference between total N uptake at maturity and total N uptake at anthesis was also positive for most of the N-efficient cultivars while it was negative for most of the N-inefficient cultivars (data not shown).

The relation between N uptake and anthesis date (AD), plant height (PH) and stem circumference (CIRC) was low under low-N stress (data not shown) implying that these traits were less important for N-uptake efficiency in this group of cultivars. However, there was a

strong relationship between N uptake and total aboveground biomass at physiological maturity (BIOH) (r ranged at different environments from 0.78** to 0.92**).

Table 6. Linear correlation coefficients (r) between grain yield (GY, t ha^{-1}) and N uptake at anthesis (BIONF), N uptake after anthesis (BIONH-F), stover N at physiological maturity (STONH), total N uptake in the aboveground biomass at physiological maturity (NUP), N utilization (NUT), N harvest index (NHI) and anthesis date (AD) in the testing environments differing in levels of N stress. Harare 2003 (Z03N1, Z03N2, Z03N3), Kenya 2003 (K03N1, K03N2, K03N3) and Harare 2004 (Z04N1, Z04N2, Z04N3).

Trait	Environment								
	Z03N1	Z03N2	Z03N3	K03N1	K03N2	K03N3	Z04N1	Z04N2	Z04N3
BIONF	0.14	0.38	0.17	0.17	0.52*	-0.45^{+}	-0.01	0.11	0.27
BIONH-F	0.64**	0.04	0.14	0.62*	0.83**	0.70**	0.74**	0.57*	0.53*
STONH	0.02	-0.25	-0.08	0.23	0.64**	-0.43^{+}	0.18	0.03	0.13
NUP	0.72**	0.45^{+}	0.29	0.75**	0.88**	0.65**	0.71**	0.67**	0.69**
NUT	0.82**	0.76**	0.49^{+}	0.75**	-0.15	0.77**	0.82**	0.61*	0.72**
NHI	0.79**	0.79**	0.44^{+}	0.64**	0.36	0.84**	0.69**	0.74**	0.62*
AD	-0.35	-0.55*	0.07	-0.14	-0.02	-0.24	-0.38	-0.57*	-0,05
GY (t ha^{-1})	2.89	8.35	12.66	6.32	8.39	10.57	3.37	6.40	11.13

$^{+}$, *, ** Significant at P<0.1, 0.05 and 0.01, respectively (each correlation with n = 16 points).

Significant differences (P<0.01) were observed among the cultivars for N utilization and N harvest index. Nitrogen-utilization efficiency was also significantly positively related with grain yield under severe low-N stress (Table 6) implying that the N-efficient cultivars generally had better N-utilization efficiency under severe low-N stress as compared to the N-inefficient cultivars. The N-efficient and less responsive cultivars like cultivar 1 and cultivar 2 had high N-utilization efficiency under low-N conditions, but a lower N uptake under high-N conditions as compared to the responsive cultivars. However, the relatively N-efficient and responsive cultivar, cultivar 15, and the N-inefficient and responsive cultivar, cultivar 7, had high N utilization under high-N condition (Figure 4B) indicating the crossover interaction for N utilization under different environments. Generally cultivars with medium N utilization had a high N uptake (Figure 4A, B). The relation between grain yield and N utilization was also positive in all environments except at K03N2. However, mean N utilization progressively declined as the mean N uptake in the total aboveground biomass increased (Figure 3).

Nitrogen harvest index was positively related to grain yield at all environments (Table 6). Most of the cultivars with high N utilization, like cultivar 16 and cultivar 12, also showed a

high N harvest index under low-N conditions while the responsive cultivar, cultivar 7, had a high N harvest index under high-N conditions (Figure 4C). The N-inefficient cultivar, cultivar 13, was among the cultivars with high N uptake (Figure 4A), but it was among the cultivars with low N harvest index (Figure 4C) indicating the importance of N partitioning for grain yield performance under all conditions.

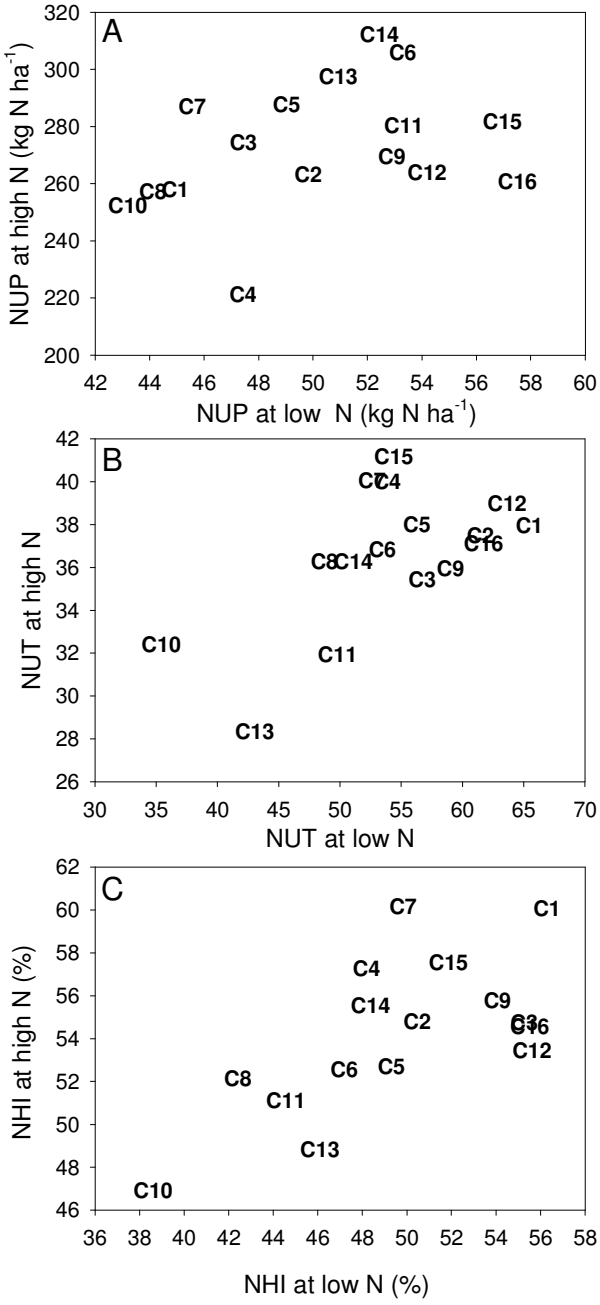

Figure 4. Relationships between (A) N uptake (NUP kg N ha^{-1}), (B) N utilization (NUT, kg grain/kg N uptakes) and (C) N harvest index (NHI, %) of contrasting N-efficient maize cultivars at Harare high-N and Harare low-N (means of Harare 2003 and 2004).

DISCUSSION

Grudloyma et al. (2003) reported a strong relationship between grain yields of hybrids under high-N and low-N when yield reduction was about 40%. Bänziger et al. (1997) and Presterl et al. (2003) reported that genetic correlation between grain yields under low-N and high-N is generally positive but decreases with increasing relative yield reduction under low-N. The result of this study showed that, generally, the linear correlation coefficient decreased as low-N stress intensity increased, indicating little relationship among cultivar performance across low-N and high-N levels. The relationships between grain yields under medium and high N conditions were high for the Harare 2003 and 2004 experiments (r = 0.54* and r = 0.62*, respectively) and for the Kiboko experiments (r = 0.79**). However, the correlation (r) between grain yields at Harare under high-N and severe low-N stress averaged 0.28 (average of 2003 and 2004). The loss of association under severe low-N stress indicates that different factors were contributing to grain yield under severe low-N stress and high-N levels. This may also indicate the importance of N-efficient cultivars, particularly in areas where N is the limiting factor for maize production. The close relationship between grain yields of low-N and high-N experiments at Kiboko in 2003 was due to the fact that Kiboko low-N stress was less as compared to the Harare low-N experiments (yield reduction of 40% versus 70 – 77% at Harare). This was reflected by a higher N uptake in the total aboveground biomass in the Kiboko low-N treatment as compared to Harare low-N, reflecting better mineralization of N in the soil and higher availability of N to the plant under Kiboko low-N than under Harare low-N conditions. The relatively dissimilarity of Kiboko high-N and Harare high-N (Figure 1) may also indicate that environmental factors other than N might have contributed to the difference in performance of some of the cultivars at the two sites.

Simonds (1991) stated that prolonged selection for performance of high yield potential under non-limiting environments has generally led to unconscious selection for high responsiveness to N supply. In this study the commercial hybrid, cultivar 7 had the highest regression coefficient (Table 4) whereas it was among the N-inefficient cultivars under low-N stress conditions (Figure 2). This may indicate that traditional commercial hybrids/cultivars selected under high-N conditions may fail to fit the actual production environments of the fields of the majority of the farmers in sub-Saharan Africa where low-N is one of the most limiting factors for maize production (Sibale and Smith, 1997; Bänziger et al., 2000).

Some of CIMMYT N-efficient hybrids/cultivars had above average response reflecting that selection for improved performance under low-N has also increased grain yield under high-N conditions, which is consistent with the results of Lafitte and Edmeades (1994b). This may be the reflection of the CIMMYT breeding strategy, which combines selection criteria based on performance under managed low-N stress and non-limiting N levels. Lafitte and Edmeades (1994c) and Lynch (1998) indicated that focussing only on improved performance under low-N may improve yield under low-N conditions but may reduce the responsiveness; however, selection that exploits both low-N and high-N environments may increase grain yield under both low-N and high-N conditions. A similar trend was observed for our results (Figure 2).

The results of this study indicate that N-uptake and N-utilization efficiencies were factors contributing to N efficiency, implying that both N-uptake efficiency and N-utilization efficiency are important traits for improved performance at low-N. This is in contrast to the results of Moll et al. (1982) who reported that N-utilization efficiency is more important than N-uptake efficiency under low-N conditions, and Kamprath et al. (1982) who reported N-uptake efficiency is more important than N-utilization efficiency under low-N conditions. Presterl et al. (2002) reported positive relationships between grain yield and N-uptake efficiency and N-utilization efficiency under low-N conditions, but the authors mainly related N efficiency to N-uptake efficiency. However, the results of this study are in agreement with the results of Wiesler et al. (2001) for temperate maize and Ortiz-Monasterio et al. (2001) for wheat who reported that both N-utilization efficiency and N-uptake efficiency are important traits for improved performance under low-N conditions. It seems that variation exists for both traits among maize cultivars (Figure 4A, B) and both traits contribute to N efficiency. Moll et al. (1982) and Ortiz-Monasterio et al. (2001) indicated that causes of variation in N efficiency in terms of component factors might differ between levels of N supply and among genotypes, as was also reflected in this study. The results of this study also showed that there are cultivars which combine good N uptake and N utilization. These cultivars are relatively N-efficient and responsive. Another group of cultivars, that are N-efficient but less responsive, have high N utilization but generally do not have high N uptake under high-N conditions indicating the importance of N uptake for responsiveness (Figure 4).

Although N uptake before anthesis was mainly positively related to N efficiency, the correlation was low as compared to N uptake after anthesis (Table 6). Nitrogen uptake after anthesis was strongly related to N efficiency especially under low-N conditions indicating that

the N-efficient cultivars might have maintained more active roots for N absorption during grain filling as compared to the N-inefficient cultivars and this may be one of the factors for better grain yields in the N-efficient cultivars. Similar results were reported in temperate maize and oilseed rape (Wiesler et al., 2001) and in tropical maize (Akintoye et al., 1999). However, some N-efficient cultivars had also negative N uptake after anthesis (data not shown) indicating N utilization was of major importance in these cultivars. The strong relationship between N-utilization efficiency and grain yield under low-N conditions means that the N-efficient cultivars produced more grain per N available in the plant than the N-inefficient cultivars. Sattelmacher et al. (1994) indicated that partitioning of N within the plant and efficient utilization of N at the cellular level are contributing factors for N-utilization efficiency under limited N condition. Ta and Weiland (1992) showed genotypic variation in translocation of stored N in the vegetative part to the sink. This may imply that cultivars which combine both N-uptake efficiency and N-utilization efficiency may be more efficient under low-N than cultivars which accumulate more N but do not translocate it to the sink. For instance, the N-inefficient cultivar, cultivar 13, had relatively high N uptake, but very low N harvest index (Figure 4A, C). This may also indicate the importance of N harvest index for N efficiency.

In conclusion, the results of this study show that both N-uptake efficiency and N-utilization efficiency contributed to improved performance of CIMMYT tropical mid-altitude maize cultivars under low-N conditions. Nitrogen uptake after anthesis was also more related to N efficiency than N uptake before anthesis.

CHAPTER 3

ROOT-SYSTEM SIZE, MINERAL NITROGEN DEPLETION AND LEAF TRAIT DIFFERENCES AMONG CONTRASTING NITROGEN-EFFICIENT TROPICAL MID-ALTITUDE MAIZE CULTIVARS UNDER DIFFERENT LEVELS OF NITROGEN

ABSTRACT

Root and leaf traits may be associated with N efficiency of crops. This study assessed differences in root-system size and leaf traits among contrasting N-efficient tropical mid-altitude maize cultivars from CIMMYT. Sixteen cultivars were evaluated under a range of N levels at Harare, Zimbabwe in 2003 and 2004, and Kiboko, Kenya in 2003. Root capacitance, leaf area, leaf senescence and leaf chlorophyll concentration were measured for all cultivars, while root-length density and mineral-N depletion within 0 – 90 cm soil depth were estimated for two cultivars differing in N efficiency. Significant differences ($P<0.01$) were observed among the cultivars for total root-system size and leaf traits. The N-efficient cultivars generally had greater root-length density in the subsoil (as estimated for two cultivars differing in N efficiency using the soil-core method), greater leaf longevity and higher leaf chlorophyll concentration compared with the N-inefficient cultivars. Also, the N-efficient cultivar depleted more mineral-N in the subsoil than the N-inefficient cultivar. However, total root-system size did not relate to N efficiency indicating that selection for improved performance under low-N did not increase total root-system size (as measured by capacitance) in tropical maize.

Key words: Chlorophyll, Leaf senescence, mineral-N, N efficiency, Root-length density, Root capacitance, *Zea mays* L.

INTRODUCTION

A breeding program aimed at developing varieties with higher N efficiency, above average grain yield under conditions of low N availability or suboptimal N supply (Graham, 1984; Sattelmacher et al., 1994), could alleviate environmental and economical problems associated with the use of inorganic fertilizers (van Beem and Smith, 1997). Different researchers reported differences for N efficiency among maize genotypes across a range of N levels (Kamprath et al., 1982; Moll et al., 1982; Wiesler et al., 2001; Horst et al., 2003; Bänziger et al., 2005). Grain yield is an important criterion for selecting for improved performance under low-N in maize but there are limitations to its use due to large genotype-by-environment interactions. There is a need to identify traits less prone to alterations by the environment (Sibale and Smith, 1997). Positive relationships have been reported between N efficiency and delayed leaf senescence, and leaf chlorophyll concentration (Bänziger et al., 1997; Mi et al., 2003; Horst et al., 2003), and between N efficiency and root-system size and morphology (O'Toole and Bland, 1987; Wiesler and Horst, 1993 and 1994b).

Horst et al. (2003) indicated that N-efficient cultivars under low-N were characterized by maintenance of N uptake during the reproductive growth phase through continued root growth, as well as greater leaf area duration, chlorophyll concentration and photosynthetic activity of the leaves. Wiesler et al. (2001) reported that yield of oilseed rape cultivars under conditions of low-N supply was correlated with N uptake during the reproductive growth phase and increased photosynthetic activity of leaves at the end of flowering. Similar results were reported by Mi et al. (2003) who found that stay-green hybrid maize was better in yield than an early senescing hybrid under N limited conditions.

Rajcan and Tollenaar (1999a, b) argued that if N uptake is maintained during grain filling, less N will be mobilized from vegetative organs, which may result in increased leaf area duration (delayed leaf senescence). Ma and Dwyer (1998) indicated that prolonged maintenance of green leaf area for photosynthate production during grain filling and the ability to take up available soil N later in grain filling are characteristics of maize hybrids with greater N efficiency. Increased grain yield under low-N combined with continued N uptake during grain filling and delayed leaf senescence were also found by Bänziger et al. (1999).

Morphological responses to N availability vary among genotypes (van Beem and Smith, 1997). Wiesler and Horst (1993, 1994a, b) reported significant differences among maize

cultivars in nitrate depletion, particularly in the subsoil, due to differences in root-length density in the subsoil at silking. The different capabilities of the cultivars to utilize nitrate particularly from the subsoil were positively correlated with shoot N uptake at maturity and root-length density in the subsoil layers at silking.

Kling et al. (1997) concluded that high yielding genotypes with increased grain filling under low-N conditions may develop fine and deep root systems in response to N stress and hence maintain N uptake and photosynthesis longer. They argued that, although a larger root system may compete for photosynthates and reduce maximum yield potential, reduced losses due to N stress, drought and lodging provide a net benefit in the lowland tropical environments of Africa where maize yields average only 1 ha^{-1}. However, Oikeh (1996), Oikeh et al. (1999) and van Beem and Smith (1997) reported that the size of the root system alone did not always relate well with grain yield (N efficiency) of maize cultivars.

CIMMYT has identified maize cultivars differing in N efficiency (Friesen et al., 2002; Bänziger et al., 2005). The objective of this study was to establish the relationship between root and leaf parameters and N efficiency.

MATERIALS AND METHODS

Germplasm, experimental site and trial management

The cultivars used, the experimental site, N levels, field experimental design and trial management practices were described in detail in Chapter 2. Briefly, sixteen contrasting N-efficient cultivars, including single-cross and three-way-cross hybrids were used in the study (Table 1).

The cultivars were tested in Zimbabwe (Harare) and Kenya (Kiboko) under three N levels, low, medium and high N at both sites resulting in nine environments: Harare 2003 low-N (Z03N1), Harare 2003 medium-N (Z03N2), Harare 2003 high-N (Z03N3), Kiboko low-N (K03N1), Kiboko medium-N (K03N2), Kiboko high-N (K03N3), Harare 2004 low-N (Z04N1), Harare 2004 medium-N (Z04N2) and Harare 2004 high-N (Z04N3). The two locations represented mid-altitude agro-ecologies of sub-Saharan Africa. The choice of N applications at each station was intended to create a range of relevant N levels, and depended on soil type, cropping history, fertilizer recommendation and experience of researchers at each station. A full description of the testing environments is given in Table 2. Nitrogen

applications were in split for high-N treatments (N3) but otherwise given solely at planting. Other agronomic management practices were applied uniformly for all the experiments based on the recommendations at each location. Alpha (0,1) lattice experimental designs with four replications (Patterson and Williams, 1976) were used for each experiment.

Table 1. Maize cultivars/hybrids used for the study.

No.	Cultivar (Hybrid)	Source	Performance under low-N
1	CML444/CML445//CML440	CIMMYT-Zimbabwe	Good
2	CML395/CML444//CML440	CIMMYT-Zimbabwe	Good
3	CML202/CML395//CML205	CIMMYT-Zimbabwe	Poor
4	SC515	Seed-CO-Zimbabwe	Poor
5	CML395/CML444//CML442	CIMMYT-Zimbabwe	Good
6	CML444/CML197//CML443	CIMMYT-Zimbabwe	Good
7	SC633	Seed-CO-Zimbabwe	Poor
8	CML181/CZL01005//CZL01006	CIMMYT-Zimbabwe	Best among QPM[1]
9	CML181/CML182//CML176	CIMMYT-Zimbabwe	Best among QPM[1]
10	CML144/(16304/6303Q)-B-6-1-3-3-B*6	CIMMYT-Mexico	Poor (QPM[1])
11	CML247//CML254	CIMMYT-Mexico	Good
12	CML78/CML373	CIMMYT-Mexico	Good
13	CML264/CML311//CML334	CIMMYT-Mexico	Poor
14	CML442/CML444//[MSRXPL9]C1F2-205-1(OSU23i)-1-1-X-1-X-B-B	CIMMYT-Kenya	Good
15	LPSC4F273-2-2-1-B-B-B/CML202//CML384	CIMMYT-Kenya	Good
16	CML312/CML247//CML78	CIMMYT-Kenya	Good

[1] Quality Protein Maize

Table 2. Fertilizer application (N1, N2, N3) and cropping history of the testing environments in Harare 2003 and 2004 (Z03, Z04) and Kiboko 2003 (K03).

Environment	Applied N (kg ha^{-1})	Cropping history and residue management
Z03N1	0	Depleted, residue removed
Z03N2	0	Previously fertilized, residue incorporated
Z03N3	168	Previously fertilized, residue incorporated
K03N1	18	Depleted, residue removed
K03N2	18	Previously fertilized, residue removed
K03N3	90	Previously fertilized, residue incorporated
Z04N1	0	Depleted, residue removed
Z04N2	0	Previously not fertilized, residue partially incorporated
Z04N3	168	Previously fertilized, residue incorporated

Root parameters

Total root-system size of the plant was estimated using electrical root capacitance (a BK Precision 810A; instrument set at the 200 nF level) (van Beem and Smith, 1997). Measurements were recorded at anthesis (RC1) and two weeks after anthesis (RC2). The measurements were recorded between 07:00 and 10:00 am with the soil at field capacity (i.e., after rain and/or irrigation). A copper probe was inserted into the soil between the centre two rows. The positive and negative electrodes of the root capacitance meter were attached, respectively, to the copper probe and the stem of the plant at 6 cm above the soil surface (van Beem and Smith, 1997, 1998; Manske et al., 2001). Root capacitance readings were recorded for six alternate plants in the two centre rows and the average was calculated for each plot.

Root samples were taken at anthesis for two cultivars (cultivar 6 and 7) differing in N efficiency using a manual soil auger (Regular Auger, 5.2 cm internal diameter, Forestry Suppliers, INC.). The samples were taken at intervals of 0 – 30 cm, 30 – 60 cm and 60 – 90 cm between two plants within a row for all replications. Three samples were taken at each soil layer and mixed for root length estimation in each layer and plot. At Harare 2003, the samples were kept in a freezer before the soil was washed from the roots using tap water and a US standard 425 µm testing sieve. The soil/root samples were soaked in water over-night before washing. For Kiboko and Harare 2004, samples were not kept in the freezer but were immediately soaked in water to disperse the soil which was then washed from the roots

following the same procedure. The clean roots were collected and root-length density was estimated for each layer using the line intersect method of Tennant (1975).

Mineral-N (NO_3^--N and NH_4^+-N) analysis in the soil

Soil samples were taken up to a depth of 90 cm at the intervals of 0 – 30 cm, 30 – 60 cm and 60 – 90 cm in the plots of two cultivars (cultivar 6 and 7) at planting, prior to the second N application, at 50 % anthesis and at physiological maturity for all experiments. The soil samples were taken using a Tube Sampler Soil Probe (Model L, 1.9 cm internal diameter, Forestry Suppliers, INC.) at Harare Station while a Puerckhauer Type soil sampler and hammer (Hornetz et al., 2000) was used at Kiboko. Six samples were taken for each layer and mixed for mineral-N (NH_4^+ and NO_3^-) determination in each layer and plot. The samples were immediately put into a coolbox in the field, and then transferred, within 2 hours, to a freezer (at $\leq$ -10^{o}C) for storage until analysis. Samples were removed from the freezer 12 hours before extraction and thawed at room temperature. Twenty-five grams of soil was taken from each sample, shaken in 100 ml 0.5 M KCl for one hour, and filtered through filter paper (Whatman filter paper, number 5). The samples from Kiboko (Kenya) were extracted at the ICRAF Laboratory in Nairobi. Then the frozen filtrates were packed in a cool box with dry ice and sent to the Institute of Plant Nutrition Laboratory, University of Hannover, Germany, where they were analyzed for mineral NH_4^+ and NO_3^- using a Technicon Auto Analyzer II (BRAN and LÜBBE, Hamburg, Germany). The samples from Harare 2004 were extracted at the University of Zimbabwe and analyzed at the Institute of Plant Nutrition, University of Hannover following the same procedure. Sub-samples were also taken from each soil sample for determination of gravimetric moisture content. The bulk density of the soil was 1.4 g cm^{-3}, 1.5 g cm^{-3} and 1.5 g cm^{-3} for 0 – 30 cm, 30 – 60 cm and 60 – 90 cm depth, respectively at Harare, while the bulk density of the soil was 1.5 g cm^{-3}, 1.6 g cm^{-3} and 1.6 g cm^{-3} for 0 – 30 cm, 30 – 60 cm and 60 – 90 cm depth, respectively, at Kiboko. Mineral-N per hectare for each layer was calculated following procedures described in ICRAF (1994).

Leaf parameters

Three weeks after planting, the fifth leaf was marked on six alternate plants in each plot. Later the mark was shifted to the tenth leaf. Marked plants were used to record total number of leaves, number of green leaves, chlorophyll concentration, and leaf area at anthesis. Total number of leaves (LN) and leaf area were determined at anthesis only. The number of green

leaves (GL) and leaf chlorophyll concentration (CHL) were determined at anthesis (GL1, CHL1), and 14 (GL2, CHL2) and 28 (GL3, CHL3) days after anthesis. A leaf was regarded as green when at least 50% of the leaf area was green. The ratio between green leaf count at each stage and total leaf number (GL1%, GL2% and GL3%) was calculated. Chlorophyll content was measured using a SPAD-502 chlorophyll meter (Minolta, Japan) and averaging recordings from alternate leaves, from the bottom to the top of the six plants. The same leaves were used for leaf area determination. Leaf area was calculated using the formula maximum width x length x 0.75, and then leaf area per plant and leaf area index (LAI) were calculated.

Leaf senescence was estimated on a scale of 0 (0% of the plot leaf area senescent) to 10 (100% of the plot leaf area senescent) at anthesis (SEN1) and 14 (SEN2), 28 (SEN3) and 42 (SEN4) days after anthesis. SEN4 was recorded at Harare 2004 only.

Statistical analyses

Within each experiment, lattice-adjusted cultivar means were calculated using the PROC MIXED procedure of SAS (SAS, 2001), with cultivars as a fixed factor and replicate and incomplete blocks within replicates as random factors. Across-experiment analysis was conducted from lattice adjusted cultivar means using cultivars as fixed factor and experiment as random factor (Bänziger et al., 2002). Although three N levels were used at both sites, N availability in the soil differed across the sites and seasons. Therefore, each N level was considered as different environment in across-experiment analysis (Bänziger et al., 1999; Bänziger et al., 2002). Differences in root-length density and mineral-N depletion at each layer (0 – 30 cm, 30 – 60 cm and 60 – 90 cm) between the two contrasting N-efficient cultivars were tested using pair-wise t-tests. Correlations of N uptake in the aboveground biomass with total root-system size and chlorophyll content were calculated. Similarly, the correlation between and N-utilization efficiency (ratio of dry grain and total N in the aboveground biomass) and leaf senescence under low-N conditions was calculated.

RESULTS

Significant cultivar differences ($P<0.01$) were recorded for most of the traits. The cultivar-by-environment interaction was also significant for most traits indicating different ranking of cultivars in different environments (Table 3). Grain yield performance across the N environments was discussed in detail in Chapter 2. Low-N stress was most severe at Harare

low-N conditions (Z03N1 and Z04N1). The N-efficient cultivars had higher grain yields than the N-inefficient ones under severe low-N stress (Figure 1). In the following section the relations between grain yield and root-system size, mineral-N depletion and leaf traits are presented.

Table 3. Analysis of variance for root capacitance at anthesis (RC1, nF) and 14 days after anthesis (RC2, nF), leaf senescence score at anthesis (SEN1), 14 days after anthesis (SEN2), 28 days after anthesis (SEN3) and 42 days after anthesis (SEN4), chlorophyll concentration at anthesis (CHL1), 14 days after anthesis (CHL2), and 28 days after anthesis (CHL3), stem circumference (CIRC, cm) and grain yield (GY, t ha^{-1}) of maize cultivars differing in N efficiency.

	RC1	RC2	LAI	SEN1	SEN2	SEN3	SEN4	CHL1	CHL2	CHL3	CIRC	GY (t ha^{-1})
Mean	43.4	35.9	3.9	0.6	2.0	2.7	3.1	43.0	42.7	41.1	7.2	7.79
CV%	16.8	15.9	10.0	53.5	27.1	22.0	15.0	7.8	8.8	9.8	6.3	14.05
G	**	**	**	**	**	**	ns	**	**	**	**	**
E	**	**	**	**	**	**	**	**	**	**	**	**
G x E	**	**	**	**	**	**	**	**	**	**	ns	**

**, ns- significant at $P < 0.01$ and non-significant, respectively.

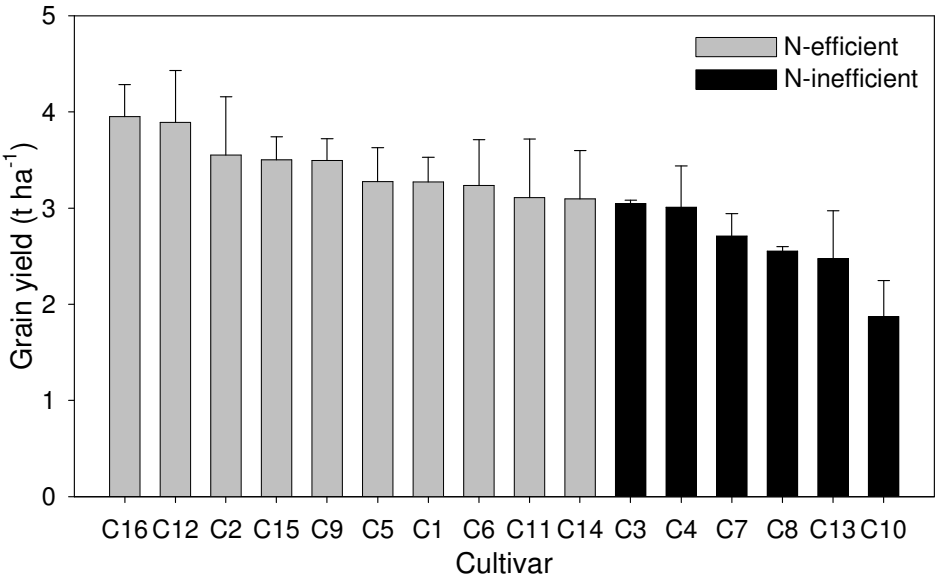

Figure 1. Mean grain yield (mean ± SE, n = 2) of efficient and inefficient cultivars at Harare low-N in 2003 and 2004 (Z03N1 and Z04N1).

Total root-system size and root-length density

Generally, a larger root mass was measured at flowering than two weeks after flowering for all cultivars in all environments. The root-system size increased with soil N level (data not shown). No significant positive correlation between total root-system size (RC1 and RC2) and

grain yield was observed in any of the environments; occasionally, even negative correlations were observed (Table 4). Similarly, no significant correlation existed between total aboveground biomass at flowering (BIOF) and grain yield, or stem circumference and grain yield (Table 4). Moreover, the associations between N uptake in the total aboveground biomass at physiological maturity and root capacitance readings (RC1 and RC2) were low (r = -0.23 to 0.13) under severe N stress, indicating no relationship between total root-system size and total amount of mineral-N acquired under N-deficient conditions. The N-inefficient cultivar 7 was among the cultivars with high root capacitance reading while the N-efficient cultivar 6 had a lower root capacitance reading than cultivar 7 (at Z03N1 and Z04N1; data not shown).

Genotype-by-environment interactions were non-significant for root-length density at any soil depth (data not shown). The root-length density progressively declined for both cultivars with soil depth. Generally root-length densities of less than 1 cm cm^{-3} were recorded in all layers. Pair-wise t-tests showed significant genotypic differences between the cultivars 6 and 7 in root-length density at 0 – 30 cm ($P<0.01$) and 60 – 90 cm ($P<0.1$) soil depth. The two cultivars did not differ significantly at 30 – 60 cm soil depth. The N-efficient cultivar 6 had a greater root-length density than the N-inefficient cultivar 7 in the 0 – 30 cm and 60 – 90 cm soil layers (Figure 2) indicating that the core (soil) and the electrical capacitance methods of root-system size estimation gave different results.

Table 4. Simple linear correlations of root capacitance at anthesis (RC1) and 14 days after anthesis (RC2), stem circumference (CIRC) and total biomass at anthesis (BIOF) with grain yield in different environments and mean grain yield (GY, t ha^{-1}) at each environment.

	Environment								
Trait	Z03N1	Z03N2	Z03N3	K03N1	K03N2	K03N3	Z04N1	Z04N2	Z04N3
RC1	-0.39	-0.53*	0.22	0.12	0.34	0.34	-0.34	-0.23	0.23
RC2	-0.51*	-0.35	0.24	0.03	0.39	-0.09	-0.31	-0.25	0.12
CIRC	-0.23	-0.34	0.18	0.10	0.31	0.24	-0.44^{+}	-0.09	0.05
BIOF	-0.01	0.34	0.24	-0.42	-0.05	-0.62*	-0.17	0.07	0.26
GY (t ha^{-1})	2.89	8.35	12.66	6.32	8.39	10.57	3.37	6.40	11.13

$^{+}$,*, **- Significant at $P<0.1$, 0.05 and 0.01, respectively (n = 16).

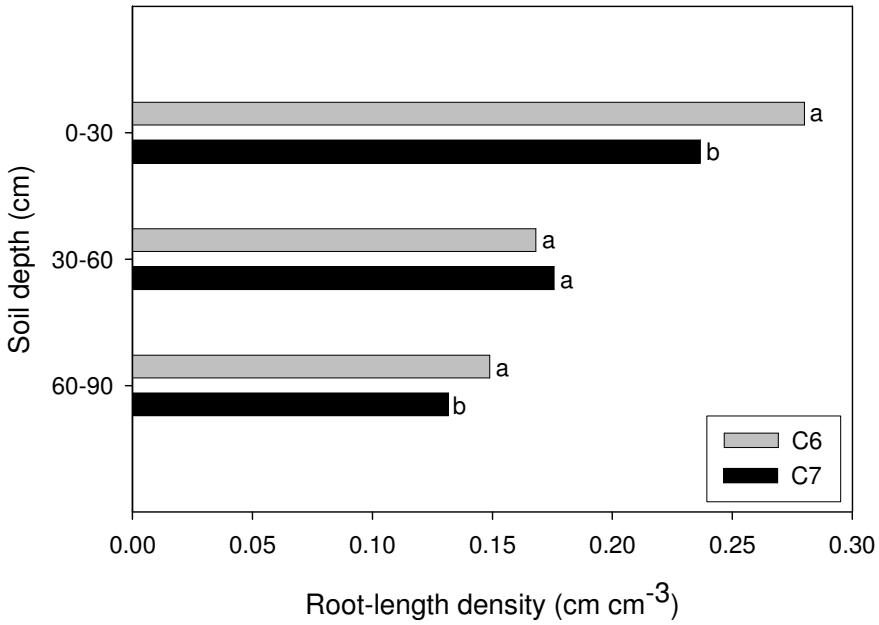

Figure 2. Root-length densities (cm cm^{-3}) across environments at different soil profiles at anthesis for an efficient cultivar (6) and an inefficient cultivar (7). Pair-wise t-tests for the two cultivars were significant at 0 – 30 cm ($P<0.01$) and 60 – 90 cm ($P<0.1$) but not significant at 30 – 60 cm.

Mineral-N depletion in the soil

Soil mineral-N contents in the plots of cultivars 6 and 7 were highest at N3. Mineral-N was present as NH_4^+ and NO_3^- (data not shown). Total mineral-N in the soil profile (0 – 90 cm depth) at planting averaged 84, 80 and 119 kg N ha^{-1} for Z04N1, Z04N2 and Z04N3 and 39, 88 and 61 kg N ha^{-1} for K03N1, K03N2 and K03N3, respectively. Mineral-N in the soil at 40 days after planting averaged 54, 83 and 103 kg N ha^{-1} for Z04N1, Z04N2 and Z04N3 and 15, 34 and 53 kg N ha^{-1} for K03N1, K03N2 and K03N3, respectively. The distribution of mineral-N in the soil layers across the experiments averaged 34%, 35% and 31% (average at planting and 40 days after planting) for 0 – 30 cm, 30 – 60 cm and 60 – 90 cm, respectively, indicating that some leaching occurred. More mineral-N was detected in the soils at Harare than at Kiboko indicating differences in soil type and management of the soils over the years. However, the crop was more stressed in Z04N1 than K03N1 (Table 4; GY). Spot application of 18 kg N ha^{-1} at planting and higher N mineralization might have contributed to better performance of the crop in K03N1.

The N-efficient cultivar 6 depleted significantly ($P<0.1$) more mineral-N in the subsoil (60 – 90 cm) than the N-inefficient cultivar 7 across the environments. This is consistent with the greater root-length densities in the subsoil layer (Figure 3). No significant differences between the two cultivars were observed for mineral-N depletion at 0 – 60 cm.

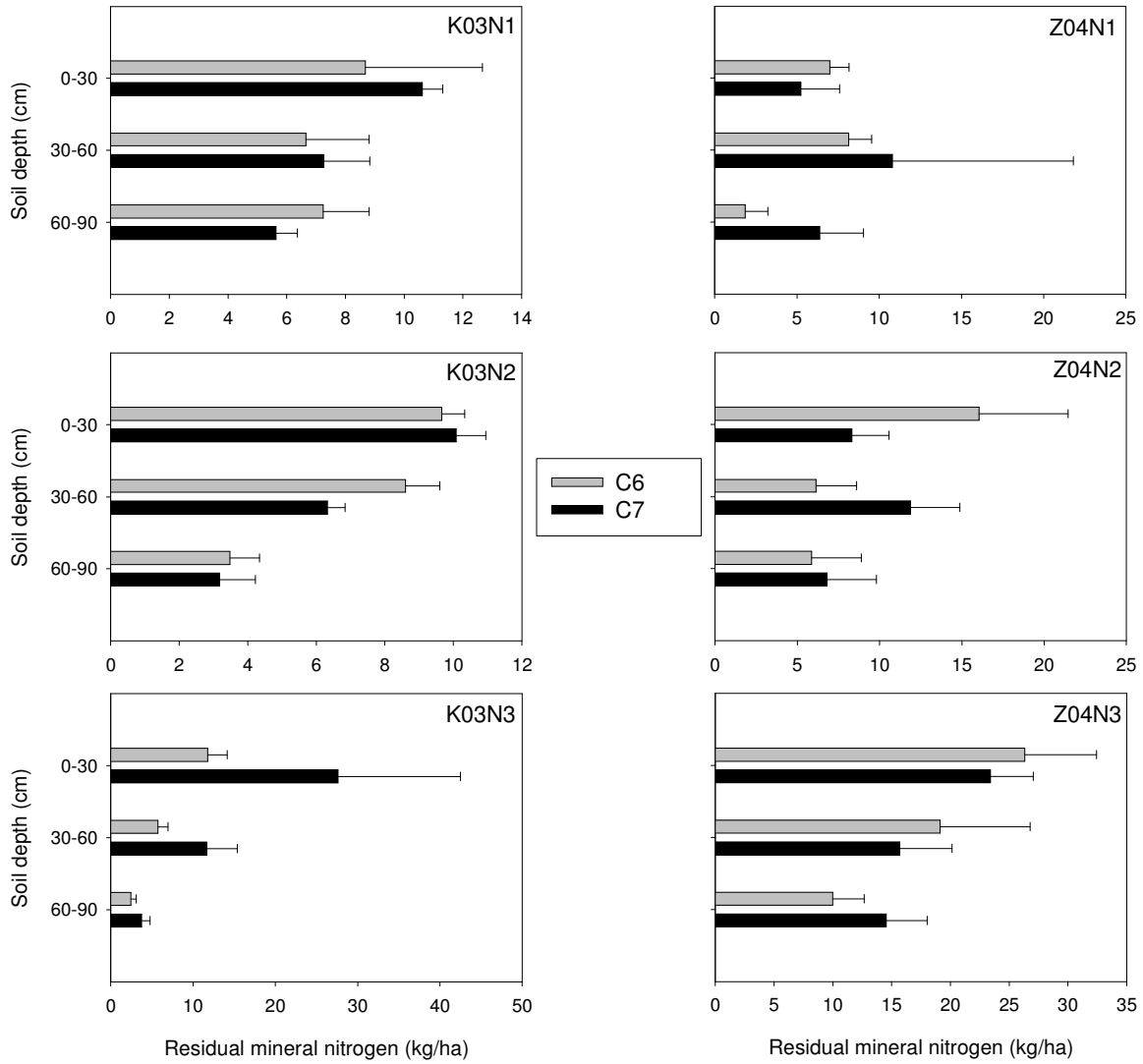

Figure 3. Residual mineral-N (mean ± SE, n = 4) in the soil at physiological maturity under two contrasting N-efficient cultivars at different N environments in Kenya, Kiboko in 2003 and Zimbabwe, Harare 2004. Pair-wise t-tests for the two cultivars across the environments (six environments) showed no significant difference at 0 – 60 cm, but significant difference at 60 – 90 cm ($P<0.1$).

Leaf characteristics

Significant genotypic differences ($P<0.01$) were observed for most of the leaf traits studied (Table 3). The correlation between grain yield and leaf number (LN), leaf area index (LAI), and number of green leaves (GL) was weak. However, in some of the environments and observation dates, positive correlations between grain yield and percent of green leaves (GL%) were observed. Also, correlations between leaf senescence and grain yield were negative in most cases while correlations between chlorophyll concentration and grain yield were mostly positive (Table 5). The association between grain yield and leaf senescence score became more negative under low-N conditions during the grain filling period indicating that

stay-green cultivars were more efficient than early senescing cultivars. Similarly, the relationship between N-utilization efficiency and leaf senescence at 28 days after anthesis was negative (r = -0.40) under Harare low-N conditions (Z03N1 and Z04N1) indicating the importance of leaf longevity for N-utilization efficiency.

All cultivars showed senescence under low-N conditions but the cultivars differed in the progress of the senescence during grain filling. Generally leaf senescence score was high for the N-inefficient cultivars (Figure 4). The N-inefficient cultivar 7 had the highest average leaf senescence score. Low leaf senescence scores were recorded for the most N-efficient cultivars, cultivar 16 and cultivar 12, confirming the negative relationship between leaf senescence and grain yield. However, some N-efficient cultivars had moderate leaf senescence scores (Figure 4).

Chlorophyll concentration decreased with N level. Chlorophyll concentration decreased between anthesis (CHL1) and 28 days after anthesis (CHL3) at low-N, but was relatively unchanged at high-N (data not shown). Generally the N-inefficient cultivars had lower chlorophyll concentrations than the N-efficient cultivars (Table 5 and Figure 5). The lowest mean chlorophyll concentration at low-N was measured for the N-inefficient cultivar 10. The relatively N-efficient cultivar 6 was among the cultivars with high leaf chlorophyll content. However, some N-efficient cultivars had a medium chlorophyll concentration. For example, cultivar 12 had a comparatively low chlorophyll concentration indicating other internal factors than a high chlorophyll concentration contributed to the N efficiency of this cultivar (Figure 5). The difference in chlorophyll concentration at 28 days after anthesis (CHL3) was mainly dependent on chlorophyll concentration at anthesis (CHL1). However, differences in chlorophyll loss were also observed among the cultivars.

Table 5. Simple linear correlations of total leaf number (LN), number of green leaves at anthesis (GL1), 14 days after anthesis (GL2) and 28 days after anthesis (GL3), percent of green leaves (GL1%, GL2% and GL3%), leaf senescence at anthesis (SEN1), 14 days after anthesis (SEN2), 28 days after anthesis (SEN3) and 42 days after anthesis (SEN4) and chlorophyll concentration at anthesis (CHL1), 14 days after anthesis (CHL2) and 28 days after anthesis (CHL3) with grain yield in different environments and mean grain yield (GY, t ha^{-1}) at each environment.

	Environment								
Trait	Z03N1	Z03N2	Z03N3	K03N1	K03N2	K03N3	Z04N1	Z04N2	Z04N3
LN	-0.20	-0.38	0.07	-0.20	-0.09	-0.07	0.06	-0.23	-0.07
LAI	0.18	0.14	-0.08	-0.05	0.38	-0.01	-0.13	0.23	0.20
GL1	0.53*	-0.01	0.19	0.05	0.31	0.08	0.17	0.22	0.36
GL2	-0.01	-0.34	0.06	0.13	0.51*	-0.06	0.05	0.02	0.32
GL3	-0.07	0.03	0.18	0.16	0.63**	0.38	0.11	0.35	0.28
GL1%	0.69**	0.31	0.09	0.20	0.48	0.16	0.10	0.50*	0.44^{+}
GL2%	0.13	-0.10	-0.02	0.24	0.62*	0.01	0.04	0.16	0.44^{+}
GL3%	0.07	0.37	0.06	0.26	0.74**	0.49^{+}	0.08	0.51*	0.39
SEN1	-0.47^{+}	-0.22	-0.33	-0.26	-0.34	-0.48^{+}	0.13	0.01	-0.41
SEN2	-0.60*	0.00	-0.02	-0.38	-0.81**	-0.56*	-0.48^{+}	-0.36	-0.54*
SEN3	-0.74**	-0.29	-0.21	-0.50*	-0.86**	-0.28	-0.44^{+}	-0.52*	-0.46+
SEN4							-0.52*	-0.34	-0.66**
CHL1	0.80**	0.59*	0.43^{+}	0.39	0.68**	0.27	0.35	0.52*	0.56*
CHL2	0.80**	0.42	0.52*	0.51*	0.64**	0.40	0.60*	0.68**	0.63**
CHL3	0.68**	0.64**	0.55*	0.53*	0.71**	0.49^{+}	0.35	0.84**	0.55*
GY (t ha^{-1})	2.89	8.35	12.66	6.32	8.39	10.57	3.37	6.40	11.13

$^{+}$,*, **- Significant at P<0.1, 0.05 and 0.01, respectively (n = 16).

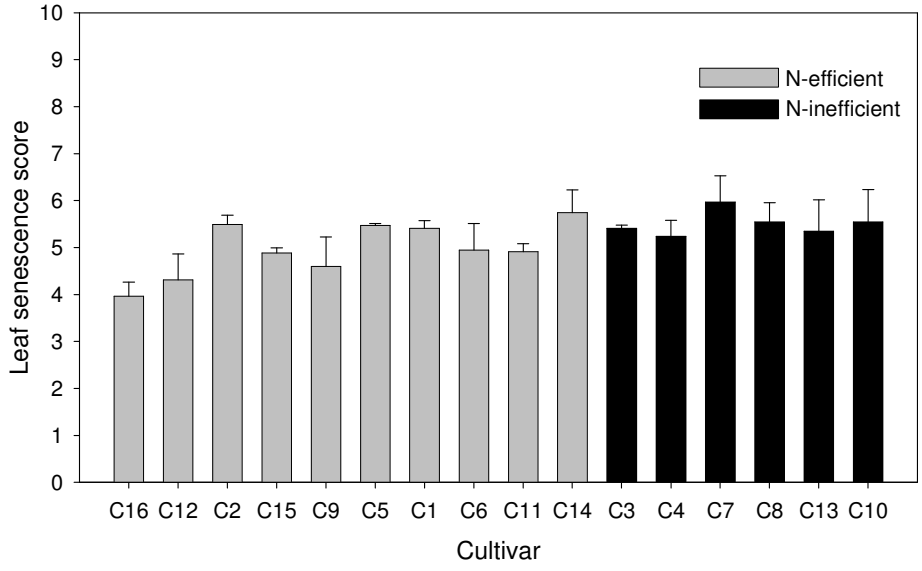

Figure 4. Leaf senescence score (mean ± SE, n = 2) (SEN3, 28 days after anthesis) for maize cultivars differing in N efficiency across low-N stress conditions of Harare 2003 and 2004 (Z03N1 and Z04N1).

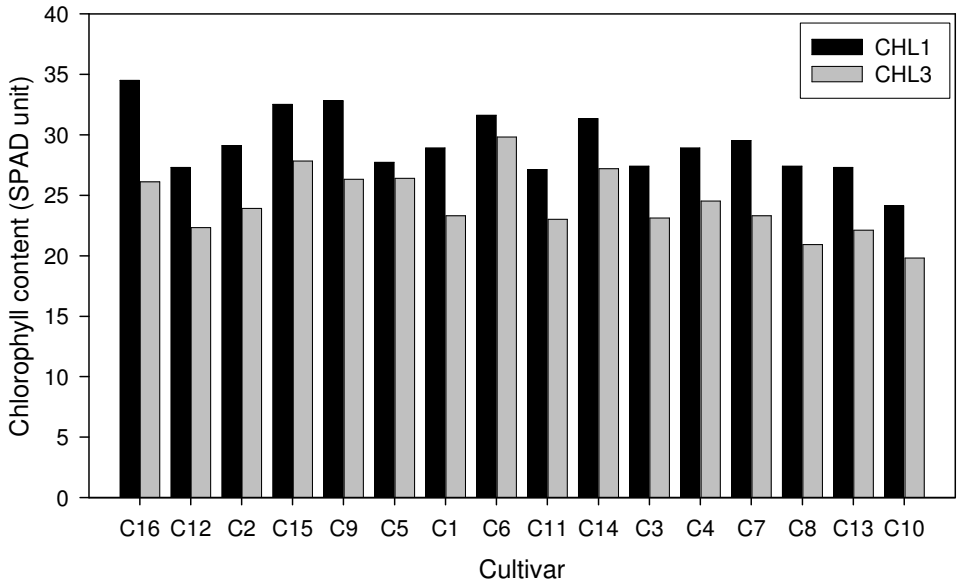

Figure 5. Mean chlorophyll concentrations across low-N stress conditions of Harare 2003 and 2004 (Z03N1 and Z04N1) at anthesis (CHL1) and 28 days after anthesis (CHL3) in cultivars differing in N efficiency.

DISCUSSION

Various root and leaf traits have been investigated for their relationship with N efficiency in maize (Wiesler and Horst, 1994 a, b; Oikeh, 1996; van Beem and Smith, 1997; Bänziger et al., 1999). Insignificant and negative correlations between grain yield and total root-system size, as measured by electrical capacitance at flowering and during grain filling period, showed no relationship between root-system size and N efficiency in our study. A similar result was reported in temperate maize (van Beem and Smith, 1997).

Spatial distribution of the roots, and in particular root-length density in the subsoil, may have been more important than total root-system size. For the two cultivars assessed, the N-efficient cultivar 6 developed relatively more roots in the subsoil (60 – 90 cm) than the N-inefficient cultivar 7, and depleted the soil more of mineral-N. Since the N-inefficient cultivar 7 was among the cultivars with a high total root-system size, cultivar 7 might have developed more horizontally distributed roots whereas the N-efficient cultivar 6 might have developed more vertically oriented fine roots and exploited more mineral-N in soil layers deeper than 60 cm.

The root-length density estimated in this study was less than previously reported in temperate maize (Wiesler and Horst, 1994 a, b). However, a similar quantity of root-length density was reported in the subsoil in tropical maize (Heuberger, 1998). This may imply that differences

in root-length densities in the subsoil between cultivars may be of greater importance in tropical than in temperate maize.

Wiesler and Horst (1993, 1994b) studied root-length density and nitrate depletion of temperate maize at 0 – 150 cm soil depth. Consistent with our results, they reported significant cultivar differences in root-length density and nitrate depletion in the subsoil whereas no significant differences were found in the topsoil. The authors also found that N depletion after flowering was mainly restricted to the soil layers below 60 cm and that significant difference existed among genotypes in subsoil depletion. Oikeh et al. (1999) reported similar results in the tropics when they compared an N-efficient maize cultivar with a local sorghum cultivar.

It may not be correct to assume that all mineral-N depletion in the soil was due to plant uptake. Mineral-N availability in the soil is affected by biological immobilization and mineralization, clay mineral and organic matter fixation, gaseous losses of N through denitrification, and leaching of mineral-N beyond the rooting zone (Wiesler, 1997). The latter point may also imply that cultivars with active post-flowering root growth may exploit more leached N from deeper soil depths than investigated in this study and that this may be one mechanism for increased N efficiency.

Research results indicate that, although leaf senescence initiation before physiological maturity is subject to environmental factors, it is also under genetic regulation (Lafitte and Edmeades, 1994a; Gan and Amasino, 1997; Noh et al., 1999; Paponov and Engels, 2003). The genotypic differences in leaf senescence and a negative relationship between leaf senescence and N efficiency reported in both temperate (Wiesler et al., 2001) and tropical maize (Lafitte and Edmeades, 1994a; Bänziger et al., 1997; Bänziger et al., 1999) are confirmed by the results of the present study. Efficient cultivars were generally characterized by a higher longevity of green leaves during the grain filling period. However, some relatively N-efficient cultivars, such as cultivar 1, 2 or 5, showed moderate leaf senescence. This may indicate that leaf senescence may not be used as single selection criteria for improving performance under low-N conditions. Bänziger and Lafitte (1997) reported that when grain yield data was combined with secondary traits selection efficiency was improved by 14% on average over selection for grain yield alone. However, they indicated that direct selection for grain yield was superior to selection for single secondary trait.

Negative relationships (r = -0.80** in 2003 and r = -0.40 in 2004) were observed between total N uptake in the aboveground biomass at physiological maturity and leaf senescence score, and between leaf senescence score at 28 days after anthesis and N uptake after anthesis (r = -0.67** in 2003 and r = -0.46^{+} in 2004) under Harare severe low-N stresses. This indicates that cultivars that continue to absorb N from the soil after anthesis may have longer leaf longevity than cultivars that do not, and that better post-anthesis root growth, leaf longevity and continued N uptake are related.

Paponov and Engels (2003) argued that higher photosynthesis at low-N could be attributed not only to a higher N allocation to the leaves or less N remobilization from the leaves to the grains, but also to higher utilization of internal N for photosynthesis. This might have contributed for improved performance of some of the cultivars at the low-N conditions in this study. For instance, the N-efficient cultivar 12 had a medium chlorophyll content indicating that higher utilization of internal N for photosynthesis might have contributed to the better performance of this cultivar under low-N conditions. Moreover, chlorophyll content in general was positively correlated with N efficiency under low-N stress, implying that the N-efficient cultivars had relatively higher chlorophyll concentrations in the leaves compared to the N-inefficient cultivars. Similar results were reported by Wiesler et al. (2001) and Sibale and Smith (1997). The positive relationship between N uptake after anthesis and chlorophyll content at 14 days after anthesis (r = 0.63**, in 2003 and r = 0.40 in 2004 at Harare low-N conditions) indicates that chlorophyll content after flowering may be influenced by active root growth and N uptake.

We conclude that root-length density in the subsoil seems to be more important for N efficiency than total root-system size. Active root growth and continued N uptake (as estimated for two contrasting N-efficient cultivars), enhanced leaf longevity and thus increased photosynthetic capacity during flowering and grain filling seem to significantly contribute to N efficiency of cultivars.

CHAPTER 4

NITROGEN EFFICIENCY AND DRY MATTER PARTITIONING IN TROPICAL MID-ALTITUDE MAIZE (*ZEA MAYS* L.) GERMPLASM UNDER DIFFERENT LEVELS OF NITROGEN STRESS

ABSTRACT

Under limited nitrogen (N) supply, grain yield of maize (*Zea mays* L.) can be limited by dry matter partitioning to the grain. In this study the relationships between N efficiency and dry matter partitioning and yield components were assessed. Sixteen contrasting N-efficient cultivars were evaluated under a range of N levels at Harare, Zimbabwe, in 2003 and 2004 and Kiboko, Kenya, in 2003 for harvest index, ears per plant, anthesis-silking interval, kernel number per ear, kernel weight, kernels per row, kernel row number and grain carbon/nitrogen (C/N) ratio. Yield components and dry matter partitioning varied significantly ($P<0.01$) among the cultivars. Harvest index was higher under high-N than low-N conditions, ranging from 16% to 37% at Harare under severe low-N stress to 30% to 52% at Kiboko under high-N conditions. There was also considerable genetic variability under all N conditions and a strong relationship between N efficiency and dry matter partitioning. Significant cultivar-by-environment interaction indicated that increasing harvest index under high-N conditions may not increase harvest index under low-N conditions. The N-efficient cultivars were characterized by a reduced anthesis-silking interval, higher dry matter production during grain filling, higher kernel number and relatively higher grain C/N ratio under limited N supply. This may imply a higher photoassimilate supply in the N-efficient cultivars during and after flowering under low-N conditions.

Key words: Grain C/N ratio, Harvest index, N efficiency, Yield components, *Zea mays* L.

INTRODUCTION

Inefficient transfer of assimilates to the ear sink limits yield in most indigenous tropical maize germplasm (Benti, 1988; Benti et al., 1993). Competition for assimilates may also exist between ear and stem (Edmeades et al., 2000). Maize breeders have explored various ways to shorten tropical maize and increase harvest index and grain yield. Since the major dwarfing genes in maize are associated with some undesirable morphological traits, the breeders have usually employed recurrent selection to achieve shorter plant height within high yielding adapted germplasm (Johnson et al., 1986; Dowswell et al., 1996). Progress has also been achieved in identifying cultivars with a better harvest index under high fertility conditions (Dowswell et al., 1996). However, the majority of farmers in tropical environments, particularly in sub-Saharan Africa, produce maize under low soil fertility conditions (Lafitte and Edmeades, 1994a; Bänziger et al., 1997; Zambezi and Mwambula, 1997; Kumwenda et al., 1997; Bänziger and Meyer, 2002; Bänziger et al., 2005). Cultivars with better harvest index under high-N conditions may fail to give better grain yield under the poor soil fertility conditions of farmer fields (Bänziger et al., 2000).

There is no doubt that significant changes in the partitioning of photosynthates in crop plants, expressed as harvest index, have been an important aspect of improving grain yield (Sinclair, 1998), under both adequate and N-limited conditions. Rajcan and Tollenaar (1999a) reported that greater dry matter accumulation and partitioning during grain filling contributed to the better performance of a newly developed hybrid compared to an old hybrid under low-N stress. Similarly, after evaluating maize populations selected for tolerance to mid-season drought under a range of N levels, Bänziger et al. (2002) reported that decreased ear and kernel abortion and increased assimilate supply during grain filling contributed to N efficiency. Bänziger et al. (1997) and Edmeades et al. (2000) also indicated that selection for a short anthesis-silking interval, an indicator of partitioning of currently formed assimilates to the ear at flowering, contributed significantly to improved grain yield (N efficiency) under low-N.

CIMMYT has identified cultivars with improved grain yield performance under low-N conditions (Friesen et al., 2002; Bänziger et al., 2005). However, changes made in assimilate partitioning and traits related to assimilate partitioning in CIMMYT cultivars selected for N efficiency have not been well documented. Therefore, the objective of this study was to assess

changes made in traits related to partitioning of assimilates among contrasting N-efficient cultivars and their relation with N efficiency.

MATERIALS AND METHODS

The cultivars used in the experiment, description of experimental sites, N levels, field experimental design and trial management practices were presented in detail in Chapter 2. Briefly, sixteen contrasting N-efficient cultivars from CIMMYT and Seed-Co International were evaluated (Table 1). The cultivars were tested under three N levels (low, medium and high N) at two sites resulting in nine environments (Table 2). The sites represent mid-altitude environments in sub-Saharan Africa. The trials were conducted at Kiboko, Kenya (2^{o}10'S and 37^{o}40'E, 975 meter above sea level) in the rainy season (April-August) of 2003 and Harare, Zimbabwe (17^{o}49'S, 31^{o}1'E and 1478 m above sea level) in the summer seasons (Nov.-April) of 2003 and 2004. The trials were irrigated to field capacity at planting using sprinkler irrigation. A second irrigation of 20 – 30 mm was applied 6 – 7 days after planting to facilitate germination. Thereafter, trials were irrigated to field capacity whenever soil moisture was less than 40% of field capacity. The trials were kept free of pests using pesticides when needed.

The experimental designs were alpha (0,1) lattice designs (Patterson and Williams, 1976) with incomplete block sizes of four plots and four replications. A plot size of 4 m length x 4.5 m width with six rows for each entry was used. Spacing was 0.75 m and 0.25 m between rows and plants, respectively. In each plot 68 plants (53,333 plants per hectare) were maintained after thinning. Anthesis date (AD) for each plot was recorded when 50% of the plants in the two central rows shed pollen. On the following day, twelve plants were harvested from an area of 2.25 m^2 in the central four rows. An area of 5.65 m^2, corresponding to 32 plants in the central four rows, was also harvested immediately after physiological maturity. During both harvests, two border plants in each row were excluded to avoid border effects. Three and eight representative plants were chopped at anthesis and harvest, respectively, for dry matter determination and N analysis. A quarter of the harvested cobs (after shelling) was also chopped with the sample plants at harvest. Homogenized sub-samples of stover and grain were taken, weighed, oven dried and used for stover and grain dry matter determination, respectively. All biomass was dried to constant weight at 80^{o}C in an oven for 72 hours before weighing. The ground stover and grain samples were analyzed for carbon (C) and N at the

Institute of Plant Nutrition, University of Hannover, Germany, using a CNS analyzer (Vario EL, Elementar Analysis Systems, Hanau, Germany).

Table 1. Maize cultivars/hybrids used for the study.

No.	Cultivar (Hybrid)	Source	Performance under low-N
1	CML444/CML445//CML440	CIMMYT-Zimbabwe	Good
2	CML395/CML444//CML440	CIMMYT-Zimbabwe	Good
3	CML202/CML395//CML205	CIMMYT-Zimbabwe	Poor
4	SC515	Seed-CO-Zimbabwe	Poor
5	CML395/CML444//CML442	CIMMYT-Zimbabwe	Good
6	CML444/CML197//CML443	CIMMYT-Zimbabwe	Good
7	SC633	Seed-CO-Zimbabwe	Poor
8	CML181/CZL01005//CZL01006	CIMMYT-Zimbabwe	Best among QPM[1]
9	CML181/CML182//CML176	CIMMYT-Zimbabwe	Best among QPM[1]
10	CML144/(16304/6303Q)-B-6-1-3-3-B*6	CIMMYT-Mexico	Poor (QPM[1])
11	CML247//CML254	CIMMYT-Mexico	Good
12	CML78/CML373	CIMMYT-Mexico	Good
13	CML264/CML311//CML334	CIMMYT-Mexico	Poor
14	CML442/CML444//[MSRXPL9]C1F2-205-1(OSU23i)-1-1-X-1-X-B-B	CIMMYT-Kenya	Good
15	LPSC4F273-2-2-1-B-B-B/CML202//CML384	CIMMYT-Kenya	Good
16	CML312/CML247//CML78	CIMMYT-Kenya	Good

[1] Quality Protein Maize

Aboveground biomass at anthesis (BIOF), aboveground biomass at physiological maturity (BIOH), dry matter accumulation after anthesis (BIOFH), harvest index (HI, ratio between grain yield and total aboveground biomass), grain C/N ratio (ratio between C and N in the grain), ears per plant (EPP, ratio of ears with at least one kernel to stand count at harvest) and anthesis-silking interval (ASI, days between 50% anthesis and 50% silking) were calculated. Similarly, kernel number per ear (KN), kernel weight (KW), kernels per row (KPR) and kernel row number (KRN) were determined. Number of rows per ear was counted for six representative ears. For determination of grain yield, all ears from the harvested area were shelled and grain weight and moisture percentage were recorded. Grain yield was adjusted to 12.5% moisture.

Table 2. Fertilizer application (N1, N2, N3) and cropping history of the testing environments in Harare 2003 and 2004 (Z03, Z04) and Kiboko 2003 (K03).

Environment	Applied N (kg ha^{-1})	Cropping history and residue management
Z03N1	0	Depleted, residue removed
Z03N2	0	Previously fertilized, residue incorporated
Z03N3	168	Previously fertilized, residue incorporated
K03N1	18	Depleted, residue removed
K03N2	18	Previously fertilized, residue removed
K03N3	90	Previously fertilized, residue incorporated
Z04N1	0	Depleted, residue removed
Z04N2	0	Previously not fertilized, residue partially incorporated
Z04N3	168	Previously fertilized, residue incorporated

Within each experiment, lattice-adjusted cultivar means were calculated using the PROC MIXED procedure of SAS (SAS, 2001) with cultivars as a fixed factor and replicate and incomplete blocks within replicates as random factors. Across experiment analysis was conducted from lattice-adjusted cultivar means using cultivar as fixed factor and experiment as random factor (Bänziger et al., 2002). Although three N levels were used at both sites, N availability in the soil differed across the sites and seasons. Therefore, each experiment was considered as a different environment in the across experiment analysis (Bänziger et al., 1999; Bänziger et al., 2002). The significance for cultivar mean square was tested against genotype-by-environment interaction (G x E) mean square as error term while the significance of G x E mean square was tested against the pooled error. Simple linear correlations were calculated to determine relationships between traits. The relationships between N harvest index (NHI, percent of total N in the aboveground biomass partitioned to the grain) and harvest index (HI), kernels per ear (KN) and leaf senescence score (where 0 = no senescence and 10 = 100% senescence) and KN and chlorophyll concentration (SPAD unit, mean chlorophyll concentration in the green leaves at anthesis) were also calculated.

RESULTS

Anthesis-silking interval and yield components

All cultivars belonged to the intermediate to late maturity group at both sites. However, they significantly (P<0.01) differed in AD (Table 3). Mean AD was delayed by four days in the severe low-N stress experiments at Harare compared to the high-N experiments (data not shown). AD was either negatively related or not related to grain yield in all environments (Table 4) indicating that late maturing cultivars were rather less yielding in this set of cultivars. ASI was negatively associated with grain yield in all experiments except at K03N3. The association was more negative under medium-N and severe low-N stress as compared to high-N supply (Table 4) indicating that the N-efficient cultivars, meaning higher yielding cultivars at yield-limiting N supply, had a shorter ASI as compared to the N-inefficient cultivars under low-N stress.

The cultivars varied significantly (P<0.01) in EPP, KN, KW, KRN and KPR (Table 3). KN increased as N level in the soil increased (data not shown). Mean KN was lowest (226) at Z03N1 (Harare 2003 low-N) and highest (497) at Z04N3 (Harare 2004 high-N). In general, the associations between grain yield and KN, KPR and EPP were high (Table 4). Even though the association between grain yield and KRN was positive under low-N conditions, it was not as close as the relationship between grain yield and KPR, indicating KPR was more indicative of N efficiency than KRN. KW was also not related to grain yield under low-N conditions but it was positively associated with grain yield under medium and high N conditions (Table 4). The correlation coefficients (r) of KPR, KN and EPP with ASI under severe low-N stresses at Harare were -0.39, -0.57* and -0.32, respectively, indicating that the N-inefficient cultivars were characterized by a long ASI. The highest mean KNs, (328, 304 and 304) under severe low-N stress at Harare were recorded for the N-efficient cultivars (cultivars 16, 12 and 9). The N-inefficient cultivars 7 and 4 had low KN (190 and 209) under severe low-N stress at Harare, but cultivar 7 was among the cultivars with the highest KN under high-N conditions. KPR was also greater for the N-efficient cultivars compared to the N-inefficient cultivars under low-N stress (data not shown).

Table 3. Mean days to anthesis (AD), anthesis-silking interval (ASI), ears per plant (EPP), kernels per ear (KN), kernel weight (KW, mg), kernel row number (KRN) and kernels per row (KPR) of contrasting N-efficient maize cultivars. Means across over all N environments.

Cultivar	AD	ASI	EPP	KN	KW	KRN	KPR
1	67	1.8	0.99	392	326	14	28
2	68	2.0	0.98	399	345	14	28
3	69	3.1	0.98	374	313	14	27
4	67	3.7	0.90	341	369	13	27
5	71	2.2	0.99	423	341	14	30
6	72	2.7	1.09	376	327	13	28
7	68	2.7	0.96	395	390	15	26
8	69	2.0	0.97	426	305	15	28
9	68	2.0	1.10	428	274	14	30
10	76	2.2	1.05	342	248	15	23
11	76	2.3	1.10	350	306	13	27
12	68	2.2	1.00	448	340	16	29
13	72	2.0	1.03	384	305	15	26
14	72	3.1	0.96	402	367	13	30
15	75	1.2	1.19	387	300	13	30
16	70	2.2	1.04	425	316	15	29
Mean	70	2.3	1.02	393	323	14	28
CV%	1.5	47.7	7.72	11.6	8.2	5.0	11.5
$LSD_{0.05}$	1.0	0.8	0.08	37	21	0.5	2.4
G	**	**	**	**	**	**	**
E	**	**	**	**	**	**	**
G x E	**	**	**	**	**	**	**

**- significant at P<0.01.

Table 4. Linear correlations between grain yield (GY) and anthesis date (AD), anthesis-silking interval (ASI), ears per plant (EPP), kernels per ear (KN), kernel weight (KW), kernel row number (KRN) and kernels per row (KPR) under different environments (Zimbabwe 2003 (N1, N2, N3), Kenya 2003 (N1, N2, N3) and Zimbabwe 2004 (N1, N2, N3)).

Trait	Z03N1	Z03N2	Z03N3	K03N1	K03N2	K03N3	Z04N1	Z04N2	Z04N3
AD	-0.35	-0.55*	0.07	-0.14	-0.02	-0.24	-0.38	-0.57*	-0.05
ASI	-0.41	-0.40	-0.23	-0.30	-0.10	0.14	-0.31	-0.56*	-0.22
EPP	0.68**	-0.12	0.18	0.20	0.13	0.08	0.41	0.23	0.05
KN	0.91**	0.86**	0.08	0.81**	0.76**	0.81**	0.85**	0.75**	0.57*
KW	-0.07	0.61*	0.44^{+}	0.49^{+}	0.63**	0.74**	0.15	0.63**	0.59*
KRN	0.59*	0.20	-0.36	0.27	0.31	0.17	0.20	0.37	-0.07
KPR	0.93**	0.79**	0.40	0.70**	0.68**	0.70**	0.83**	0.64**	0.75**
GY (t ha^{-1})	2.89	8.35	12.66	6.32	8.39	10.57	3.37	6.40	11.13

$^{+}$, *, **- Significant at P<0.1, 0.05 and 0.01, respectively (each correlation with n = 16).

Harvest index and aboveground biomass

Significant differences (P<0.01) among the cultivars were observed for BIOF, BIOH and HI (Table 5). Differences in the traits among the cultivars differed between environments i.e., levels of N stress (significant (P<0.01) genotype-by-environment interaction). HI decreased as soil N availability decreased, ranging from 16% to 37% under low-N conditions (at Z03N1) to 30% to 52% under high-N conditions (at K03N3) (data not shown).

Table 5. Mean aboveground biomass at flowering (BIOF, t ha^{-1}) and at harvest (BIOH, t ha^{-1}), harvest index (HI, %), grain carbon/nitrogen (grain C/N) ratio and grain yield (GY, t ha^{-1}) of contrasting N-efficient maize cultivars. Means over all N environments

Cultivar	BIOF	BIOH	HI	Grain C/N	GY (t ha^{-1})
1	5.7	15.8	40.1	38.5	11.3
2	5.9	17.2	38.0	39.8	11.0
3	6.0	15.3	37.4	37.3	10.0
4	5.2	14.0	39.5	38.6	9.2
5	6.2	17.6	38.7	41.0	13.5
6	6.8	17.6	37.5	41.6	12.7
7	5.8	16.4	42.8	38.8	12.8
8	5.7	16.0	37.9	39.2	11.3
9	5.7	16.9	38.6	37.8	10.5
10	6.9	15.0	29.0	34.4	8.7
11	6.3	16.8	32.7	36.0	9.1
12	5.8	18.3	40.6	39.3	12.2
13	6.2	17.6	32.9	33.7	8.7
14	6.6	18.3	37.3	37.7	13.4
15	6.7	17.5	38.2	37.8	13.5
16	5.1	16.3	40.3	37.1	10.4
Mean	6.0	16.7	37.6	38.0	11.1
CV%	13.1	12.0	8.6	8.9	14.1
$LSD_{0.05}$	0.6	1.4	2.6	2.3	0.9
G	**	**	**	**	**
E	**	**	**	**	**
G x E	**	**	**	**	**

**- significant at P<0.01.

There was a positive association between grain yield and HI in all environments reflecting that cultivars that partitioned more assimilate to grain yield were higher yielding (Table 6). The strength of the relationship between grain yield and HI increased as N level in the soil decreased indicating that increased partitioning of assimilates was more important under severe N stress. Cultivar 7 was among the cultivars with the highest grain yield and HI under

high-N but it was among the cultivars with lowest grain yield and HI under severe low-N stress indicating genotype-by-environment interaction of cultivars in translocation of assimilates to the economic yield in different environments (Figure 1). Cultivar 12 and cultivar 16 had the highest HI under low-N conditions.

Table 6. Linear correlation between grain yield and aboveground biomass at anthesis (BIOF), aboveground dry matter accumulation after anthesis (BIOFH), total dry matter accumulation at physiological maturity (BIOH), harvest index (HI) and grain carbon/nitrogen ratio (grain C/N ratio) at different environments (Zimbabwe 2003 (N1, N2, N3), Kenya 2003 (N1, N2, N3) and Zimbabwe 2004 (N1, N2, N3)).

Trait	Z03N1	Z03N2	Z03N3	K03N1	K03N2	K03N3	Z04N1	Z04N2	Z04N3
BIOF	-0.01	0.34	0.24	-0.42	-0.05	-0.62*	-0.17	0.07	0.26
BIOFH	0.81**	0.33	0.35	0.83**	0.91**	0.85**	0.86**	0.80**	0.86**
BIOH	0.83**	0.55*	0.40	0.75**	0.89**	0.79**	0.62*	0.76**	0.82**
HI	0.88**	0.79**	0.50*	0.80**	0.67**	0.71**	0.80**	0.75**	0.66**
Grain C/N	0.59*	0.65**	0.29	0.57*	-0.30	0.61*	0.54*	0.26	0.50*
GY (t ha^{-1})	2.89	8.35	12.66	6.32	8.39	10.57	3.37	6.40	11.13

*, **- Significant at P<0.05 and 0.01, respectively (each correlation with n = 16).

Significant differences (P<0.01) among the cultivars were observed in grain C/N ratio (Table 5). Grain C/N ratio was constantly positively related to grain yield under severe low-N conditions (Table 6) indicating that cultivars which accumulated relatively more C in the grain at limited N supply were more N-efficient than cultivars which accumulated less C. The relation between N harvest index and HI was close (r ranged at different N environments from 0.55* to 0.97**) indicating that both traits were important for high grain yield under all N environments (data not shown).

Grain yield was not associated with biomass accumulation before anthesis (BIOF) under low-N conditions at both sites (Table 6) implying that dry matter accumulation before anthesis was less important for N efficiency. However, accumulation of dry matter after anthesis (BIOFH) was closely related to grain yield under severe low-N stress conditions in both years (Table 6) indicating that cultivars which had a better photosynthetic capacity after anthesis performed better under low-N conditions. Total biomass at physiological maturity (BIOH) was also positively related to grain yield.

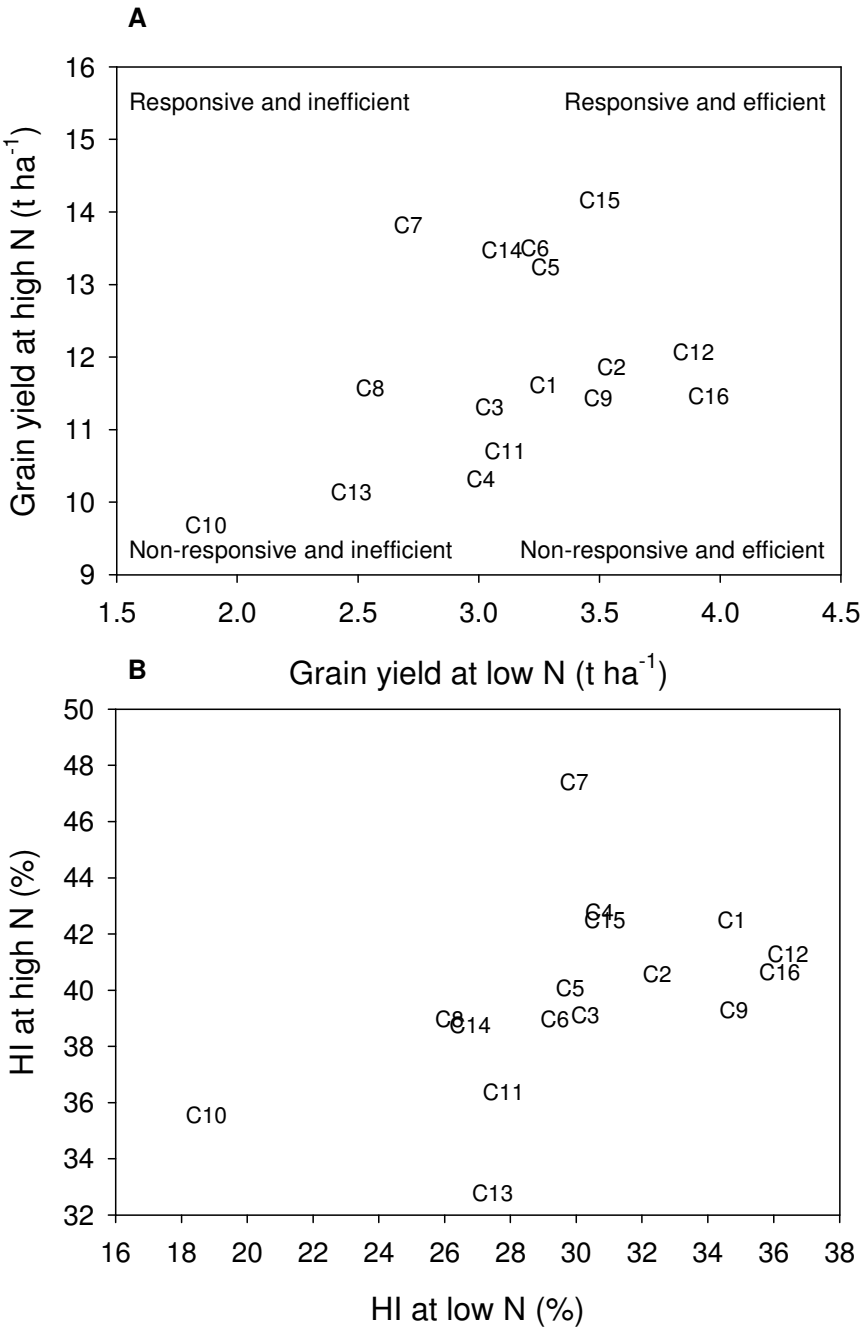

Figure 1. Mean grain yield (t ha^{-1}) and harvest index (HI, %) at Harare high-N (Z03N3 and Z04N3) and at Harare low-N (Z03N1 and Z04N1) of contrasting N-efficient cultivars.

DISCUSSION

In this study CIMMYT tropical mid-altitude maize cultivars with contrasting N efficiency were compared for dry matter accumulation and partitioning of biomass under gradients of N fertility. The results indicated that partitioning of assimilates is influenced by soil-N supply. The cultivars varied significantly for yield components and assimilate partitioning at all N levels and the ranking of the cultivars for these traits differed across the environments.

Sibale and Smith (1997) reported that genotypic effects and N level influence KN with KN increasing as soil N level increases. This is consistent with our results. N-Inefficient cultivars had less EPP and KN under low-N stress conditions compared to the efficient cultivars, indicating poor pollination and/or more abortion of ears and ovules/kernels in the N-inefficient cultivars. Nitrogen deficiency may limit KN as less biomass is partitioned to reproductive structures during flowering (Lemcoff and Loomis 1986; Andrade et al., 1999) or as carbon assimilation is reduced (Uhart and Andrade, 1995). Abortion of ovules, kernels and ears occur in the period one week before until two weeks after silking under stress conditions (Bänziger et al., 2002) which might have resulted in low grain yield in the N-inefficient cultivars as compared to the efficient cultivars in this study. The negative relationship ($r = -0.65^{**}$, in 2003) between KN and leaf senescence score at anthesis and positive relationship ($r = 0.79^{**}$, in 2003 and $r = 0.38^{ns}$ in 2004) between KN and leaf chlorophyll concentration at anthesis under severe low-N conditions (Z03N1 and Z04N1) also suggests the view that source capacity at flowering affects KN.

The strong negative relationship between ASI and KN and positive association between grain yield and KPR under severe low-N stress suggests that N-efficient cultivars had better silk development and pollination than the N-inefficient cultivars. There have been several reports that rapid ear and silk growth under stress conditions are indications of better partitioning of photosynthates to the developing ear (Lemcoff and Loomis, 1986; Edmeades et al., 1993; Lafitte and Edmeades, 1994a; Oikeh, 1996; Bänziger and Lafitte, 1997; Westgate, 1997; Mugo et al., 2003). Messmer et al. (2004) indicated that ear and silk development and ASI are strongly related and under genetic control.

Bänziger et al. (2002) found an increase of KW under low-N stress in maize populations selected for drought tolerance as a result of greater carbohydrate availability to kernels in the N-efficient populations. However, in this study KW was mainly associated with grain yield under medium and high N conditions and was not related to grain yield under severe low-N stress conditions. Since the cultivars included in this study were selected from germplasm of different origins, genetic differences in kernel size and weight which are not related to selection for N efficiency may have influenced the result. The higher KNs in the N-efficient cultivars under low-N may also have influenced the result. Below (1997) indicated that the weight of an individual kernel is a function of the kernel number and the assimilate supply during grain filling. Borras and Otegui (2001) reported that KW was increased between 0.09

to 0.28 mg kernel^{-1} per unit decrease in kernel number per plant depending on stand density and genotype. Lafitte and Edmeades (1995) also reported that the correlation between grain yield and hundred kernels weight was low under low-N as compared to high-N conditions, which is consistent with our result.

Although HI decreased as the severity of low-N stress increased, there was a close positive relationship between grain yield and HI under severe low-N stress implying that high HI under low-N stress was related to N efficiency. Dry matter accumulation before anthesis was not related to grain yield under severe low-N stress, but biomass accumulation after anthesis and total biomass at harvest were, indicating that biomass accumulation during and after flowering are of major importance for N efficiency. Rajcan and Tollenaar (1999a, b) reported that higher rates of dry matter accumulation and partitioning during grain filling contributed to better performance of a recent hybrid as compared to an older hybrid under low-N stress. Similarly, Blum (1997) concluded that mobilization of assimilates to the grain during grain filling is crucial for stress tolerance.

Bänziger et al. (2002) reported that individual kernel N content decreased in N-efficient populations. The result of this study indicated a constant close relationship between grain yield and grain C/N ratio under low-N conditions. N-efficient cultivars assimilated and partitioned more carbon to the grain with limited N compared to the inefficient cultivars. The close relation between N harvest index and HI indicates that both traits are important for N efficiency.

In conclusion, this study established a strong relationship between N efficiency and dry matter partitioning. Significant cultivar-by-environment interactions indicated that high HI under high-N conditions may not imply high HI under low-N conditions. The N-efficient cultivars were characterized by a shorter anthesis-silking interval, higher dry matter production during grain filling, higher kernel number and relatively increased grain C/N ratio under limited N supply. N-efficient cultivars seem to assimilate more carbon in particular during and after flowering under low-N conditions.

CHAPTER 5

PROTEIN QUANTITY AND QUALITY, AND AGRONOMIC PERFORMANCE OF QUALITY PROTEIN MAIZE AND NORMAL ENDOSPERM MAIZE UNDER DIFFERENT LEVELS OF NITROGEN STRESS

ABSTRACT

The majority of farmers in sub-Saharan Africa produce maize under conditions of low soil nitrogen (N) fertility. Quality protein maize (QPM), which contains more essential amino acids, lysine and tryptophan, than normal endosperm maize is being promoted in areas where maize is a major source of dietary proteins. This study examines the effect of N stress on protein quantity and quality, grain yield and other agronomic traits of a selection of QPM and non-QPM cultivars. Three QPM and 13 non-QPM cultivars were planted under a range of N levels at Harare, Zimbabwe in 2003 and 2004 and at Kiboko, Kenya in 2003. Significant genotypic differences were observed for grain protein content, endosperm protein, lysine and tryptophan contents, grain yield, and susceptibility to ear rot. The quantity of grain protein and endosperm lysine, tryptophan and protein contents was influenced by N level in the soil for both QPM and non-QPM cultivars. QPM cultivars maintained their superiority over non-QPM cultivars in lysine and tryptophan contents in all environments. QPM cultivars had a higher quality index than non-QPM cultivars in all environments reflecting the stable effect of the opaque-2 gene for protein quality across soil-fertility gradients and sites. On average, endosperm tryptophan was 45% higher and lysine was 27% higher in QPM cultivars than in non-QPM cultivars. The best QPM cultivar outyielded a non-QPM commercial cultivar under severe low-N stress at Harare indicating the possibility of developing N-efficient QPM cultivars that may combine high yield potential and good protein quality under low-N conditions.

Key words: Cultivar, Lysine, Nitrogen environment, Tryptophan, *Zea mays* L.

INTRODUCTION

Despite the impressive rise in food production in recent decades, over 800 million people of the world consume less than 2,000 calories a day, live a life of permanent hunger and are chronically undernourished (Conway and Toenniessen, 1999; Reeves, 1999). It is estimated that 37% of sub-Saharan African people are undernourished (Reeves, 1999).

Maize is the most important staple cereal in sub-Saharan Africa comprising more than 30% of the diets of about 100 million people in this region (CIMMYT, 2001; CIMMYT, 2002). Although normal endosperm maize cultivars with improved yield potential have been developed for the region, their grain contains low concentrations of the two essential amino acids, lysine and tryptophan (CIMMYT, 2001). Normal maize contains about 50% prolamines (zein), which comprises almost no lysine and tryptophan. Other protein groups, albumins, globulins and glutamins, constitute the remaining 50% of the maize protein (National Research Council, 1988; Bajarnason and Vasal, 1992; Singh, 2003). Consequently, normal endosperm maize has a low biological value, between 40 and 57% (Bressani, 1992 as cited in Bhatnagar et al., 2003). This can lead to malnutrition, particularly among children, pregnant women and the ill in countries where maize is the staple food and often a significant source of protein (Conway and Toenniessen, 1999; CIMMYT, 2002; Pixley et al., 2002).

According to Scott et al. (2003), if one amino acid is deficient in a diet, other amino acids cannot be used efficiently. However, more protein could be made available in maize-based diets by changing the relative levels of certain amino acids without altering the protein content of the grain. Quality protein maize (QPM), the product of a long term breeding effort by CIMMYT, has relatively higher contents of tryptophan and lysine than normal maize (Bjarnason et al., 1976; Motto, 1979; Vasal, 1980; Vasal, et al., 1984; CIMMYT, 1999; Vasal, 2000; Vasal, 2001; CIMMYT, 2002). QPM has a biological value of 90% indicating its more balanced content of essential amino acids necessary for normal growth and development of monogastric animals including humans (National Research Council, 1988). Pixley and Bjarnason (1993) reported that the best QPM cultivar had comparable grain yield to normal endosperm maize and exceeded the normal endosperm check by 48% and 60% for tryptophan concentration in the grain and tryptophan concentration in grain protein, respectively.

Grain protein content is subject to strong genotype-by-environment interactions (Bjarnason and Vasal, 1992; Vasal, 2001). Pixley et al. (2002) evaluated different QPM cultivars at 13

tropical locations on four continents under non-limiting soil nitrogen (N) conditions and reported that protein and tryptophan content of grain and tryptophan content of protein were generally stable across the environments. However, the majority of farmers in sub-Saharan Africa produce maize under conditions of low-N stress on depleted infertile soils (Lafitte and Edmeades, 1994a; Bänziger et al., 2000; Bänziger and Meyer, 2002; Bänziger et al., 2005). The effects of limiting N conditions on protein quality and quantity have not been studied. Ear rot is also among the biotic stresses limiting maize production in the mid-altitude ecologies (Tilahun et al., 2002). Evaluation of QPM cultivars for resistance to this biotic stress will enable identification of cultivars that are adapted to mid-altitude areas.

The purpose of this study was to evaluate and compare QPM and normal endosperm maize cultivars for protein quality and quantity, grain yield and other important agronomic traits under a range of N levels.

MATERIALS AND METHODS

Germplasm, testing environment and trial management

Three QPM and 13 normal endosperm maize (non-QPM) cultivars were included in the study. A full description of the cultivars is given in Table 1. Both types of cultivars included single-cross and three-way-cross hybrids.

The cultivars were tested in Zimbabwe (Harare) and Kenya (Kiboko) under three N levels (low, medium and high N) at both sites resulting in nine environments: Harare 2003 low-N (Z03N1), Harare 2003 medium-N (Z03N2), Harare 2003 high-N (Z03N3), Kiboko low-N (K03N1), Kiboko medium-N (K03N2), Kiboko high-N (K03N3), Harare 2004 low-N (Z04N1), Harare 2004 medium-N (Z04N2) and Harare 2004 high-N (Z04N3). The two locations represent mid-altitude agro-ecology of sub-Saharan Africa. The choice of N application rates at each station was intended to create a range of relevant N levels, and depended on soil type, cropping history, fertilizer recommendation and experience of researchers at each station. A full description of the testing environments is given in Table 2. Nitrogen was applied in split under high-N conditions (N3), and otherwise given solely at planting. Other agronomic management practices were applied uniformly for all the experiments based on the recommendations at each center. Alpha (0,1) lattice experimental designs with four replications (Patterson and Williams, 1976) were used in each experiment.

A plot size of 4 m length by 4.5 m width with six rows per plot was used. Spacing was 0.75 m and 0.25 m between rows and plants, respectively. A plant density of 53,333 plants per hectare was kept after thinning.

Table 1. Maize cultivars/hybrids used for the study.

No.	Cultivar (Hybrid)	Source	Performance under low-N
1	CML444/CML445//CML440	CIMMYT-Zimbabwe	Good
2	CML395/CML444//CML440	CIMMYT-Zimbabwe	Good
3	CML202/CML395//CML205	CIMMYT-Zimbabwe	Poor
4	SC515	Seed-CO-Zimbabwe	Poor
5	CML395/CML444//CML442	CIMMYT-Zimbabwe	Good
6	CML444/CML197//CML443	CIMMYT-Zimbabwe	Good
7	SC633	Seed-CO-Zimbabwe	Poor
8	CML181/CZL01005//CZL01006	CIMMYT-Zimbabwe	Best among QPM[1]
9	CML181/CML182//CML176	CIMMYT-Zimbabwe	Best among QPM[1]
10	CML144/(16304/6303Q)-B-6-1-3-3-B*6	CIMMYT-Mexico	Poor (QPM[1])
11	CML247//CML254	CIMMYT-Mexico	Good
12	CML78/CML373	CIMMYT-Mexico	Good
13	CML264/CML311//CML334	CIMMYT-Mexico	Poor
14	CML442/CML444//[MSRXPL9]C1F2-205-1(OSU23i)-1-1-X-1-X-B-B	CIMMYT-Kenya	Good
15	LPSC4F273-2-2-1-B-B-B/CML202//CML384	CIMMYT-Kenya	Good
16	CML312/CML247//CML78	CIMMYT-Kenya	Good

[1] Quality Protein Maize

Table 2. Fertilizer application (N1, N2, N3) and cropping history of the testing environments in Harare 2003 and 2004 (Z03, Z04) and Kiboko 2003 (K03).

Environment	Applied N (kg ha^{-1})	Cropping history and residue management
Z03N1	0	Depleted, residue removed
Z03N2	0	Previously fertilized, residue incorporated
Z03N3	168	Previously fertilized, residue incorporated
K03N1	18	Depleted, residue removed
K03N2	18	Previously fertilized, residue removed
K03N3	90	Previously fertilized, residue incorporated
Z04N1	0	Depleted, residue removed
Z04N2	0	Previously not fertilized, residue partially incorporated
Z04N3	168	Previously fertilized, residue incorporated

Measurements

An area of 5.65 m^2, corresponding to 32 plants in the central four rows, was harvested immediately after physiological maturity for grain yield and grain N analysis at harvest. During harvests, two border plants at the ends of each row were excluded to avoid border effects. Grain moisture percent (MOI %) was estimated using a Dickey-John multi grain moisture tester. Grain yield (GY t ha^{-1}) was calculated using shelled grain and adjusted to 12.5% moisture. Grain rot percentage (Grain rot %) of the ears was estimated for each plot at harvest. Days from effective planting date to anthesis (AD) were determined for the two central rows.

Grain sub-samples were taken and oven dried at 80^{o}C for 72 hours, milled using analytical mills and analyzed for grain N content at the Plant Nutrition Institute Laboratory, University of Hannover, Germany using a CNS analyzer (Vario EL, Elementar Analysis Systems, Hanau, Germany). To get protein contents, N values were multiplied by 6.25 (Horwitz, 1980). Then, grain protein content per grain (GP g kg^{-1}) was calculated.

Eight plants were sib-mated plant-to-plant for two QPM and two non-QPM cultivars in each plot to avoid crosses between QPM and non-QPM cultivars in the grain of those plants. At harvest the F_2 grain was shelled from the middle of five ears for all sibbed plots. Then 20 seeds were taken at random and analyzed for total protein, tryptophan and lysine content in the endosperm at the CIMMYT Cereal Quality Laboratory (Mexico D.F., Mexico) using the

standard procedure described by Villegas et al. (1984). Lysine was analyzed only for Kiboko 2003 and Harare 2004 experiments. Quality index (QI) was calculated as the ratio of tryptophan content to total protein content in the endosperm, expressed as a percentage.

Statistical analyses

Within each experiment, lattice-adjusted cultivar means were calculated using the PROC MIXED procedure of SAS (SAS, 2001) with cultivars as a fixed factor and replicate and incomplete blocks within replicates as random factors. Across-experiment analysis was conducted from lattice adjusted cultivar means using cultivars as fixed factor and experiment as random factor (Bänziger et al., 2002). Although, three N levels were used at both sites, N availability in the soil differed across the sites and seasons. Therefore, each experiment was considered as different environment in the across experiment analysis (Bänziger et al., 1999; Bänziger et al., 2002). The significance of the genotype mean square was tested against genotype-by-environment interaction (G x E) mean square while the significance of that G x E mean square was tested against the pooled error. The same procedure was followed for statistical analysis for endosperm N, protein, tryptophan and lysine contents. Cultivars were considered as fixed factor while replicates were considered as random factors. Simple linear correlation coefficients were calculated to determine relationships between the traits.

RESULTS

Protein quantity and quality

Significant genotypic differences ($P<0.01$) among the cultivars were observed for grain protein content, which averaged 80 g kg^{-1} and ranged from 72 g kg^{-1} to 89 g kg^{-1} across the environments (Table 3). QPM cultivars had average grain protein and endosperm protein contents comparable to non-QPM cultivars overall (Table 3 and 4) and in each environment (data not shown for grain protein). However, grain protein and endosperm protein yields varied among environments. Grain protein content decreased as soil-N supply decreased (Figure 1). Furthermore, generally there was a negative relationship between grain yield and grain protein content in each environment (data not shown) and the relationship between mean grain yields and mean grain protein across environments is shown in Figure 2.

Table 3. Mean anthesis date (AD, days after planting date to 50% anthesis), grain yield (GY, t ha^{-1}), moisture percent (MOI %), grain rot percent (grain rot %), and grain protein content (GP g kg^{-1}) of 16 maize cultivars averaged over 9 nitrogen fertility environments.

Cultivar	AD	GY (t ha^{-1})	MOI%	Grain rot %	GP (g kg^{-1})	Type
1	67	7.63	21.3	3.52	78.0	non-QPM
2	68	8.05	22.3	2.39	78.8	non-QPM
3	69	6.99	22.8	1.39	79.3	non-QPM
4	67	6.77	21.9	3.27	77.2	non-QPM
5	71	8.57	24.3	2.36	73.2	non-QPM
6	72	8.20	24.2	1.33	72.3	non-QPM
7	68	9.01	22.6	5.53	76.8	non-QPM
8	69	7.75	23.5	10.04	78.3	QPM
9	68	7.79	20.1	4.18	81.7	QPM
10	76	5.52	24.9	2.93	89.3	QPM
11	76	6.95	28.0	4.74	87.2	non-QPM
12	68	9.02	24.0	11.20	77.8	non-QPM
13	72	7.27	24.2	13.59	88.5	non-QPM
14	72	8.59	22.9	5.12	79.0	non-QPM
15	75	8.42	22.8	0.95	77.8	non-QPM
16	70	8.06	23.7	7.59	80.2	non-QPM
Mean	70	7.79	23.3	5.01	79.7	
CV%	1.54	14.05	9.47	68.36	9.61	
$LSD_{0.05}$	0.99	0.88	1.33	4.52	4.48	
G	**	**	**	**	**	
E	**	**	**	**	**	
G x E	**	**	**	**	**	

**- Significant at P<0.01

Severe N stress (Z03N1 and Z04N1) reduced endosperm protein content by 32% and 44% at Harare in 2003 and 2004, respectively, in relation to high-N conditions (Z03N3 and Z04N3). Similarly, endosperm protein content was reduced by 45% under low-N (K03N1) relative to high-N (K03N3) at Kiboko, Kenya (Table 5). Significant genotypic differences (P<0.01) were observed for endosperm tryptophan and lysine content and quality index (Table 4). Endosperm tryptophan and lysine contents were higher for QPM cultivars than non-QPM cultivars in all environments (Table 5). On average, endosperm tryptophan was 45% higher and lysine was 27% higher in QPM cultivars than non-QPM cultivars. The N-efficient QPM cultivar, cultivar 9, had the highest average endosperm tryptophan and lysine contents.

Similarly, endosperm protein quality index, QI was higher for QPM cultivars than for non-QPM cultivars (Table 5).

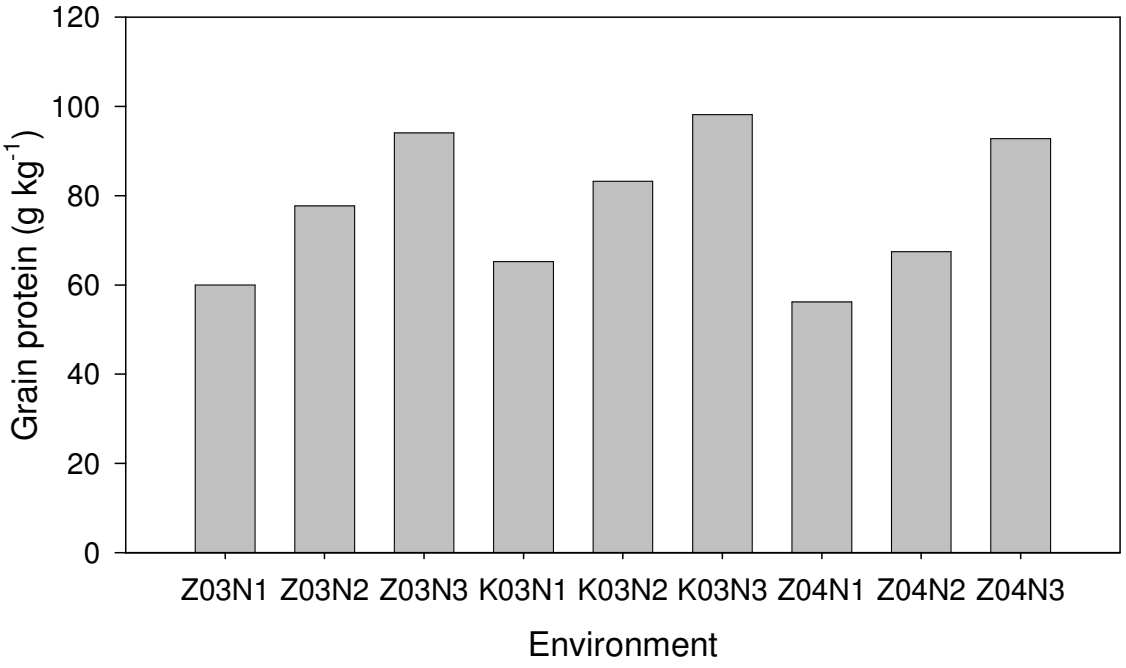

Figure 1. Grain protein content (g kg^{-1}) across N fertility environments (Harare 2003 N1, N2 and N3, Kiboko 2003 N1, N2 and N3, and Harare 2004 N1, N2 and N3).

On average, the QPM cultivars had approximately 26% more lysine in the endosperm protein than non-QPM cultivars. Similarly, endosperm protein quality index, QI (percentage of the ratio between endosperm tryptophan content and total endosperm protein) was higher for QPM cultivars than for non-QPM cultivars. The QPM cultivars had 34% more tryptophan in the endosperm protein (across environments) than non-QPM cultivars (Table 5).

Both tryptophan and lysine contents of the endosperm (g kg^{-1}) were increased for all the cultivars as the N level in the soil increased (Table 5). Endosperm lysine and tryptophan contents were highly correlated (r = 0.92**; across the environments). Protein quality index (QI) was higher under low-N than under high-N conditions for all cultivars (QPM and non-QPM). QI relatively tended to decrease as N level in the soil increased (Table 5) and was higher in QPM cultivars than in non-QPM cultivars in all environments. The genotype-by-environment interaction for QI was non-significant implying that the ranking of the cultivars was similar across environments.

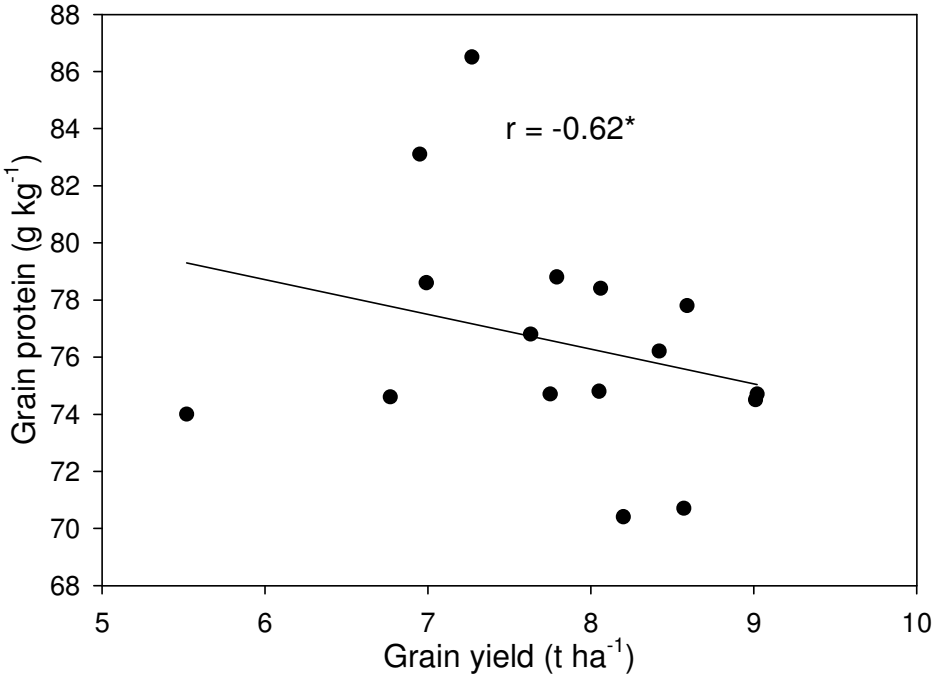

Figure 2. Relationship between mean grain yield, t ha^{-1} (mean of 9 environments) and mean grain protein content, g kg^{-1} (mean of 9 environments) in 16 contrasting N-efficient CIMMYT tropical mid-altitude maize cultivars.

Table 4. Mean endosperm tryptophan (Try), lysine, nitrogen (N), protein (all in g kg^{-1}) and quality index (QI) of two quality protein maize (QPM) and two non-QPM cultivars across environments.

Cultivar	Try	Lysine	N	Protein	QI	Remark
6	0.35	1.82	10.9	68.0	0.56	non-QPM
7	0.41	2.14	11.6	72.5	0.60	non-QPM
8	0.54	2.51	10.8	67.7	0.81	QPM
9	0.55	2.53	12.0	75.3	0.74	QPM
Mean	0.46	2.25	11.3	70.9	0.68	
CV%	10.86	12.45	16.69	16.69	10.47	
G	**	**	*	*	**	
E	**	**	**	**	**	
G x E	**	ns	ns	ns	ns	

*, **, -Significant at P<0.05 and P<0.01, respectively

Table 5. Endosperm tryptophan, lysine and protein contents (all in g kg^{-1}), and quality index (QI, %) of quality protein maize (QPM) and normal endosperm maize (non-QPM) cultivars in different environments, Harare 2003 N1, N2, N3 (Z03N1, Z03N2, Z03N3), Kiboko 2003 N1, N2, N3 (K03N1, K03N2, K03N3) and Harare 2004 N1, N2, N3 (Z04N1, Z04N2, Z04N3)

Cultivar	Type	Amino acid	Z03N1	Z03N2	Z03N3	K03N1	K03N2	K03N3	Z04N1	Z04N2	Z04N3	Mean	$LSD_{0.05}$
6	non-QPM	Tryptophan	0.33	0.36	0.41	0.32	0.35	0.44	0.30	0.31	0.36	0.35	
7	non-QPM		0.40	0.38	0.45	0.39	0.47	0.53	0.33	0.35	0.44	0.41	
8	QPM		0.42	0.63	0.64	0.44	0.62	0.68	0.43	0.40	0.58	0.54	
9	QPM		0.50	0.64	0.63	0.50	0.58	0.64	0.42	0.46	0.57	0.55	0.04
6	non-QPM	Lysine				1.45	1.53	1.94	1.88	1.97	2.14	1.82	
7	non-QPM					1.69	2.15	2.35	2.01	2.12	2.52	2.14	
8	QPM					1.85	2.70	2.89	2.15	2.29	3.15	2.51	
9	QPM					2.06	2.55	2.83	2.14	2.52	3.06	2.53	0.22
6	non-QPM	QI	0.60	0.49	0.44	0.74	0.55	0.48	0.75	0.56	0.43	0.56	
7	non-QPM		0.57	0.60	0.51	0.74	0.57	0.56	0.67	0.67	0.48	0.60	
8	QPM		0.89	0.87	0.72	0.88	0.81	0.78	0.85	0.82	0.68	0.81	
9	QPM		0.73	0.75	0.67	0.90	0.73	0.70	0.81	0.72	0.67	0.74	0.04
6	non-QPM	Nitrogen	9.60	11.90	15.00	7.00	10.80	14.80	6.40	8.90	13.50	10.90	
7	non-QPM		11.70	10.30	14.30	8.50	13.50	15.30	7.90	8.40	14.40	11.60	
8	QPM		7.60	11.50	14.20	8.10	12.40	13.80	8.10	7.90	13.70	10.80	
9	QPM		10.80	13.70	15.20	8.90	12.80	14.70	8.50	10.20	13.70	12.00	0.88
6	non-QPM	Protein	60.00	74.40	93.40	43.80	67.70	92.40	40.10	55.80	84.40	68.00	
7	non-QPM		73.30	64.10	89.50	52.90	84.60	95.60	49.60	52.50	90.10	72.50	
8	QPM		47.70	72.00	89.00	50.80	77.40	86.10	50.80	49.10	85.80	67.70	
9	QPM		67.60	85.30	94.90	55.70	79.70	92.00	53.00	63.80	85.70	75.30	5.49

Agronomic performance

Nitrogen efficiency (grain yield under low-N stress) varied among QPM cultivars in almost the same magnitude as among non-QPM cultivars. The best QPM cultivar, cultivar 9, was among the high yielding cultivars under severe low-N stress conditions (Z03N1 and Z04N1), outyielding the N-inefficient non-QPM cultivar 7 by 29% under severe low-N stress at Harare (Z03N1 and Z04N1). However, QPM cultivars yielded less than the best non-QPM cultivars in all environments (Chapter 2).

Cultivars varied significantly ($P<0.01$) in their response to grain rot (Table 3). The severity was almost nil at Kiboko but was mainly observed at Harare where ear rot among some cultivars reached relatively high levels while most showed few symptoms. The relationship between grain yield and percent grain rot (average $r = -0.15$) was weak in all environments. QPM cultivars showed comparable ear rot ratings to the non-QPM cultivars, even though there was cultivar variation within each cultivar group (Table 3). The highest grain rot percentage was recorded for a non-QPM cultivar.

QPM cultivars had similar grain moisture percentage to non-QPM cultivars at harvest (Table 3) indicating that QPM cultivars were similar to non-QPM cultivars in dry-down at physiological maturity.

DISCUSSION

The maize endosperm is particularly dedicated to the accumulation of starch and protein, which are the primary sinks for carbon and N compounds (Balconi et al., 1997). There is considerable genotypic variation in grain protein content in tropical maize (Feil et al., 1993). Our results confirm this for both QPM and non-QPM cultivars. In agreement with other researchers (Ortiz-Monasterio et al., 2001; Bjarnason and Vasal, 1992), we found that protein concentration in the grain decreased as grain yield increased. Duvick (1997) evaluated hybrids released from 1934 to 1991 in the USA and reported a linear increase in grain starch percent and a linear decrease in grain protein percent as grain yield increased over the years. However, since genetic variation for grain protein exists in maize (Pixley and Bjarnason, 1993), it may be possible to increase grain yield without decreasing protein content if protein content is monitored during selection.

QPM cultivars had protein concentrations in the grain comparable to non-QPM cultivars. Although QPM cultivars were superior to non-QPM cultivars in protein quality in all environments, the contents of N, protein, tryptophan and lysine in the grain endosperm were influenced by soil N level in both QPM and non-QPM cultivars. Protein, tryptophan and lysine contents of the grain endosperm increased markedly as available N in the soil increased. This shows that all protein fractions in the grain are reduced when N in the soil is limiting.

Protein quality index (QI) tended to decline for both QPM and non-QPM cultivars as the N level in the soil increased. This suggests that zein increased more under high-N conditions, where grain yields are also higher, as compared to other protein fractions (albumins, globulins and glutamins) which contain tryptophan and lysine. Vasal (2001) confirmed that zein synthesis could be manipulated by N fertilization and genetic means and that a positive relationship existed between the zein content and grain yield. However, zein was less increased in QPM cultivars as compared to non-QPM cultivars under high-N conditions. This is reflected in the large difference between QI values of QPM cultivars and non-QPM cultivars under high-N conditions. In addition, QPM cultivars had greater lysine and tryptophan per endosperm protein contents than non-QPM cultivars in all environments. This is consistent with the results of Pixely et al. (1993) and Bhatnagar et al. (2003) who reported the superiority of QPM cultivars over non-QPM cultivars for grain protein quality (lysine and tryptophan contents) under non-limiting soil fertility conditions.

Pixley (2001) reported that yields of currently available QPM germplasm for mid-altitude ecologies of eastern and southern Africa are competitive with some regional cultivars although they are lower than that of the best available non-QPM cultivars. Even though the best QPM cultivar outyielded a non-QPM commercial cultivar under low-N stress conditions in our study, non-QPM cultivars were the best in all environments. Further investments are required in long term breeding programs to bring yields of QPM cultivars on par with the best normal endosperm cultivars adapted to mid-altitude areas of eastern and southern Africa.

Although the QPM cultivars were not the best for ear rot resistance compared to ear rot resistance among non-QPM cultivars, the results of this study indicated that QPM cultivars had better resistance to ear rot than some of the non-QPM cultivars. Bhatnagar et al. (2003) also found QPM cultivars that were significantly less susceptible to aflatoxin than non-QPM

checks in southern USA. Genetic variability seems to exist in both QPM and non-QPM cultivars for ear rot resistance.

In conclusion, protein, lysine and tryptophan contents in grains of both QPM and non-QPM cultivars were influenced by the available N level in the soil. Moreover, QPM cultivars maintained their superiority over non-QPM cultivars in lysine and tryptophan contents in all environments. The results of this study suggest that it is possible to develop N-efficient cultivars that may combine high yield potential and protein quality at all levels of soil fertility. This is an important finding given the need to improve protein availability in maize based diets in sub-Saharan Africa.

GENERAL DISCUSSION

Ceccarelli (1989) stated that differences for grain yield observed in the absence of stress are largely unrelated with differences observed in presence of severe stress. He further stated, however, when environments with average yields (sub-optimum) are defined as stress environments, the same genotype may be high yielding under both optimum and sub-optimum conditions. The results of the present study also showed that the high yielding cultivars under high-N conditions were also the high yielding under medium-N. However, crossover genotype-by-environment interaction was occurred under severe low-N conditions. On average, the N-efficient cultivars (average of 10 cultivars) outyielded the commercial cultivars (average of two cultivars) by 22% under severe low-N stress conditions (Chapter 2). This indicated that physiological mechanisms contributing to grain yield under severe low-N conditions are at least partially different from those under high-N.

In this study crop-physiological mechanisms related to improved performance under low-N conditions were assessed under field conditions (Chapter 2, 3 and 4). In addition, gene effects under contrasting N environments (Chapter 1), and protein quality and quantity across N environments (Chapter 5) were studied. The most important results and conclusions of these field studies are discussed in the following section.

Gene effects under contrasting N environments and implication for maize breeding

Cooper and Byth (1996) argued that the association between quantitative characters measured in two separate environments is a function of the degree to which the same genes influence genetic variation in both environments. In the present study the relationships between grain yields under high-N and severe low-N stress were low (Chapter 1, 2) indicating a change in the ranking of the cultivars across the N levels.

Although GCA (additive effects) constitutively contributed to grain yield across high-N and low-N conditions, the relative contribution of SCA (non-additive effects) on progeny performance increased under low-N conditions. Betran et al. (2003a) and Diallo et al. (2003) also reported a greater importance of non-additive gene effects for grain yield under low-N conditions as compared to high-N conditions. Gallais and Hirel (2004) found that genetic variability in maize is expressed differently under high-N and low-N conditions. They indicated that Quantitative Trait Loci (QTLs) detected for grain yield at high-N were different from those detected at low-N. This indicates the necessity to select hybrid combinations under

both managed low-N stress and optimum-N conditions to identify maize cultivars which can give good yield across N levels. However, the contribution of additive gene effects was mostly higher than non-additive gene effects for anthesis date, plant and ear height, and ear rot under both high-N and low-N conditions. Similarly, the contribution of additive gene effects was higher than non-additive gene effects for gray leaf spot (under high-N conditions, where it was scored) and leaf senescence (under low-N conditions, where it was scored) (Chapter 1). This may imply that selection for these traits could be conducted at early stage of inbred line development (Lafitte and Edmeades, 1995; Betran et al., 2003b).

In all trials in 2002 the contribution of additive gene effects was more important for grain yield than non-additive gene effects under both high-N and low-N conditions. Severe drought in 2002 in southern Africa might have influenced the results. Betran et al. (2003a) also found that contribution of additive gene effects was more important than non-additive gene effects under drought conditions. On the other hand, preliminary research results on ASI (anthesis-silking interval) QTL indicate that most of the genomic regions identified under low-N are similar to those identified under drought (Beck et al., 1996). This may suggest that testing the inbred lines under managed drought stress and selection of the hybrid combinations under both managed drought and low-N stress conditions may enhance hybrid performance under both stresses in the tropics. Other researchers also indicated that good performance across stress levels can be achieved in tropical maize cultivars (Bänziger et al., 1999; Bänziger et al., 2005; Betran et al., 2003a; Zaidi et al., 2003).

Traits contributing to nitrogen efficiency

In this study significant cultivar differences ($P<0.01$) were recorded for N efficiency. Both N-uptake and N-utilization efficiencies were also strongly related to grain yield under low-N conditions (Chapter 2). This is in agreement with the results of Wiesler et al. (2001) and Ortiz-Monasterio et al. (2001) who found that both N-uptake and N-utilization efficiencies are important traits for N efficiency. However, Kamprath et al. (1982) and Presterl et al. (2002) reported that N-uptake efficiency is more important than N-utilization efficiency under low-N conditions. Other researchers attributed genetic differences in N efficiency in maize to differences in utilization of accumulated N for grain production (Moll et al. 1982; Lafitte and Edmeades, 1994b). Considering the managed severe low-N stress conditions under which the cultivars were tested and the number of contrasting N-efficient cultivars included in the

present study, it seems that both N-uptake and N-utilization efficiencies contribute to improved grain yield under low-N conditions.

Generally, delayed leaf senescence and higher leaf chlorophyll concentration were positively related to grain yield, particularly under low-N conditions, implying that improvement of the source capacity during flowering and grain filling might have contributed to N efficiency (Chapter 3 and 4). Genetic differences for delayed leaf senescence and leaf chlorophyll concentration were also reported by other researchers (Lafitte and Edmeades, 1994a; Gan and Amasino, 1997; Noh et al., 1999; Paponov and Engels, 2003; Messmer et al., 2004). Delayed leaf senescence may be responsible for higher root growth and N uptake (Chapter 2 and 3). In addition, the negative relationship between N uptake and leaf senescence and positive relationship between chlorophyll concentration and N uptake under low-N conditions (chapter 3) may indicate that active root growth in the subsoil and better N uptake have contributed to delayed leaf senescence and higher leaf chlorophyll concentration in the N-efficient cultivars. The delayed leaf senescence and higher photosynthetic capacity in the N-efficient cultivars under low-N conditions probably also contributed to higher dry matter production, better silk development and pollination, and higher kernel set in the N-efficient cultivars (Chapter 3 and 4). This also resulted in a better harvest index (HI) and N-utilization efficiency in the N-efficient cultivars under low-N conditions as compared to the N-inefficient ones (Chapter 2 and 4).

The relationships between leaf senescence and N uptake or active root growth in the subsoil, and photosynthetic efficiency still need to be clarified. In addition, the genetic variability for these traits needs to be investigated. On the other hand, some N-efficient cultivars had moderate leaf senescence score under low-N conditions (Chapter 3) indicating that leaf senescence score may not be useful as a single selection criteria for N efficiency. However, when it is combined with grain yield data in the selection process, it improves the breeding progress (Bänziger and Lafitte, 1997). There is no doubt that high early leaf senescence negatively affects grain yield by reducing photosynthetic capacity, but cultivars which mobilize the N in the leaf to the grain at the later stage of the crop may be N-efficient. This suggests that selection against early leaf senescence but for enhanced late senescence may improve the breeding progress for N efficiency.

The superiority of one of the N-efficient cultivars in root-length density (as measured by soil-core method at anthesis) and mineral-N depletion of the subsoil (60 – 90 cm) at physiological

maturity as compared to an N-inefficient cultivar may indicate that the N-efficient cultivar had higher active root growth in the subsoil and higher N uptake during and after flowering than the N-inefficient cultivar. On the other hand, total root-system size (as measured by root capacitance at anthesis and two weeks after anthesis) was not positively related to N efficiency. This suggests continued root growth in the subsoil after flowering or cultivar differences in root morphology are more important for N efficiency than total root-system size in the soil (Chapter 3). In agreement with the present result, Heuberger (1998) found inconsistent relationship between root capacitance readings and N efficiency in tropical maize. She also reported differences among the hybrids for angle of the roots suggesting vertical root growth may contribute to N efficiency.

Manske et al. (2001) concluded that root systems (depth of penetration) in cereals are largely influenced by additive genes. This indicates that there is potential for the selection of maize cultivars with a higher root-length density in the subsoil. However, selection of maize cultivars for higher root-length density in the subsoil under field conditions needs time and cost effective techniques that may allow to measure root growth at different soil depths. Root capacitance is time and cost effective for estimation of total root-system size in the soil, but it is not possible to estimate root-system size at different soil depths and crop stages using root capacitance. This shows the need for better techniques for root studies under field conditions.

Nitrogen efficiency and protein quality

Total endosperm protein, lysine and tryptophan contents (Chapter 5) were reduced under low-N stress conditions for both QPM and non-QPM cultivars, but the QPM cultivars maintained their superiority over non-QPM cultivars in endosperm lysine and tryptophan contents under all N environments indicating that there was no specific loss of protein quality in QPM cultivars because of N efficiency. Generally increasing grain yield under limited N supply resulted in high C/N ratio in the grain (Chapter 4) and reduced protein percent in the grain (Chapter 5). Thus, the strategy of changing the relative levels of lysine and tryptophan in maize grains may be the right strategy to improve protein availability in maize based diets in sub-Saharan Africa.

Based on grain yield performance under low-N and high-N conditions, the contrasting N-efficient cultivars were grouped into four groups; namely, inefficient and responsive, efficient and responsive, efficient and less responsive, and inefficient and non-responsive (Chapter 2

and 4). This confirms the feasibility of breeding for N efficiency (Bänziger et al., 2000). In general, it seems that different interrelated mechanisms such as better root growth in the subsoil, better N uptake, delayed leaf senescence, better photosynthetic capacity under limited N conditions, and better partitioning of the assimilates to the economic yield contributed to better performance of maize cultivars under low-N conditions (Chapter 2, 3 and 4). Use of some of these traits (high number of EPP, reduced ASI and slow senescence under limited N conditions) in selection of maize cultivars under field conditions, in addition to grain yield, may improve the breeding progress in the development of N-efficient cultivars (Bänziger and Lafitte 1997; Betran et al., 2003b). The relatively good performance of some of the maize cultivars under both low-N and high-N conditions (efficient and responsive) and the difference in the magnitude of gene effects under contrasting N environments (Chapter 1 and 2) also indicate that selection under both low-N and high-N conditions may increase the chance to select for favourable genes which contribute to high grain yield under both conditions. Better understanding of the physiological and genetic basis of N efficiency in the future may help in the development of more N-efficient cultivars. Thus, integration of research activities of crop and soil management, plant nutrition, maize breeding and plant molecular biology is crucial in developing maize cultivars which adapt to low-N conditions (Cakmak, 2001) and enhancing sustainable agriculture and food security in sub-Saharan Africa.

Nitrate leaching and N deficiency are common phenomena, particularly in humid areas, in the tropical environments. It seems that early vigorous growth and the capacity to capture nitrate in the topsoil at the beginning of the season, active root growth in the subsoil at later stage of the crop and better N uptake, slow leaf senescence but which translocate carbon and N from the vegetative part to the economic yield, higher photosynthetic efficiency, and reasonable grain yield under both low-N and high-N conditions are main attributes of an N-efficient ideotype. The results of the present study also suggest that these traits are contributing to N efficiency. However, it may be challenging to develop cultivars which possess all these traits. Plant molecular biology may assist in developing N-efficient cultivars in the future.

SUMMARY

Different physiological mechanisms may be associated with high grain yield in favourable environments and high grain yield in unfavourable environments. CIMMYT has identified N-efficient maize cultivars (cultivars with the ability to realize an above average grain yield under low-N conditions). However, no information on the underlying mechanisms is available for these cultivars. The objectives of the present field studies were: (1) to estimate the magnitude of gene effects and combining ability under contrasting N environments, (2) to study the crop-physiological basis of N efficiency under field conditions, and (3) to study protein quality and quantity under a range of N levels.

Six hundred and thirty five experimental inbred lines (S_2 – S_7) were crossed in different crossing designs (Diallels, North Carolina Design II and Line x Tester cross) and evaluated under high-N and low-N conditions at CIMMYT-Zimbabwe for the genetic study. For physiological, and protein-quality and quantity studies sixteen tropical mid-altitude maize cultivars (quality protein maize, QPM and non-QPM) differing in N efficiency from CIMMYT and Seed-CO International were evaluated under three N levels (low-N, medium-N and high-N) at Harare, Zimbabwe in 2003 and 2004 and Kiboko, Kenya in 2003. The main results of these studies are summarized below:

(1) Low-N stress reduced grain yield by 64% as compared to high-N conditions across all the genetic study trials indicating a high severity of the low-N stress. Some crosses had consistent specific combining ability (SCA) effects under both high-N and low-N conditions while the SCA effects of other crosses changed under high-N and low-N conditions. On average, SCA sum of squares, indicative of non-additive gene effects, explained 51% of the total sum of square among the crosses under low-N conditions but only 36% under high-N conditions. Pair-wise t-test also showed significant differences ($P<0.05$) between the proportion of SCA sum of square for grain yield under high-N and low-N conditions. On the other hand, the contribution of general combining ability (GCA) sum of squares, indicative of additive gene effects, was higher than SCA sum of square (non-additive gene effects) for most of the secondary traits under both high-N and low-N conditions. This indicated the necessity of selecting hybrid combinations under both low-N stress and optimum-N conditions to increase grain yield under both high-N and low-N conditions.

(2) Severe N stress under low-N at Harare (average of Z03N1 and Z04N1) reduced grain yield by 74% as compared to high-N conditions (Harare high-N conditions, Z03N3 and Z04N3). Significant ($P<0.01$) genotypic differences were observed for grain yield, and N-uptake and N-utilization efficiencies. Grain yields ranged from 1.5 – 4.3 t ha^{-1} at Z03N1 and 10.6 – 14.9 t ha^{-1} at Z03N3, both at Harare in 2003. Significant ($P<0.01$) genotype-by-environment interactions were observed. The strong relationships between grain yields and N-uptake and N-utilization efficiencies under low-N conditions across the years and sites indicated that both N-uptake and N-utilization efficiencies contributed to improved performance under low-N conditions.

(3) Significant differences ($P<0.01$) were observed among the cultivars for total root-system size and leaf traits. The root-system size increased with soil N level. No significant positive relationships between total root-system size and grain yields were observed in any of the environments. In addition, the associations between N uptake in the total aboveground biomass at physiological maturity and total root-system size (as measured by root capacitance) were low ($r = -0.23$ to 0.13) under severe low-N stress conditions. However, the N-efficient cultivar 6 had a higher root-length density (as estimated using the soil-core method) and depleted mineral-N in the subsoil more than the N-inefficient cultivar 7 indicating that higher root-length density in the subsoil contributed to N efficiency. The N-efficient cultivars generally had greater leaf longevity and higher leaf chlorophyll concentration compared with the N-inefficient cultivars.

(4) The cultivars significantly ($P<0.01$) varied in ears per plant (EPP), kernel number per ear (KN), kernels per row (KPR), dry matter accumulation after flowering (BIOFH), harvest index (HI) and grain C/N ratio. Significant ($P<0.01$) genotype-by-environment interactions were observed. The N-efficient cultivars had a reduced anthesis-silking interval, a higher dry matter production during grain filling, a higher KN and relatively an increased grain C/N ratio at limited N supply. In addition, the results indicated that N-efficient cultivars had better dry matter partitioning to the reproductive structures than the N-inefficient cultivars under low-N conditions.

(5) Significant ($P<0.01$) genotypic differences were observed among the cultivars for grain-protein content, and endosperm lysine, tryptophan and protein contents. The quantity of grain protein, and endosperm lysine, tryptophan and protein contents decreased as the N level in the soil decreased. However, the endosperm tryptophan and lysine contents were higher for QPM

cultivars than for non-QPM cultivars in all environments. Genotype-by-environment interaction for quality index (percentage of the ratio between endosperm tryptophan content and total endosperm protein) was non-significant indicating the stable effect of the opaque-2 gene for protein quality across the N levels. Although the best QPM cultivar outyielded a non-QPM commercial cultivar under low-N stress conditions, non-QPM cultivars were the best in all environments. This may suggest that further investments are required in long term breeding programs to bring yields of QPM cultivars on par with the best normal endosperm hybrids adapted to mid-altitude areas of eastern and southern Africa.

In general, the results of these field studies indicate that different interrelated mechanisms contribute to N efficiency. The N-efficient cultivars were characterized by better N-uptake and N-utilization efficiencies, greater leaf longevity, higher chlorophyll concentration in the leaves, higher root-length density in the subsoil (as estimated for two contrasting N-efficient cultivars), and more dry matter production and partitioning during and after flowering under limited N supply. There is a possibility to develop N-efficient cultivars that may combine high grain yield and protein quality under low-N conditions.

REFERENCES

Akintoye, H.A., J.G. Kling and E.O. Lucas. 1999. Nitrogen-use-efficiency of single, double and synthetic maize grown at four N levels in three ecological zones of West Africa. Field Crops Research 60:189-199.

Andrade, F.H., C. Vega, S. Uhart, A. Cirilo, M. Cantarero and O. Valentinuz. 1999. Kernel number determination in maize. Crop Science 39: 453-459.

Aquino, P., F. Carrion, R. Calvo and D. Flores. 2001. Selected maize statistics. pp. 45-57. In: Pingali, P.L. (ed.). CIMMYT 1999-2000 World Maize Facts and Trends. Meeting World Maize Needs: Technological Opportunities and Priorities for the Public Sector. CIMMYT, Mexico, D.F.

Asfaw Negasa, Abdisa Gemeda, Tesfaye Kumsa and Gemechu Gedeno. 1997. Agro-ecological and socio-economical circumstances of farmers in East Wellega zone of Oromia. Research Report No. 32, IAR, Addis Abeba, Ethiopia.

Balconi, C., D. Bosio, E. Rizzi, L. Nembrini and M. Motto. 1997. Effects of organic and inorganic N supplies on protein and carbohydrates accumulation in high and low protein maize endosperms grrown in vitro. Maydica 42: 257-264.

Baker, R.J. 1978. Issues in diallel analysis. Crop Science 18 (4): 533-536.

Bänziger, M., F.G. Betran and H.R. Lafitte. 1997. Efficiency of high N selection environments for improving maize for low N target environments. Crop Science 37:1103-1109.

Bänziger, M. and H.R. Lafitte. 1997. Efficiency of secondary traits for improving maize for low N target environments. Crop Science 37:1110-1117.

Bänziger, M., G.O. Edmeades and H.R. Lafitte. 1999. Selection for drought tolerance increases maize yields across a range of N levels. Crop Science 39: 1035-1040.

Bänziger, M., G.O. Edmeades, D. Beck and M. Bellon. 2000. Breeding for Drought and N Stress Tolerance in Maize: From Theory to Practice, Mexico, D.F., CIMMYT.

Bänziger, M., G.O. Edmeades and H.R. Lafitte. 2002. Physiological mechanisms contributing to the increased N stress tolerance of tropical maize selected for drought tolerance. Field Crops Research 75: 223-233.

Bänziger, M. and J. D. Meyer. 2002. Collaborative maize variety development for stress prone environments in Southern Africa. pp. 269-296. Farmers, Scientists and Plant Breeding. CAB International, London.

Bänziger, M., P.S. Setimela, D. Hodson and B. Vivek. 2005. Breeding for drought tolerance in maize adapted to southern Africa. In: New Directions for a Diverse Planet : Proceedings of the 4th International Crop Science Congress, Brisbane, Australia Sep. 26-Oct. 1, 2004. www.cropscience.org.au.

Basford, K.E. and M. Cooper. 1998. Genotype-by-environment interactions and some considerations of their implications for wheat breeding in Australia. Aust. J. Agric. Res. 49:153-174.

Beck, D.L., J. Betran, M. Bänziger, G.O. Edmeades, J.M. Ribaut, M. Willcox, S.K. Vasal and A. Ortega. 1996. Progress in developing drought and low soil N tolerance in maize. pp. 85-111. In: D. Wilkinson (ed.). Proceedings 51st Annual Corn and Sorghum Research Conf., Chicago, 10-11 Dec. 1996. ASTA, Washington, DC.

Below, F.E. 1997. Growth and productivity of maize under nitrogen stress. pp. 235-240. In: Edmeades, G.O., M. Bänziger, H.R. Mickeison, and C.B. Pena-Valdivia (eds.). Developing Drought and Low-N Tolerant Maize: Proceedings of a Symposium, Elbatan, Mexico, March 25-29, 1996. CIMMYT, Mexico, D.F.

Below, F.E., P.S. Brandau, R.J. Lambert and R.H. Teyker. 1997. Combining ability for N use efficiency in maize. pp. 316-319. In: Edmeades, G.O., M. Bänziger, H.R. Mickeison, and C.B. Pena-Valdivia (eds.). Developing Drought and Low-N Tolerant Maize: Proceedings of a Symposium, Elbatan, Mexico, March 25-29, 1996. CIMMYT, Mexico, D.F.

Benti Tolessa. 1988. Genetic improvement of maize in Ethiopia: Strategies and progress made. pp. 47-60. In Towards Self Sufficiency: Proceedings of the Second Eastern, Central and Southern Africa Regional Maize Workshop, March 15-21, 1987, CIMMYT, Harare, Zimbabwe.

Benti Tolessa, Tasew Gobezayew, Mosisa Worku, Yigzaw Desalegne, Kebede Mulatu and Gezahegne Bogale. 1993. Genetic improvement of maize in Ethiopia: A review. pp. 13-22. In: Benti Tolessa and J.K. Ransom (eds.). Proceedings of the First National Maize Workshop of Ethiopia. May 5-7, 1992, IAR/CIMMYT, Addis Ababa, Ethiopia.

Betran, F.J., D. Beck, M. Bänziger and G.O. Edmeades. 2003a. Genetic analysis of inbred lines and hybrid grain yield under stress and non-stress environments in tropical maize. Crop Science 43: 807-817.

Betran, F.J., D. Beck, M. Bänziger and G.O. Edmeades. 2003b. Secondary traits in parental inbred and hybrids under stress and non-stress environments in tropical maize. Field Crops Research 83: 51-65.

Bhatnagar, S., F.J. Betran and D.K. Transue. 2003. Agronomic performance, aflatoxin accumulation and protein quality of subtropical and tropical QPM hybrids in southern U.S. Maydica 48: 113-124.

Bjarnason, M., W.G. Pollmer and D. Klein. 1976. Inheritance of modified endosperm structure and lysine content in opaque-2 maize: I. Modified endosperm structure. Cereals Research Communications 4 (4): 401-410.

Bjarnason, M., and S.K. Vasal. 1992. Breeding of quality protein maize. pp. 181-216. In: J. Janick (ed.). Plant Breeding, John Wiley & Sons, Inc., UK.

Borras, L. and M.E. Otegui. 2001. Maize kernel weight response to postflowering source-sink ratio. Crop Science 49: 1816-1822.

Blum, A. 1997. Constitutive traits affecting plant performance under stress. pp. 131-135. In: Edmeades, G.O., M. Bänziger, H.R. Mickeison and C.B. Pena-Valdivia (eds.). Developing Drought and Low-N Tolerant Maize: Proceedings of a Symposium, Elbatan, Mexico, 25-29 March 1996. CIMMYT, Mexico, D.F.

Byerlee, D. and P.W. Heisey. 1996. Past and potential impacts of maize research in sub-Saharan Africa: Critical assessment. Food Policy 21 (3): 255-277.

Cakmak, I. 2001. Plant nutrition research: Priorities to meet human needs for food in sustainable ways. pp. 4-7. In: Horst et al. (eds.). Plant Nutrition- Food Security and Sustainability of Agro-ecosystems. Kluwer Acadamic Publishers. The Netherlands.

Ceccarelli, S. 1989. Wide adaptation: How wide? Euphytica 40(3):197-205.

Ceccarelli, S., and S. Grando. 1991. Environment of selection and type of germplasm in barley breeding for low yielding conditions. Euphytica 57 (3): 207-219.

Ceccarelli, S., S. Grando and J. Hamblin. 1992. Relationship between barley grain yield measured in low and high yielding environments. Euphytica 64(1-2): 49-58.

Ceccarelli, S. 1996. Positive inerpretation of genotype-by-environment interactions in relations to sustainability and biodiversity. pp. 467-486. In: Cooper M. and G.L. Hammer (eds.). Plant Adaptation and Crop Improvement. CAB International and IRRI, UK.

CIMMYT. 1999. The improvement and promotion of quality protein maize in selected developing countries, CIMMYT, Mexico, D.F.

CIMMYT. 2001. The improvement and promotion of quality protein maize in selected developing countries, CIMMYT, Mexico, D.F.

CIMMYT. 2002. The development and promotion of quality protein maize in sub-Saharan Africa, Zimbabwe. CIMMYT, Harare.

Conway, G. and G. Toenniessen. 1999. Feeding the world in the twenty-first century. Nature, 402: 55-58.

Cooper, M. and D.E. Byth. 1996. Understanding plant adaptation to achieve systematic applied crop improvement: A fundamental challenge. pp. 5-23. In: M. Cooper and G.L. Hammer (eds.). Plant Adaptation and Crop Improvement. CAB International and IRRI, UK.

Corbett, J.D. 1998. Classifying maize production zones in Kenya through multivariate cluster analysis. pp. 15-25. In: Hassan, R.M. (ed.) Maize Technology Development and Transfer in Kenya: A GIS Application for Research Planning in Kenya. CAB International, New York.

Crossa, J., H.G. Gauch and R.W. Zobel. 1990. Additive main effects and multiplicative interaction analysis of two international maize cultivar trials. Crop Science 30: 493-500.

Dass, S., Y.P. Dang, A.K. Dhawan, N.N. Singh and S. Kumar. 1997. Morpho-physiological basis for tolerant maize genotypes in India. pp. 107-111. In: Edmeades, G.O., M. Bänziger, H.R. Mickeison and C.B. Pena-Valdivia (eds). Developing Drought and Low N Tolerant Maize: Proceedings of a Symposium, Elbatan, Mexico, March 25-29, 1996. CIMMYT, Mexico, D.F.

Delacy, I.H., K.E. Basford, M. Cooper, J.K. Bull and C.G. Mcharen. 1996. Analysis of multi-environment trials: An historical perspective. pp. 39-124. In: Cooper, M. and G.L.

Hammer (eds.). Plant Adaptation and Crop Improvement. CAB International and IRRI, UK.

Dhillon, B.S. and W.J. Pollmer. 1978. Combining ability analysis of an experiment conducted in two contrasting environments. EDV in Medizin und Biologie 9 (3/4): 109-111.

Diallo, A.O., W. Muasya and H. de Groote. 2003. Combining ability of early maize inbred lines and yield responses of their single cross hybrids tested under drought, low N and optimum conditions. pp. 36-37. In: Conrad, J., D. Fox, K. Harrington and M. Listman (eds.). Book of Abstracts: Arnel R. Hallauer International Symposium on Plant Breeding. CIMMYT, 17-22 August 2003, Mexico City. Mexico, D.F.

Dowswell, C.R., R.L. Paliwal and R.P. Cantrell. 1996. Maize in the Third World. West Views Press, INC, Colorado, USA.

Duvick, D.N. 1997. What is yield? pp. 332-335. In: Edmeades, G.O., M. Bänziger, H.R. Mickeison and C.B. Pena-Valdivia (eds). Developing Drought and Low-N Tolerant Maize: Proceedings of a Symposium, Elbatan, Mexico, March 25-29, 1996. CIMMYT, Mexico, D.F.

Ebdon, J.S. and H.G. Gauch. 2002. Additive main effects and multiplicative interaction analysis of national turfgrass performance trials: Interpretation of genotype x environment interaction. Crop Science 42:489-496.

Eberhart, S.A. and W.A. Russell. 1966. Stability parameters for comparing varieties. Crop Science 6 : 36-40.

Edmeades, G.O., J. Bolanos, M. Hernandez and S. Bello. 1993. Causes for silk delay in a lowland tropical population. Crop Science 33: 1029-1035.

Edmeades, G.O., J. Bolanos, A. Elings, J.M. Ribaut, and M. Bänziger. 2000. The role and regulation of the anthesis-silking interval in maize. pp. 43-73. In: Physiology and Modeling Kernel Set in Maize: CSSA Special Publication No. 29. Crop Science Society of America and American Society of Agronomy, Madison, USA.

Edmeades, G., M. Bänziger, H. Campos and J. Schussler. 2003. Improving tolerance to abiotic stresses in staple crops: a random or planned process? pp. 12-13. In: Conrad, J., D. Fox, K. Harrington and M. Listman (eds.). Book of Abstracts: Arnel R. Hallauer International Symposium on Plant Breeding, 17-22 August 2003, Mexico City. CIMMYT , Mexico, D.F.

Falconer, D.S. 1990. Introduction to Quantitative Genetics. John Wiley & Sons, INC., New York.

Federer, W.T. 1977. Experimental Design, Theory and Application. McMillan, New York.

Fehr, W.R. 1991. Principles of Cultivar Development: Theory and Technique. Iowa State University, USA.

Feil, B., R. Thiraporn and P. Stamp. 1993. Can maize cultivars with low mineral nutrient concentrations in the grains help to reduce the need for fertilizers in third world countries? pp. 295-299. In: Randall, P.J. (ed.), Genetic Aspects of Plant Mineral Nutrition. Kluwer Acadamic Publishers. The Netherlands.

Fox, P.N., J. Crossa and I. Romagosa. 1997. Multi-environment testing and genotype x environment interaction. In: Kempton, R.A. and P.N. Fox (eds.). Statistical Methods for Plant Variety Evaluation. Chapman and Hall, London.

Friesen, D.K., S.R. Waddingiton, A.O. Diallo and K. Kanampiu. 2002. Breeding and agronomic approaches to managing abiotic stresses in maize. pp. 214-222. In: Mandefro Nigussie, D. Tanner, and S. Twumasi-Afriyie (eds.). Enhancing the Contibution of Maize to Food Security in Ethiopia: Proceedings of the Second National Maize Workshop of Ethiopia, Addis Abeba, 12-16 November 2001. CIMMYT and EARO, Addis Abeba.

Gallais, A. and B. Hirel. 2004. An approach to the genetics of nitrogen use efficiency in maize. Journal of Experimental Botany 55 (396): 295-306.

Gan, S. and R.M. Amasino. 1997. Making sense of senescence: Molecular genetic regulation and manipulation of leaf senescence. Plant Physiology 113: 313-319.

Gauch, H.G. 1992. Statistical Analysis of Regional Yield Trials: AMMI Analysis of Factorial Designs. Elsevier, New York.

Graham, R.D. 1984. Breeding for nutritional characteristics in cereals. pp. 57-102. In: Tinker, P. B.and Lauchli, A. (eds.). Advances in Plant Nutrition 1.

Griffing, B. 1956. Concept of general and specific combining ability in relation to diallel crossing systems. Australian J. Biol. Sci. 9: 463-493.

Grudloyma, P., S. Prasitwattanaseree, M. Pumklom and W. Duangjam. 2003. Identification of drought and low N tolerant maize germplasm in Thailand. pp. 40-41. In: Conrad, J., D.

Fox, K. Harrington and M. Listman (eds.). Book of Abstracts: Arnel R. Hallauer International Symposium on Plant Breeding. 17-22 August 2003, Mexico City. CIMMYT , Mexico, D.F.

Hallauer, A.R. and J.B. Miranda Fo. 1988. Quantitative genetics in maize breeding. Iowa State University Press, Ames, IA.

Heisey, P.W. and W. Mwangi. 1997. Fertilizer use and maize production. pp. 193-211. In: Byerlee D. and C.K. Eicher (eds.). Africa's Emerging Maize Revolution. Lynne Rienner Publishers, INC., London.

Heuberger, H.T. 1998. Nitrogen efficiency in tropical maize- indirect selection criteria with special emphasis on morphological characterstics. PhD Thesis, University of Hannover. Hannover, Germany.

Hornetz, B., C.A. Shisanya, N. Kanata and M. Gitonga. 2000. Studies on the ecophysiology of locally suitable cultivars of food crops and soil fertility monitoring in the semi-arid areas of south east Kenya: A final report on collaborative research project between Kenyatta University, Kenya and the University of Trier, Germany. University of Trier, Germany.

Horst, W.J., T. Behrens, H. Heuberger, M. Kamh, G. Reidenbach and F. Wiesler. 2003: Genotypic differences in nitrogen use-efficiency in crop plants. pp. 75-92. In: Lynch, J.M., J.S. Schepers and I. Ünver (eds.): *Innovative Soil-Plant Systems for Sustainable Agricultural Practices* . OECD.

Horwitz, W. 1980. Official Methods of Analysis. The Association of Official Analytical Chemists. Benjamin Franklin Station, Washington, DC.

ICRAF. 1994. Field Moist Soils-Speciality Analyses. ICRAF, Nairobi, Kenya.

Johnson, E.C., K.S. Fisher, G.O. Edmeades and A.F.E. Palmer. 1986. Recurrent selection for reduced plant height in lowland tropical maize. Crop Science 26: 253-260.

Kamprath, E.J., R.H. Moll and N. Rodriquez. 1982. Effects of N fertilization and recurrent selection on performance of hybrid populations of corn. Agronomy Journal 74: 955-958.

Kling, J.G., S.O. Oikeh, H.A. Akintoye, H.T. Heuberger and W.J. Horst. 1997. Potential for developing N use-efficient maize for low input agricultural systems in the moist savannas of Africa. pp. 490-501. In: Edmeades, G.O., M. Bänziger, H.R. Mickeison

and C.B. Pena-Valdivia (eds.). Developing Drought and Low-N Tolerant Maize: Proceedings of a Symposium, Elbatan, Mexico, 25-29 March 1996. CIMMYT, Mexico, D.F.

Kumwenda, J.D.T., S.R. Waddington, S.S. Snapp, R.B. Jones and M.J. Blackie. 1997. Soil fertility management in southern Africa. pp. 157-172. In: Beyer D. and C.K. Eicher (eds.). Africa's Emerging Maize Revolution. Lynne Rienner Publishers, INC. Colorado.

Laegreid, M., O.C. Bockman and O. Kaarstad. 1999. Agriculture, Fertilizers and the Environment. CBI Publishing, U.K.

Lafitte, H.R. and G.O. Edmeades. 1994a. Improvement for tolerance to low soil N in tropical maize. I. Selection criteria. Field Crops Research 39:27-38.

Lafitte, H.R. and G.O. Edmeades. 1994b. Improvement for tolerance to low soil N in tropical maize II. Grain yield, biomass production and N accumulation. Field Crops Research 39: 15-25.

Lafitte, H.R. and G.O. Edmeades. 1994c. Improvement for tolerance to low soil N in tropical maize. III. Variation in yield across environments. Field Crops Research 39:27-38.

Lafitte, H.R. and G.O. Edmeades. 1995. Association between traits in tropical maize inbred lines and their hybrids under high and low soil N. Maydica 40: 259-267.

Lamkey, K.R. and J.W. Edwards. 1999. Quantitative genetics of heterosis. pp. 31-48. In: Coors J.G. and S. Pandey (eds.). The Genetics and Exploitation of Heterosis in Crops. Crop Science Society of America, Inc., Wisconsin, USA.

Lemcoff, J.H. and R.S. Loomis. 1986. Nitrogen influences on yield determination in maize. Crop Science 26: 1017-1022.

Lynch, J. 1998. The role of nutrient-efficient crops in modern agriculture. pp. 241-261. In: Rengel, Z. (ed.). Nutient Use in Crop Production. Haworth Press, Inc. New York.

Ma, B.L. and L.M. Dwyer. 1998. Nitrogen uptake and use of two contrasting maize hybrids differing in leaf senescence. Plant and Soil 199: 283-291.

Manske, G.G.B., J.I. Ortiz-Monasterio and P.L.G. Vlek. 2001. Techniques for measuring genetic diversity in roots. pp. 208-218. In: Reynods, M.P., J.I. Ortiz-Monasterio and

A. McNab (eds.). Application of Physiology in Wheat Breeding. CIMMYT, Mexico, D.F.

Menkir, A., B. Badu-Apraku, C. The and A. Adepoju. 2003. Evaluation of heterotic patterns of IITA's lowland white maize inbre lines. Maydica 48: 161-170.

Messmer, R., Y. Fracheboud, M. Bänziger, P. Stamp and J. M. Ribaut. 2004. Genetic analysis of drought tolerance in tropical maize. pp. In: Book of Abstracts. Resilient Crops for Water Limited Environments. CIMMYT/Rockfeller Foundation Workshop, May 24-28, 2004, CIMMYT, Mexico, D.F.

Mi, G., J. Liu, F. Chen, F. Zhang, Z. Cui and X. Liu. 2003. Nitrogen uptake and remobilization in maize hybrids differing in leaf senescence. Journal of Plant Nutrition 26 (1): 237-247.

Moll, R.H., E.J. Kamprath and W.A. Jackson. 1982. Analysis and interpretation of factors which contribute to efficiency of N utilization. Agronomy Journal 74:502-564.

Motto, M. 1979. Heritability and interrelations of seed quality and agronomic traits in a modified opaque-2 synthetic variety of maize. Maydica XXIV: 193-202.

Mugo, S. and D. Hoisington. 2002. Biotechnology for the improvement of maize for resource poor farmers: The CIMMYT approach. pp. 203-213. In: Mandefro Nigussie, D. Tanner, and S. Twumasi-Afriyie (eds.). Enhancing the Contibution of Maize to Food Security in Ethiopia: Proceedings of the Second National Maize Workshop of Ethiopia, Addis Abeba, 12-16 November 2001. CIMMYT and EARO, Addis Abeba.

Mugo, S.N., G.O. Edmeades and D.T. Kirubi. 2003. Genetic improvement for drought tolerance increases tolerance to high plant density in tropical maize under low input levels. pp. 50-51. In: Conrad, J., D. Fox, K. Harrington and M. Listman (eds.). Book of Abstracts: Arnel R. Hallauer International Symposium on Plant Breeding . 17-22 August 2003, CIMMYT, Mexico, D.F.

National Research Council. 1988. Quality Protein Maize. National Academy Press Washington. DC.

Noh, Y.S. and R.M. Amasino. 1999. Regulation of developmental senescence is conserved between Arabidopsis and Brassica napus. Plant Molecular Biology 41: 195-206.

Oikeh, S.O. 1996. Dynamics of soil N in cereal-based cropping systems in the Nigerian savanna. PhD Thesis. Ahmadu Bello University, Zaria, Nigeria.

Oikeh, S.O., J.G. Kling, W.J. Horst, V.O. Chude and R.J. Carsky. 1999. Growth and distribution of maize roots under N fertilization in plinthite soil. Field Crops Research 62:1-13.

Ortiz-Monasterio, J.I., G.G.B. Manske and M. van Ginkel. 2001. Nitrogen and phosphorus use efficiency. pp. 200-207. In: Reynods, M.P., J.I. Ortiz-Monasterio, and A. McNab (eds.). Application of Physiology in Wheat Breeding: CIMMYT, Mexico, D.F.

O'Toole, J.C. and W.L. Bland. 1987. Genotypic variation in crop plant root systems. Advances in Agronomy 41: 91-145.

Paponov, I.A. and C. Engels. 2003. Effect of nitrogen supply on leaf traits related to photosynthesis during grain filling in two maize genotypes with different N efficiency. J. Plant Nutr. Soil Sci. 166: 756-763.

Patterson, H.D. and E.R. Williams. 1976. A new class of resolvable incomplete block designs. Biometrica 63: 83-89.

Pingali, P.L. and S. Pandey. 2001. Meeting world maize needs: Technological opportunities and priorities for public sector. pp. 1-20. In: Pingali P.L. (ed.) CIMMYT 1999-2000 World Maize Facts and Trends. Meeting World Maize Needs: Technological Opportunities and Priorities for the Public Sector. CIMMYT, Mexico, D.F.

Pixley, K.V. and M.S. Bjarnason. 1993. Combining ability for yield and protein quality among modified endosperm opaque-2 tropical maize inbreds. Crop Science 33: 1229-1234.

Pixley, K.V. 2001. Quality Protein Maize: Overview and Breeding Strategies. CIMMYT, Harare, Zimbabwe.

Pixley, K.V. and M.S. Bjarnason. 2002. Stability of grain yield, endosperm modification, and protein quality of hybrid and open-pollinated quality protein maize cultivars. Crop Science 42: 1882-1890.

Presterl, T., S. Groh, M. Landbeck, G. Seitz, W.Schmidt and H. H. Geiger. 2002. Nitrogen uptake and utilization efficiency of European maize hybrids developed under conditions of low and high nitrogen input. Plant Breeding 121:480-486.

Presterl, T., G. Seitz, M. Landbeck, E. M. Thiemt, W. Schmidt and H. H. Geiger. 2003. Improving nitrogen-use efficiency in European maize: Estimation of quantitative genetic parameters. Crop Sci. 43: 1259-1265.

Rajcan, I. and M. Tollenaar. 1999a. Source : sink ratio and leaf senescence in maize: I. Dry matter accumulation and partitioning during grain filling. Field Crops Research 60: 245-253.

Rajcan, I. and M. Tollenaar. 1999b. Source : sink ratio and leaf senescence in maize: II. Nitrogen metabolism during grain filling. Field Crops Research 60: 255-265

Ransom, J.K., K. Short and S. Waddington. 1993. Improving productivity of maize under stress conditions. pp. 30-33. In: Benti Tolessa and J.K. Ransom (eds.). Proceedings of the First National Maize Workshop of Ethiopia. Addis Abeba, May 5-7, 1992. CIMMYT, Addis Abeba, Ethiopia.

Reeves, T., P. Pinstrup-Andersen and R. Pandya-Lorch. 1999. Food security and the role of agricultural research. pp. 1-5. In: Coors J.G. and S. Pandey (eds.). The Genetics and Exploitation of Heterosis in Crops. Crop Science Society of America, Inc., Wisconsin, USA.

Romagosa, I. and P.N. Fox. 1993. Genotype by environment interaction and adaptation. pp. 373-390. In: Hayward, M.D., N.O. Bosemark and I. Romagosa (eds.). Plant Breeding: Principles and Prospects. Chapman and Hall, London.

Sallah, P.Y.K., N.J. Ehlke and J.L. Geadelmann. 1997. Selection for response to low N in the La Posta population. pp. 502-507. In: Edmeades, G.O., M. Bänziger, H.R. Mickeison and C.B. Pena-Valdivia (eds.). Developing Drought and Low-N Tolerant Maize: Proceedings of a Symposium, Elbatan, Mexico, March 25-29, 1996. CIMMYT, Mexico, D.F.

Santos, M.X., M.A. Lopes, A.M. Coelho, P.E.O. Guimaraes, S.N. Parentoni, E.E.G. Gama and G.E. Franca. 1997. Drought and low N status limiting maize production in Brazil. pp. 20-23. In: Edmeades, G.O., M. Bänziger, H.R. Mickeison and C.B. Pena-Valdivia (eds.). Developing Drought and Low N Tolerant Maize: Proceedings of a Symposium, Elbatan, Mexico, March 25-29, 1996. CIMMYT, Mexico, D.F.

SAS Institute. 2001. The SAS System for Windows Release 8.02. SAS Institute INC., Cary, NC.

Sattelmacher, B., W.J. Horst and H.C. Becker. 1994. Factors that contribute to genetic variation for nutrient efficiency of crop plants. Z. Pflanzenernähr. Bodenk., 157: 215-224

Scott, M.P., E.H. Bicar, A. Darrigues, M. Lee and K.R. Lamkey. 2003. Breeding for grain quality. pp. 24-25. In: Dee J., C. Fox, K. Harrington and M. Listman (eds.). Book of Abstracts: Arnel R. Halluer International Syposium on Plant Breeding., 17-22 August 2003, CIMMYT, Mexico, D.F.

Sibale, E.M. and M.E. Smith. 1997. Relationship between secondary traits and grain yield of maize in low N soils in Malawi. pp. 245-248. In: Edmeades, G.O., M. Bänziger, H.R. Mickeison and C.B. Pena-Valdivia (eds). Developing Drought and Low N Tolerant Maize: Proceedings of a Symposium, Elbatan, Mexico, March 25-29, 1996. CIMMYT, Mexico, D.F.

Simonds, N.W. 1991. Selection for local adaptation in a plant breeding program. Theory. Appl. Genetics 82:363-367.

Singh, B.D. 2003. Plant Breeding Principles and Methods. Kalyani Publishers, New Delhi.

Singh, R.K. and B.D. Chaudhary. 1985. Biometrical Methods in Quantitative Genetic Analysis. KALIYANI Publishers. New Delhi.

Sinclair, T.R. 1998. Historical changes in harvest index and crop N accumulation. Crop Science 38(3): 638-643.

Ta, C.T. and R.T. Weiland. 1992. Nitrogen partitioning in maize during ear development. Crop Science 32: 443-451.

Tennant, D. 1975. A test of modified line intersect method of estimating root length. Journal of Ecology 63: 995-1001.

Tilahun T., G. Ayana, F. Abebe and D. Wegary. 2002. Maize pathology research in Ethiopia: A review. pp. 97 - 105. In: Mandefro Nigussie, D. Tanner, and S. Twumasi-Afriyie (eds.). Enhancing the Contibution of Maize to Food Security in Ethiopia: Proceedings of the Second National Maize Workshop of Ethiopia, Addis Abeba, 12-16 November 2001. CIMMYT and EARO, Addis Abeba.

Uhart, S.A. and F.H. Andrade. 1995. Nitrogen deficiency in maize: II. Carbon-nitrogen interaction effects on kernel number and grain yield. Crop Science 35:1384-1389

Van Beem, J.V. and M.E. Smith. 1997. Variation in N use-efficiency and root-system size in temperate maize genotypes. pp. 241-244. In: Edmeades, G.O., M. Bänziger, H.R. Mickeison and C.B. Pena-Valdivia (eds.). Developing Drought and Low N Tolerant

Maize: Proceedings of a Symposium, Elbatan, Mexico, March 25-29, 1996. CIMMYT, Mexico, D.F.

Van Beem, J. and M.E. Smith. 1998. Estimating root mass in maize using portable capacitance meter. Agron. J. 90: 566-570.

Vasal, S.K., E. Villegas, M. Bjarnason, B. Gelaw and P. Goertz. 1980. Genetic modifiers and breeding strategies in developing hard endosperm Opaque-2 materials. pp. 37-73. In: Pollmer, W.G. and R.H. Phipps (eds.). Improvement of Quality Traits of Maize for Grain and Silage Use. Martinus Nijhoff Publishers, Boston.

Vasal, S.K., E. Villegas and C.Y. Tang. 1984. Recent advances in the development of quality protein maize germplasm at Centro Internacional De Mejoramento De Maiz Y Trigo (CIMMYT). pp. 167-189. Cereal Grain Protein Improvement: International Atomic Energy Agency, Vienna.

Vasal, S.K. 2000. The quality protein story. Food and Nutrition Bulletin 21 (4): 445-450.

Vasal, S.K. 2001. High quality protein corn. pp. 85-129. In A.R. Hallauer (ed.). Speciality Corns. CRC Press, New York.

Villegas, E., E. Ortega and R. Bauer. 1984. Chemical methods used at CIMMYT for Determining Protein Quality in Cereal Grains. CIMMYT, Mexico City, Mexico.

Westgate, M.E. 1997. Physiology of flowering in maize: Identifying avenues to improve kernel set during drought. pp. 136-141. In: Edmeades, G.O., M. Bänziger, H.R. Mickeison and C.B. Pena-Valdivia (eds.). Developing Drought and Low-N Tolerant Maize: Proceedings of a Symposium, Elbatan, Mexico, March 25-29, 1996. CIMMYT, Mexico, D.F.

Wiesler, F. and W.J. Horst. 1993. Differences among maize cultivars in the utilization of soil nitrate and the related losses of nitrate through leaching. Plant and Soil 151:193-203.

Wiesler, F. and W.J. Horst. 1994a. Root growth of maize cultivars under field conditions as studied by the core and minihizotron method and relationship to shoot growth. Z. Pflanzenernähr.Bodenk. 157: 351-358.

Wiesler, F. and W.J. Horst. 1994b. Root growth and nitrate utilization of maize cultivars under field conditions. Plant and Soil 163: 267-277.

Wiesler, F. 1997. Agronomical and physiological aspects of ammonium and nitrate nutrition of plants. Z. Pflanzenernähr. Bodenk. 160: 227-238.

Wiesler, F., T. Behrens and W.J. Horst. 2001. The role of N-efficient cultivars in sustainable agriculture. The Scientific World.

Zaidi, P.H., G. Srinivasan and C. Sanchez. 2003. Gains from stress-adaptive changes with selection for mid-season drought tolerance in tropical maize. pp. 80 – 81. In: Conrad, J., D. Fox, K. Harrington and M. Listman (eds.). Book of Abstracts: Arnel R. Hallauer International Symposium on Plant Breeding ., 17-22 August 2003, CIMMYT. Mexico, D.F.

Zambezi, B.T. and C. Mwambula. 1997. The impact of drought and low soil N on maize production in the SADC region. pp. 29-34. In: Edmeades, G.O., M. Bänziger, H.R. Mickeison and C.B. Pena-Valdivia (eds). Developing Drought and Low N Tolerant Maize: Proceedings of a Symposium, Elbatan, Mexico, March 25-29, 1996. CIMMYT, Mexico, D.F.

ACKNOWLEDGMENTS

I express my sincere thanks and appreciation to my supervisors, Prof. Dr. Walter J. Horst, Dr. M. Bänziger and Dr. D. Friesen for their guidance, consistent support and fruitful discussions that enabled me to produce this work within the required period of time. They have tirelessly guided and encouraged me throughout the course of my study and writing of the dissertation.

Dr. Gunda Schulte auf'm Erley is gratefully acknowledged for her valuable comments and discussions. Dr. B. Vivek is also gratefully acknowledged for his comments and assistance in genetic data analysis. The contribution of Dr. A. Diallo, Dr. G. Srinivasan and Dr. K. Pixley is highly appreciated.

A heartfelt appreciation is due to Frau I. Dusy for all her help and giving administration support throughout my study. The administrative support of Ms. C. Samudzimu and Mr. E. Tofa (Zimbabwe), Ms. E. Irungu, Mr. A. Imbai (Kenya) is also highly acknowledged.

It would not have been possible to complete the laborious field and laboratory works without the great efforts and supports of Mr. J. Kasango, Mr. M. Muthoka, Mr. V. Eget, Mr. W. Muasya, Mr. I. Mutabai, Mr. H. Mwangi Mrs. M. Nyambura, Mr. J. Wagoba and Mr. M. Sobo (Kenya) Mr. Hartmut Wieland, Mr. Andre Specht and Mrs. Sabine Klebba-Farber (Germany), Mr. E. Nyamutowa, Mr. S. Peri, Mr. S. Mawere, Mr. W. Tegmo Mr. S. Chisoro, Mr. N. Damu, Mr. G. Chumbo, Mr. M. Massukume and Dr. P. Mapfumo (Zimbabwe), and Dr. J. Lopez (Mexico). My gratitude also goes to Mr. Abdullah Ulas, Mr. Hendrik Führs and Dr. Mahmoud Kamh for introducing me to field techniques at Göttingen (Germany). I am grateful to Dr. Dejene Eticha and Dr. Lemma Ebsa for all their supports and efforts to increase my computer literacy, and assistance in compiling the thesis.

I thank all the staff of the Institute for Plant Nutrition and CIMMYT staff in respective countries for their friendly support during the period of my study.

I express special thanks to CIMMYT and BMZ for giving me the scholarship and funding my study. I am also grateful to Mr. D. Tanner and Dr. S. Twumasi-Afriye for all their help in processing the scholarship. The contribution of EARO is appreciated for giving me study leave.

Finally, I am deeply indebted to my wife, Elsabet Asefa, for her encouragement and taking care of our sons, Melba and Kiyya, during my stay abroad.

CURRICULUM VITAE

I. PERSONAL DETAILS

Name, First name	Worku Regasa, Mosisa
Address	Institute of Plant Nutrition, Herrenhaeuser Str. 2, 30419 Hannover, Germany Tel. +49(0)5117625112, Fax 0049 511 7623611 email: mosisa.worku@pflern.uni-hannover.de
Date of birth	28 June 1962
Place of birth	West Shoa, Oromia, Ethiopia
Sex	Male
Nationality	Ethiopian

II. EDUCATION

Year	School/University	Academic Degree
Primary and Junior Secondary Education		
1970-1971	Winne-Buyama Elementary School	
1972-1975	Shennen Elementary School	Certificate
1976-1977	Tikur Inchini Junior Secondary School	Certificate
Secondary Education		
1978-1981	Ambo Secondary School	School Leaving Certificate
Tertiary Education		
1982-1985	Alemaya University, Ethiopia	BSc. in Plant Sciences
1998-1999	Alemaya University, Ethiopia	MSc. in Agronomy
2002-to-date	University of Hannover, Germany	PhD. in Horticulture

III. WORK EXPERIENCE

Employment

Jan. 1986 - Jan.1988 Junior Researcher (Cereal Crops Improvement), Asosa Research Center, Ethiopia
Jan. 1988 - Jun.1993 Assistant Researcher (Maize Improvement), Bako Research Center, Ethiopia
Jun. 1993 – Jun. 1996 Research Officer II (Maize Improvement), Bako Research Center, Ethiopia
Jun. 1996 – May 2002 Associate Researcher I (Maize Improvement), Bako Research Center, Ethiopia

Leadership

Jan. 2001 – May 2002 National Maize Research Coordinator of Ethiopia

Short Term Training Course

Jun. 1989 – Sep. 1989 Maize Improvement Course, Maize Research Institute, Zemun/Belgrade, Yugosilavia
Oct. 1993 – Nov. 1993 Maize Research and Seed Production Tour in Mexico, Guatemala and Brazil
Sep. 4 – 20, 2000 Stress Breeding Course, CIMMYT, Nairobi, Kenya
Aug. 2002- Sep. 2002 Advanced Maize Breeding Course, CIMMYT, Mexico

IV. PUBLICATIONS

Dawit M., Assefa T. and Mosisa W. 1989. Crop weed competition studies in maize at Assosa. In: Proceedings of the Third Eastern and Southern Africa Maize Workshop. CIMMYT, Mexico D.F.

Dawit M., Assefa T. and Mosisa W. 1989. Determination of optimum time and frequency of hand weeding in tef. In: Proceedings of the Seventh Annual Conference of the Ethiopian Weed Science Society of Ethiopia. IAR, Addis Ababa, Ethiopia.

Mosisa W. Behailu A. and Yigzaw D. 1992. DZ-cr-37: The most adapted tef Variety around Asosa. IAR News Letter Vol. 7, No. 1., IAR, Addis Ababa, Ethiopia.

Benti T., Tassew G., Mosisa W., Yigzaw D., Kebede M. and Gezahegn B. 1993. Genetic improvement of maize in Ethiopia. In: Proceedings of the First National Maize Workshop of Ethiopia. CIMMYT/IAR, Addis Ababa, Ethiopia.

Kebede M., Gezahegh B., Benti T., Mosisa W., Yigzaw D. and Assefa A. 1993. Maize production and research in Ethiopia. In: Proceedings of the First National Maize Workshop of Ethiopia. CIMMYT/IAR, Addis Ababa, Ethiopia.

Mosisa W., Gezahegn B. and Benti T. 1994. Commencement of hybrid maize development and production in Ethiopia. IAR News Letter Vol. 9, No. 4, IAR, Addis Ababa, Ethiopia.

Kebede M., Benti T., Legesse W., Gezahegn B., Mosisa W. and Yigzaw D. 1995. Contribution of CIMMYT germplasm to maize improvement in Ethiopia. IAR News Letter, Vol. 10, No. 1. IAR, Addis Ababa, Ethiopia

Mosisa W., Legesse W., Benti T., Kebede M. and Leta T. 1996. Heterotic patterns of some intermediate maturing maize germplasm. African Crop Science Journal, Vol. 4:4 (497-501)

Legesse W., Mosisa W., Benti T. and Kebede M. 1996. CIMMYT maize varieties perform well at Bako. IAR News Letter Vol. 11:4. IAR, Addis Ababa, Ethiopia.

Benti T., Kebede M., Legesse W., Mosisa W., and Leta T. 1996. Reflections of hybrid maize in Ethiopia. In: Proceedings of the 5th Eastern and Southern Africa Maize Workshop. CIMMYT, Mexico D.F.

Kebede M., Benti T., Legesse W., Mosisa W. and Yigzaw D. 1996. Effect of inducing stresses on ear branching in maize inbred line. In: Proceedings of the First Conference of Agronomy and Physiology Society of Ethiopia. APSE, EARO, Addis Ababa, Ethiopia.

Mosisa W., Legesse W., Benti T. and Kebede M. 1997. Performance of maize varietal and top cross hybrids. IAR News Letter, Vol. 12, No. 3. IAR, Addis Ababa, Ethiopia.

Benti T., Kebede M., Legesse W., Gezahegn B., Mosisa W., and Gemechu K. 1997. Heterosis and genetic diversity in crosses of adapted east African maize composites. In: Proceedings of International Symposium on the Genetics and Exploitation of Heterosis in Crops, CIMMYT, Mexico D.F.

Mosisa W., Legesse W., Benti T. and Gemechu K. 1998. GH-232-28: The QPM hybrid outyielded the normal commercial hybrid maize, BH-140 at Bako. Agrotopia Vol. 13 No. 1. EARO, Addis Ababa, Ethiopia.

Legesse W., Gemechu K., Mosisa W., Benti T. 1998. GXE interaction in CIMMYT quality protein maize populations. In: Proceedings of Sixth Eastern and Southern Africa Maize Workshop, CIMMYT, Addis Ababa, Ethiopia.

Aschalew G., Legesse W., Gemechu K., Wende A., Benti T., K.V. Pixley and Mosisa W. 1998. Strategies for quality protein maize (QPM) breeding and dessimination in Ethiopia. In: Proceedings of Sixth Eastern and Southern Africa Maize Workshop. CIMMYT, Addis Ababa, Ethiopia.

Mosisa Worku and Abdisa Gemeda, 1998. Recommended technology for maize production. In: Thirty Five Years of Research Experience. Bako Agricultural Research Center Bulletine No. 2. Oromia Agricultural Development Bureau, Finfinnee/Addis Ababa, Ethiopia.

Girma A., Mosisa W., Dereje B., Abdisa G. and Demissew K. 2001. Participatory analysis of off-season maize production in east and west Wellega zones of Oromia, Ethiopia. Research Report, No.37 EARO, Addis Ababa, Ethiopia

Mosisa W., Hadji T., Mandefro N., and Aberra D. 2002. Maize production trends and research in Ethiopia. In Proceedings of the Second National Maize Workshop of Ethiopia. CIMMYT/EARO, Addis Ababa, Ethiopia.

Mosisa W., Jemal A., Leta T., Hadji T., Legesse W., Kassa Y., Wonde A., Aschalaw G., Sewagegne T., Teshale A., Tamirat B., Yoseph B., Habtamu Z. 2002. Improved germplasm development for the mid and low altitude sub-humid agro-ecologies of Ethiopia. In: Proceedings of the Second National Maize Workshop of Ethiopia. CIMMYT/EARO, Addis Ababa, Ethiopia.

Hadji T., Mosisa W., Tolessa D., Mandefro N., Leta T., Hussien M., Yoseph B., Girma C., Tamirat B. and Taye H. 2002. Maize seed production in research centers. In: Proceedings of the Second National Maize Workshop of Ethiopia. CIMMYT/EARO, Addis Ababa, Ethiopia.

Leta T., Mosisa W., Jemal A., Mandefro N., S. Tuwamsi A., Kassa Y., Yoseph B. 2002. Quality protein maize research in Ethiopia. In: Proceedings of the Second National Maize Workshop of Ethiopia. CIMMYT/EARO, Addis Ababa, Ethiopia.

Mosisa W., Habtamu Z., Girma T., Benti T., Legese W., Wende A., Aschalew G. and Hadji T. 2004. Yield stability of maize genotypes across locations. In: Proceeding of 7th eastern and southern Africa Maize Conference. CIMMYT/KARI, Nairobi, Kenya.

Mosisa W., Haji T., Wende A., Legese W., Alpha D., S. Twumasi A., Aschalew G. 2004. Developing Low N-tolerant Maize varieties for mid altitude sub-humid agro-ecology of Ethiopia. In: Proceedings of 7th Eastern, Centeral and Southern Africa Maize Conference. CIMMYT/KARI, Nairobi, Kenya.

Dereje B., Mossisa W., Hadji T., Leta T., Mendefro N., Jemal A., S.Twumasi A. 2004. On farm performance of CIMMYT quality protien maize varieties in Ethiopia.In: Proceedings of 7th Eastern, Centeral and Southern Africa Maize Conference. CIMMYT/KARI, Nairobi, Kenya.

Mosisa W., Habtamu Z., Girma T., Benti T., Legese W., Wende A., Aschalew G. and Hadji T. 2004. Advances in improving harvest index of maize. In: Asfaw et al. (eds.). Proceedings of the Tenth Conference of Crop Science Society of Ethiopia (CSSE). EARO, Addis Ababa, Ethiopia.

Mosisa W., Hadji T., Legesse W., Wende A., Aschalew G. and. Danye W. 2004. S1 Selection for early maturity in an open pollinated maize variety and its effect on other important characters. In: Asfaw et al. (eds.). Proceedings of the Tenth Conference of Crop Science Society of Ethiopia (CSSE). EARO, Addis Ababa, Ethiopia.

Mosisa W., Habtamu Z., Girma T., Benti T., Legese W., Wende A., Aschalew G. and Hadji T. 2004. Genotype x environment interaction and yield stability of maize genotypes. In: Asfaw et al. (eds.). Proceedings of the Tenth Conference of Crop Science Society of Ethiopia (CSSE). EARO, Addis Ababa, Ethiopia.