

# **Genetic variability and inheritance studies for low pH tolerance in tropical and sub-tropical maize germplasm**

By

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A thesis submitted in accordance with the requirements for the degree Philosophiae Doctor in the Department of Plant Sciences, Division Plant Breeding, in the Faculty of Natural and Agricultural Sciences at the University of the Free State

Bloemfontein, South Africa

2015

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## DECLARATION

I, Kesbell K.E. Kaonga, do hereby declare that the thesis hereby submitted for qualification for the degree Philosophiae Doctor in Agriculture at the University of the Free State represents my own original, independent work and that I have not previously submitted the same work for a qualification at another university/ faculty.

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Date

## **DEDICATION**

This work is specifically dedicated to our last born daughter Deliness Kaonga, who missed parental care during the course of my study, secondly to my wife Judith Lwasha Kaonga, our son Arisai Kaonga, and our daughters Byenala and Clevereen Kaonga for their patience and hard times they may have gone through during my study period.

## ACKNOWLEDGEMENTS

To Almighty God, the Creator and the one who takes care of my life, thank you for keeping me healthy throughout my study period. It is because of You that I have been able to complete my studies. I wish to sincerely thank the Ministry of Agriculture, through the former Principal Secretary, Dr. Andrew Daudi for offering me a Government PhD Scholarship. I don't take this for granted knowing that I was among the very few beneficiaries. I would like to thank the ministry for the financial support and administrative clearance to enable me to undertake the study.

I am indebted to the University of the Free State, Department of Plant Sciences: Plant Breeding, for accepting and registering me as their student. Again I don't take this for granted because I was among the few students that were enrolled during the period. My gratitude goes to the Department of Agriculture Research Services (DARS) for administrative clearance and moral support and encouragement during the entire period of my study. Thank you Dr. A.P. Mtukuso, Dr. Banda and Human Resource staff for administrative issues during my study period.

I wish to convey my sincere gratitude and appreciation to various organisations and individuals who contributed in one way or another in terms of resources and knowledge. It is not possible to mention the names of all individuals and institutions but your valuable contributions have been fully recognised and appreciated:

- CIMMYT-Colombia through Dr. Luis Narro for the maize genotypes used in the study. Populations, released and non-released inbred lines.
- CIMMYT-Zimbabwe through Dr. Amsal Tarekegne for the maize genotypes used in the study. Populations, released and non-released inbred lines.
- IITA - Nigeria through Dr. Abebe and Dr. Apraku for the maize populations as well as the detailed description of maize genotypes which originated from their institution through CIMMYT-Zimbabwe.
- The Soils and Agriculture Engineering Research Commodity Team through Dr. W. Makumba and M. Munthali for guidance in the hydroponic nutrient solution experiment. Laboratory technicians for the nutrient solution preparations and field soil sampling and laboratory analyses.

- The Maize Research Commodity Team Technical Staff (Maize Breeding and Agronomy) for the setting up of the hydroponic nutrient solution experiment in a glasshouse transplanting and initial data collection and final data collection.
- Lilongwe Water Board for the support in distilled water when demand was high to be met by Chitedze Soils and Agriculture Engineering Lab.
- Maize technicians, research attendants and station managers for all research stations that hosted the field trials: Lunyangwa Research, Meru Research, Baka Research, Chitedze Research, Bembeke Research, Bvumbwe Research, Tsangano Research site and Chitala Research.
- Prof M.T. Labuschagne for her excellent supervision and encouragement, material and other valuable support.
- Dr. Amsal Tarekegne of CIMMYT-Zimbabwe for guidance in the breeding work in Malawi and for supervision.
- Dr. Angeline van Biljon (PhD) for supervision and support rendered on recent publications on research done on stress tolerance.
- Dr. B.M. Jumbo of CIMMYT-Kenya for accepting and showing interest to edit papers earmarked for publication from this work.
- Me. S. Geldenhuys of the Plant Breeding office, for all the communications, other administrative issues, moral support and encouragement during the period of my study.
- My fellow PhD students in the Plant Science Department for their cooperation and assistance, academically and socially.

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## ABBREVIATIONS AND SYMBOLS

AD	Days to anthesis
ADD	Agricultural Development Division
AEA	Average environmental axis
AECa	Average environment coordination abscissa
AECo	Average environment coordination ordination
Al	Aluminium
AMMI	Additive main effects and multiplicative interaction
ANOVA	Analysis of variance
ASI	Anthesis-silking interval
ATTC	Agricultural Technology Clearing Committee
B	Boron
BKA	Baka Research Station
BKE	Bembeke Research Site
BKT	Bembeke Turnoff Research Site
C	Carbon
Ca	Calcium
CIMMYT	International Maize and Wheat Improvement Center
Cl	Chlorine
CLA	Chitala Research Station
cm	Centimetre (s)
CML	CIMMYT maize line
CRD	Completely Randomised Design
Cu	Copper
CV	Coefficient of variation
CZE	Chitedze Research Station
DC	Double cross
DM	Downy mildew
DS	Days to silking
DT	Drought tolerant
DT2	Distal transition zone
ExY	Environment by year/season interaction

EH	Ear height
EPP	Ears per plot
F <sub>1</sub>	First filial generation
FAO	Food and Agriculture Organisation of the United Nations
FAOSTAT	Food and Agriculture Organisation Statistics
Fe	Iron
FeHEDTA	Ferric hydroxethylethylenediaminetriacetate.
FEWSNET	Famine early warning system net work
FSRL	Final seminal root length
GxE	Genotype by environment interaction
GxExY	Genotype by environment by year interaction
GxY	Genotype by year interaction
G	Genotype
GA	Genetic advance
GCA	General combining ability
GCV	Genotypic coefficient of variation
GDP	Gross domestic product
GEI	Genotype by environment interaction
GGE	Genotype and genotype by environment interaction
GLS	Gray leaf spot
GT	Grain texture
GWS	Genome wide selection
GY	Grain yield
h <sup>2</sup> b	Broad sense heritability
H	Hydrogen
ha	Hectare (s)
IFPRI	International Food Policy Research Institute
IITA	International Institute of Tropical Agriculture
IPCA	Interaction principal component analysis
ISRL	Initial seminal root length
K	Potassium
KAl (SO <sub>4</sub> ) <sub>2</sub>	Potassium aluminium sulphate
kg ha <sup>-1</sup>	Kilogram per hectare

L	Litre
LB	Leaf blight disease
LOX	Lipoxygenase
LSD	Least significant difference
LU	Lunyangwa Research Station
m	Metre (s)
masl	Metre (s) above sea level
Max	Maximum
Mg	Magnesium
Min	Minimum
Mn	Manganese
Mo	Molybdenum
MOA	Ministry of Agriculture
MRU	Meru Research Station
MSE	Mean square error
MSV	Maize streak virus
MT	Metric ton
MVAC	Malawi Vulnerability Assessment Committee
N	Nitrogen
NADH	Nicotinamideadehyde
NBOS	National Bureau of Statistics
NCSS	Number Cruncher Statistical System
Ni	Nickel
NSRL	Nett seminal root length
NUE	Nitrogen use efficiency
O <sub>2</sub>	Oxygen
OPV	Open-pollinated variety
P	Phosphorus
PAL	Phenylalanine ammonia lyase
PC	Principal component
PCA	Principal component analysis
PCV	Phenotypic coefficient of variation
PH	Plant height

$P_i$	Cultivar performance measure
POD	Peroxidase
QPM	Quality protein maize
QTL	Quantitative trait loci
$r$	Pearson correlation coefficient
$R^2$	Coefficient of determination
$r_{cop}$	Cophenetic correlation
RDP	Rural Development Programme
RE	Rotten ears
RFLP	Restriction fragment length polymorphism
RL	Root lodging
ROS	Reaction oxygen species
Rti	Root tolerance index
S	Sulphur
SCA	Specific combining ability
SE	Standard error
SH	Shelling percentage
SL	Stem lodging
SSA	Sub-Saharan Africa
SVD	Single value decomposition
SWT	100 seed weight
TSA	Tsangano Research Site
TSS	Total sum of squares
UN	United Nations
UPGMA	Unweighted pair-group method with arithmetic averages
US	United States
VIG	Plant vigour
$W_i$	Wricke's ecovalence
WFP	World food programme
Y	Year
Zn	Zinc
$\Sigma$	Summation
$\sigma^2_e$	Error variance

$\sigma^2g$	Genotypic variance
$\sigma^2i$	Stability variance
$\sigma^2o$	Environmental variance
$\sigma^2p$	Phenotypic variance
%	Percent
°C	Degrees Celsius

## CHAPTER 1

### General introduction

#### 1.1 Origin, importance and production constraints of maize

Maize (*Zea mays* L.) is an important crop and is favoured as well as indispensable food for over one billion people in Sub-Saharan Africa (SSA) and Latin America (Gupta *et al.*, 2009). It is a cultivated sub-species of teosinte, a wild naturally found grass with its centre of origin the Mesoamerican region, now Mexico and Central America (Mangelsdorf, 1974). It was discovered by Columbus's men in Cuba in 1492 and later introduced to Europe and Africa by explorers in 1500 as reported by Gibson and Benson (2002). It is a very popular crop but the name "maize" is not English. The genus *Zea* was derived from an old Greek name for a food grass (Mangelsdorf, 1974), while the sub-species "*mays*" derived from Spanish: *maíz* after Taíno *mahiz* (Encyclopædia Britannica, 2010). It has a number of uses and in the tropics it is grown for direct consumption by man and animals as well as various industrial uses (Powell *et al.*, 2004).

Worldwide, reports indicate that maize is cultivated on approximately eight million hectares (ha) of low pH soils (Brewbaker, 1985; Pandey and Gardner, 1992) and yields can be reduced by up to 70% under these conditions (Welcker *et al.*, 2005). Reports also indicate that on these soils, aluminium (Al) or manganese (Mn) toxicity, calcium (Ca), magnesium (Mg), phosphorus (P) and molybdenum (Mo) deficiencies are the main causes of yield reduction (Aldrich *et al.*, 1973; Granados *et al.*, 1993). In Africa, acid soils in the tropical area are estimated to cover 29% of the continent (Eswaran *et al.*, 1997). However, von Uexküll and Mutert (1995) reported that low pH soils are present all over the world with 41% in America, 26% in Asia, 17% in Africa, 10% in Europe and 6% in Australia and New Zealand. Acidity is a major constraint to maize production and other crops on tropical soils. This is because at low pH (pH<5) toxic Al<sup>3+</sup> ions are released into the soil solution, and hinder root growth thus affecting the development of the entire plant (Kochian, 1995; Kidd and Proctor, 2000). Al toxicity causes short, thick and under developed roots and

plants, thus reducing nutrient uptake and increases susceptibility to drought (Sasaki *et al.*, 1996).

## **1.2 Maize production in Malawi**

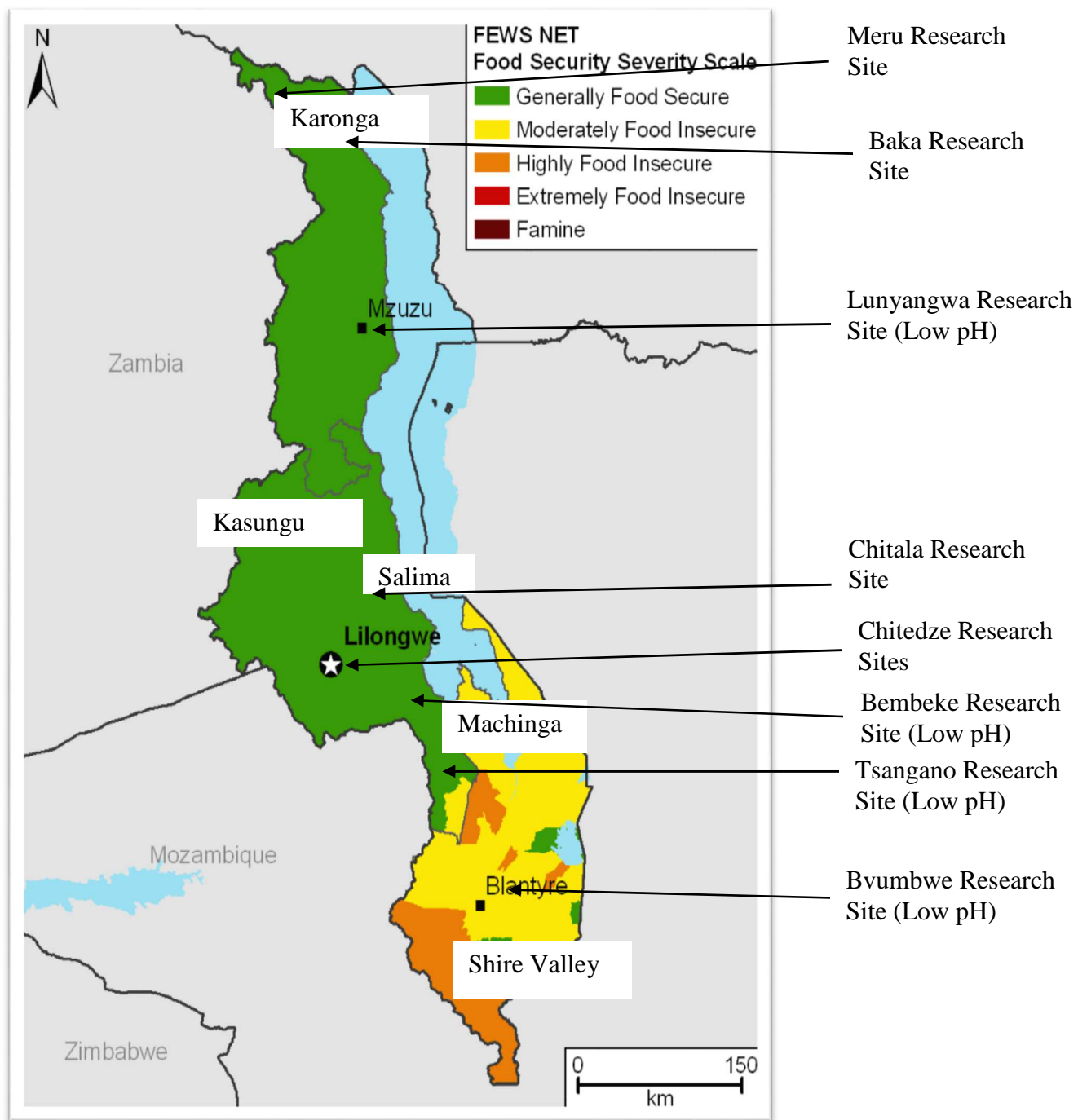
It is commonly said that “maize is life” for countries in SSA and this is true for Malawi more than any other country. The National Bureau of Statistics (NBOS) of the Government of Malawi data for 2006/07 reported that maize represented about 69% of area covered by 16 major crops grown in the country. The FAOSTAT for 2011 estimated that maize represented about 44% of the total area covered by more than 40 crops in Malawi. Other essential crops include groundnut, tobacco, cassava, sweet potato, cotton, rice, soybean, sorghum and millet. Almost 75% of maize in Malawi is cultivated in pure stands while mixed stands represent about 25%. Cultivation is mostly by resource-challenged smallholder farmers (MOA, 1994).

Malawi’s maize requirement is 2.4 million metric tons (MT) per year and in 2009 the country registered a 1.2 million MT surplus while in 2010 the country had a surplus of approximately 800 000 MT this slight reduction as compared to the previous year, probably because of drought in some districts in the southern region (FAOSTAT, 2011). In 2013 the country produced 3.6 MT representing a surplus of 1.2 MT (FAOSTAT, 2013). The country saw a record harvest in 2014 of just over 3.9 MT (GIEWS, 2015)

## **1.3 Maize agro-ecology in Malawi**

Malawi covers an area of 118 000 km<sup>2</sup> which is relatively small, yet it is endowed with diverse agro-ecology areas (Figure 1.1). About 1.2 million ha are grown to maize which is widely cultivated across the 28 districts which are grouped into eight Agricultural Development Divisions or ADDs (Karonga, Mzuzu, Kasungu, Salima, Lilongwe, Machinga, Blantyre and Shire Valley) and three regions (northern, central and southern). Approximately 57% of all maize in Malawi is cultivated in the central region, followed by the southern region (24%) and northern region (19%) (Table 1.1 and Figure 1.2). Among

the ADDs, Karonga, Mzuzu, Kasungu, and Salima combined represent 80% of all area cultivated to maize in the country (MOA, 1994; MVAC, 2013).

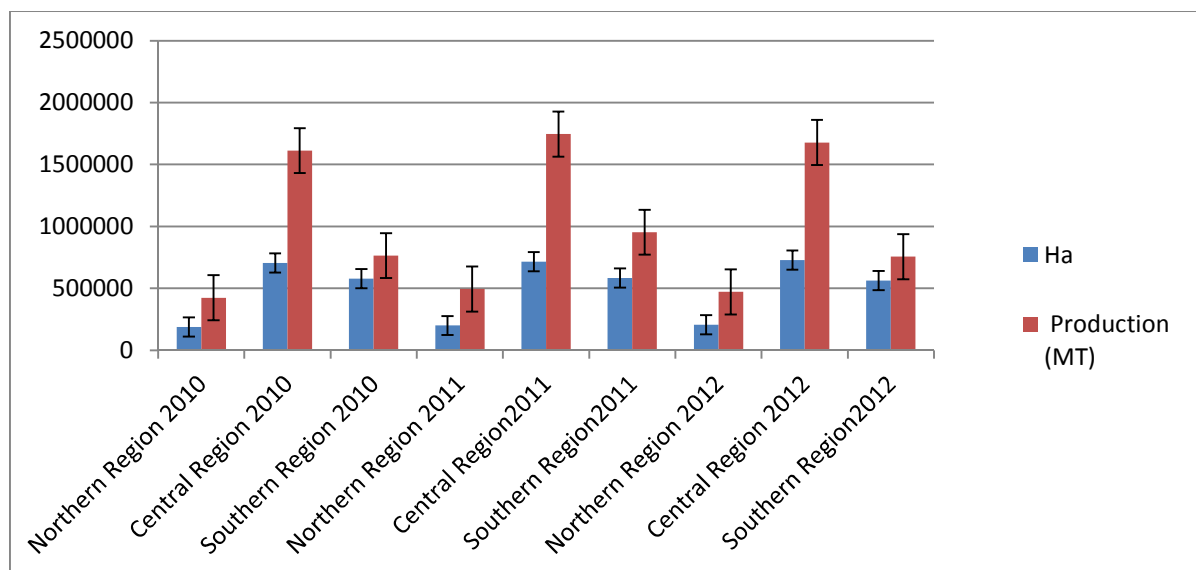


**Figure 1.1 Map of Malawi depicting eight agricultural development divisions and experimental sites at research stations**

**Table 1.1 Malawi mean maize hectarage and production comparisons for 2010 versus 2011 and 2011 versus 2012**

ADD	Area (ha)			Production (MT)			Area (ha)			Production (MT)		
	2009/10	2010/11	% change	2009/10	2010/11	% change	2010/11	2011/12	% change	2010/11	2011/12	% change
Karonga	45855	48960	6.8	116603	137578	18.0	48960	49996	2.1	137578	127381	-7.4
Mzuzu	143569	151262	5.4	307758	357446	16.1	151262	156046	3.2	357446	344552	-3.6
Kasungu	305909	308921	1.0	752808	804331	6.8	308921	323692	4.8	804331	814454	1.3
Salima	59287	60208	1.6	145859	157168	7.8	60208	56659	-5.9	157168	124322	-20.9
Lilongwe	341252	346453	1.5	714180	784013	9.8	346453	347140	0.2	784013	739271	-5.7
Machinga	287509	287976	0.2	389779	382004	-2.0	287976	276207	-4.1	382004	296374	-22.4
Blantyre	250026	254663	1.9	332359	542210	63.1	254663	256199	0.6	542210	438895	-19.1
Shire Valley	42211	42106	-0.2	42211	28594	-32.3	42106	31890	-24.3	28594	20743	-27.5
Total	1475618	1500549	18.0	2801557	3193344	87.4	1500549	1497829	-23.4	3193344	2905992	-105.3

Source: Ministry of Agriculture Crop Estimates for 2010, 2011, 2012



**Figure 1.2 Malawi mean maize hectarage and production per region from 2010 to 2012**

#### **1.4 Abiotic constraints to maize production in Malawi**

Soil acidity is prevalent in most parts of Malawi and a limiting factor in crop production. The increasing population is creating pressure on land and continuous mono-cropping and slashing and burning of crop residues during land clearing have exacerbated the problem. More acid soils are found in the high rainfall areas (>1000 mm per year) where there is moderate to high leaching, while the alkaline soils are found in low rainfall areas (< 500 mm per year). Regions with soil pH less than 5.5 have been identified in the country and according to the soils database prepared by the Soils Commodity Team, over 40% of the country has such soil. The largest hectarage of very acid soils are found in the following ADDs: Lilongwe, Mzuzu and Blantyre. Chilimba (1994) reported higher Al saturation percentages in some areas of Bembeke, Lunyangwa, Nkhatabay and Mulanje. The soil pH in the ADDs in the country is outlined in Table 1.2.

**Table 1.2 Percentage area covered by soil pH values below 5.6 in four agricultural development divisions**

ADD	Soil pH	Area % coverage
Blantyre	4.2 – 5.5	36
Kasungu	4.2 – 5.5	10
Lilongwe	4.7 – 5.5	65
Mzuzu	4.4 – 5.5	33

Source: Chilimba and Saka 1998

The well-known low pH soils are found in most parts of Bembeke, Kanyama and Mayani in Dedza; Namwera rural development programme (RDP) in Mangochi; Tsangano in Ntcheu; Mulanje RDP in Mulanje; Thyolo RDP in Thyolo; Nkhata Bay RDP in Mzuzu ADD (Lunyangwa, Ntchenechena, Mphompha, Uzumala, Mzuzu city, Mzimba central and South Mzimba) and Misuku Hills in Chitipa. High pH soils or alkaline/sodic soils are located in Shire Valley, along Lake Chilwa and Lake Malawi (Chilimba and Komwa, 2003). In such low pH soils, crop yields are limited and sometimes total crop losses occur. For instance, Munthali and Chilimba (2004) reported a yield reduction of more than 85% in low pH soils in Lunyangwa as compared to the potential yield of 8.5-10 ton ha<sup>-1</sup> for maize hybrids under normal fertility conditions.

The problem of low-soil pH can be solved by using soil amendments such as liming, although most farmers in developing countries cannot afford such amendments (Pandey *et al.*, 1994). A more sustainable solution would be to select Al tolerant maize genotypes for use in acid soils which, in the long run, is less expensive, sustainable and more environmentally friendly.

Other abiotic stresses are droughts and floods common in low-land areas of the country. Mazunda and Droppelmann (2012) reported that in a country of which its economic base is heavily dependent on agriculture, not only are the rural livelihoods affected due to the negative impacts on the agricultural sector, but non-farm and urban households are not spared either, given the strong relationship of production and prices between agriculture and the rest of the economy. According to the Malawi Vulnerability Assessment Committee (MVAC, 2010), 718 000 people were declared food insecure between March and June in eight districts in southern Malawi due to poor harvests as a result of prolonged

dry spells in the 2009/2010 season. The number of affected people is expected to increase to 1.1 million by October 2010 (FEWSNET, 2010). FEWSNET (2012) estimated that above one and a half million people would be in need of food relief between October 2012 and March 2013.

Flooding affected the country in early 2013 in such a way that the United Nations (UN) World Food Programme (WFP) in conjunction with the Government of Malawi were providing food relief to about 6 700 households which were flood victims (FEWSNET, 2013). Incidences of food shortages worsen and sharp price increases occur which reduce households' disposable incomes. It is mostly small-scale farmers and those residing in the flood-prone southern regions of the country that stay vulnerable (Selka, 2012). The economic losses as a result of climate related disasters are evident: Malawi loses 1.7% of its gross domestic product (GDP) on average every year due to the combined effects of droughts and floods. This is equivalent to approximately US\$22 million in 2005 prices (Mazunda and Droppelmann, 2012).

### **1.5 Biotic constraints to maize production in Malawi**

Economic importance maize diseases in Malawi include viral disease such as maize streak virus (MSV), fungal diseases such as leaf blight (LB) caused by *Exserohilum turcicum* (Leonard and Snugs) and gray leaf spot (GLS) caused by the pathogen *Cercospora zeae-maydis* (Tehon and Daniels) and downy mildew (DM) another fungal disease caused by the genus *Peronosclerospora*. GLS can cause yield losses of up to 60% (Ringer and Grybauskas, 1995). The most destructive disease world-wide is DM (Frederiksen and Renfro, 1977) and in Malawi two species are known to cause this disease, these are *P. philippinensis* and *P. sorghi*. Two pathotypes of *P. sorghi* have been reported, one capable of infecting both maize and sorghum and the other infecting only maize (Anaso *et al.*, 1987). The disease was first identified in maize in Mozambique (Plumb-Dhindsa and Mondjane, 1984). In Malawi its occurrence on sorghum was reported by Beck (1980) and its observation on maize was in the 2004/05 season in the Blantyre ADD where over 40 000 farm families were left food insecure especially in the Mulanje and Thyolo districts. Adenle and Cardwell (2000) reported that the tassel bracts may proliferate, resulting in a very bushy appearance, causes distortion and/or stunting of the maize plant. It frequently

occurs in areas of fields subject to flooding where the zoospores infect the growing point of the young maize plants.

Another biotic stress in maize production in Malawi is witch weed *Striga spp.* Its origin is not very clear (Holm *et al.*, 1977) and it is believed to be indigenous to tropical and sub-tropical Africa and Asia. In Malawi the most important genera for cereals is *S. asiatica* locally known as *kaufiti* and is the most widely spread in the country as opposed to other witch weeds like *S. hemonthica* and *Alectra vogelli* for legumes. Kabambe *et al.* (2008) reported that yield losses depend on level of infestation, susceptibility of the maize genotype, soil fertility and crop management practices. *Striga* seeds are shed in large numbers (over 50 000 per plant) and remain viable for long time (up to 20 years) (Ramaiah *et al.*, 1983).

## **1.6 Malawi National Maize Breeding Programme**

The Malawian National Maize Breeding Programme, with its main office at Chitedze Agricultural Research Station, was established with the aim of variety development and breeder seed production as well as seed distribution to growers. Major achievements have been reached in the development of new maize varieties and identification of improved varieties for tolerance to stresses obtained from other breeding institutions. To this effect nine maize hybrids were released in 2013, three of which are both drought and low nitrogen (N) tolerant (CIMMYT, 2013). For drought alone, the programme has released a total of five cultivars since 2009. These are Musungabanja (ZM309), Mwayi open-pollinated variety (OPV) ZM523, MH30, MH31 and MH32. In terms of dissemination, four newly released hybrids of 2013 were already selected for production by different seed companies. In terms of nutrition, two quality protein maize (QPM) varieties were released in 2008 and 2009, an OPV, Chitedze2QPM and a hybrid (MH29), respectively (Kaonga, 2009; Mviha *et al.*, 2011). There are a good number of released hybrids from the programme which are tolerant to GLS, MSV as well as LB and are distributed by seed companies.

Despite all these achievements varieties for low pH tolerance are yet to be developed. Hence the objectives of this study were:

1. To evaluate maize genotypes of diverse genetic variability for tolerance to AI as a proxy for low pH tolerance.
2. To study maize genotypes of diverse genetic variability for tolerance to low pH soils by use of phenotypic and morphological traits.
3. To study the genotype by environment interaction (GxE) and stability of the tropical and sub-tropical maize genotypes.
4. To estimate combining ability among well adapted inbred lines and low pH lines from CIMMYT-Colombia.

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## CHAPTER 2

### Literature review

#### 2.1 Importance of maize and consumption levels

Maize is the most significant cereal crop in the Gramineae family in eastern and southern Africa, representing over 29% of the total harvested area of annual food crops and 25% of total caloric intake and income (FAOSTAT, 2010). It is one of the most important food staples in SSA, providing nourishment to over 300 million resource-poor smallholders. Its cultivation spans the entire continent and it is the dominant cereal food crop in many countries accounting for 56% of the total harvested area of food crops and 30-70% of the total caloric consumption (FAOSTAT, 2007).

Consumption is high in Southern Africa; the per capita average is about 195 kg in South Africa, 181 kg in Malawi, 168 kg in Zambia and 153 kg in Zimbabwe (Hassan, 1998). According to Calba *et al.* (2001) it was estimated that for SSA to be food secure by 2050, food production should be multiplied by seven as compared to the 1995 level. This requires proper planning for increased agricultural productivity which is sustainable without or with minimal environmental degradation.

##### 2.1.1 Important abiotic factors affecting maize production

The major abiotic constraints to maize production includes drought, low N soils and low pH soils. With respect to low pH, maize is planted on approximately 8 million ha of acid soils all over the world (Brewbaker, 1985; Pandey and Gardner, 1992). Soil acidity is found to be a major yield-limiting factor for many crops and covers extensive areas of land in tropical, sub-tropical and temperate zones; with low pH occupying approximately 3.95 billion ha, about 30% of the ice free land of the world (von Uexküll and Mutert, 1995). The lower yield of crops grown in acid soil is basically due to combinations of low pH, toxicity of iron (Fe), Al and Mn as well as deficiencies of N, P, Mg and Ca. However, Al toxicity was found to be the main problem in maize production because of root growth inhibition, consequently reducing the water and nutrient uptake and its interference in different

physiological processes of crop development (Roy *et al.*, 1988). The key effect of low pH soil on the plant is a slow growing root system, accompanied by the establishment of surface roots. This negatively influences the use of soil nutrients and induces plants to be more susceptible to drought (Piñeros *et al.*, 2005; Hartwig *et al.*, 2007). Soil amelioration can be implemented by correcting the low pH soil status. However, the use of soil amendments such as liming, which is well known to increase soil pH, may have some adverse effects to the environment and have a temporary effect and are too expensive for resource challenged farmers in developing countries. The low pH change has been reported to occur only in a restricted top soil layer upon liming, while the sub-soil surface layers of the soil profile with toxic Al remain acidic (Custódio *et al.*, 2002).

## **2.2 Concept of low pH, definition and origin**

The concept of low pH first came about by a Danish chemist, Soren Peder Lauritz Sorensen in 1909. Soil pH is a measure of the acidity or basicity in soils and pH is defined as the negative logarithm of hydrogen ions ( $H^+$  or, more precisely,  $H_3O^+$  aq) in a solution. According to Brady (1990) the pH scale ranges from 0 to 14, with 7 being neutral. According to this notion, a pH below 7 denotes acidity and above 7 denotes alkalinity. Soil pH is considered a significant variable in soils as it dictates many chemical processes that take place. It significantly affects plant nutrient availability by determining the chemical forms of the nutrient. The optimum pH range for most plant species is between 5.5 and 7.0 however, some plants have adapted to thrive at pH values beyond this range.

Acid soils have a low pH because of the parent materials from which they derived or originated from through weathering and have low basic cation (Ca, Mg, K and Na) content because these elements have been reduced from the soil by leaching or via harvested crops (Granados *et al.*, 1993). Generally acid soils have low pH and contain toxic levels of Al and Mn also are deficient in Ca, Mg, P, K, and Mo (Duque-Vargas *et al.*, 1994) and occurring mainly in the form of stable Al silicate complexes, which is non-toxic to plants (Ma and Ryan, 2010). When Al solubilises and forms octahedral hexahydrate  $[Al(H_2O)_6]^{3+}$  also known as  $Al^{3+}$ , it becomes toxic to plants even at micro-molar concentration (Kochian *et al.*, 2005).

Globally, 30% of all land area is reported to be comprised of low pH soils and 50% of the world's cultivated lands are potentially acidic; thus Al toxicity is considered as one of the most significant limitations to crop production (Piñeros *et al.*, 2005). In Brazil, more than 500 million ha are reported to have acid soils, especially those covered by Savannah (Cerrado biome) vegetation (Vitorello *et al.*, 2005). The soils of these areas have high acidity (average pH 4.6), a high concentration of Al and Mn, and deficiencies of Ca<sup>2+</sup>, Mg<sup>2+</sup> as well as P. These limitations, if not corrected, can lead to remarkable reduction in crop productivity. Development of genotypes tolerant to low-soil pH has gained importance in recent years. There is great variability in low-soil pH tolerance between species and even between genotypes within species (Huang *et al.*, 2009). The mechanisms of tolerance to Al can be summarised into two classes: (i) those that eliminate absorbed Al or prevent/reduce its uptake by the roots (Al exclusion) and (ii) detoxification mechanisms, which usually act by Al complexation, followed by the transfer and storage of these complexes in vacuoles (internal tolerance) (Hartwig *et al.*, 2007).

According to Kochian *et al.* (2005), the main site of Al accumulation and toxicity is the root meristem, primarily the distal part of the transition zone. The rapid root growth inhibition after exposure signifies that the Al instantly terminates cell enlargement and elongation before interfering with cell division (Kochian *et al.*, 2005). After an adequate exposure of the root system to Al, its toxicity is manifested through a set of symptoms expressed in its continuous and increasing effect on the morphology and physiology of the roots, which involves decrease in the following: biomass; the number and length of the roots, often coupled with an increase in the mean radius and root volume; and the uptake of water and mineral nutrients, resulting in severe losses of root elongation and the subsequent productivity.

### **2.2.1 Research findings on aluminium toxicity effects**

Studies showed that the binding of Al to cell wall components changes the cation exchange capacity (Panda *et al.*, 2009). Ma *et al.* (2004) reported that visco-elasticity and other properties of the cell wall are affected, resulting in alterations that interfere with growth. Al can cause decline in the elasticity of the cell wall and stimulate the synthesis and accumulation of lignin (Peixoto *et al.*, 2007) through the activation of a peroxidase (POD)

linked to the cell wall, which is involved in the improvement of hydroxyproline-rich glycoprotein binding to phenolic acids. The enzymes activated by Al are comprised of nicotinamide dehydrogenase (NADH) oxidase, phenylalanine ammonia lyase (PAL), and lipoxygenase (LOX). NADH oxidases are responsible for the synthesis of hydrogen peroxide, which is significant for rapid polymer binding catalysed by the cell wall POD. PAL is the key enzyme in the biosynthesis of phenylpropanoids and LOX is responsible for the peroxidation of membrane polyunsaturated fatty acids resulting in the formation of hydroperoxides. These compounds are reported to be highly reactive and quickly degraded into compounds that, by the octadecanoic pathway results in the production of jasmonic acid, which functions in the lignin synthesis signalling pathway (Xue *et al.*, 2008).

Kochian *et al.* (2004a; 2004b) indicated that Al can disrupt the cytoskeletal dynamics, interacting with microtubules and actin filaments. Al can also interfere with signal transduction, particularly in the  $\text{Ca}^{2+}$  signalling pathway (Rengel and Zhang, 2003). According to Sivaguru *et al.* (2000) and Jones *et al.* (2006) Al can increase callus synthesis, blocking the plasmodesmata and preventing cell wall loosening, thus limiting the expansion of cells. The plasma membrane has a negatively charged surface, making it a sensitive target for Al toxicity. Al strongly binds to phospholipids, which leads to alterations of the lipid composition (Peixoto *et al.*, 2001), decreases membrane fluidity and increases the folding of density of lipids (Chen *et al.*, 1991a; 1991b). Al can also inhibit the  $\text{H}^+$ -ATPase in the plasma membrane, which deters the development of and maintenance of the  $\text{H}^+$  gradient (Ahn *et al.*, 2001). Therefore Al interferes with transportation of secondary ions, indirectly causing changes of ion homeostasis in root cells. Al also rapidly and effectively inhibits the influx of  $\text{Ca}^{2+}$  into cells by modulating the activity of transporters which causes alterations in the membrane potential (Kochian *et al.*, 2005). It has strong affinity for phosphate groups that makes the  $\text{Al}^{3+}$  bind to DNA, negatively affecting its template activity and chromatin structures (Silva *et al.*, 2000) and this alters the cell division process (Barceló and Poschenrieder, 2002; Kochian *et al.*, 2005).

### **2.3 Mechanisms for low pH tolerance**

It is important to note that plants have developed various mechanisms to overcome the effects of toxic Al in the soil. These mechanisms can be divided into two groups (i)

symplastic mechanisms comprising of immobilisation or neutralisation of Al within the cell and (ii) exclusion or apoplastic mechanisms that deter the Al from penetrating into the cell, by its immobilisation or neutralisation in the rhizosphere (Kochian, 1995; Samac and Tesfaye, 2003). In the symplastic mechanism, Al within the cell is reported to react with several entities such that it can form complexes with organic acids (Foy, 1988; Taylor, 1988), with proteins or other compounds (Suhayda and Haug, 1995). Internal Al is kept inactive in the cytoplasm or in the vacuoles; this is an advantage because it prevents its negative effects in many cellular processes. However, the intracellular mechanisms of tolerance are not well understood, since both tolerant and sensitive plants have an accumulation of Al when grown in soil conditions of high availability of this element. Different forms of Al can be transported into vacuoles, where it is stored without causing further damage to the cell. The exclusion mechanisms of Al are well studied (Samac and Tesfaye, 2003; Kochian *et al.*, 2004a; 2004b) and validated on the basis of genetic, physiological and molecular evidence. In these mechanisms, chelating compounds are reported to be released by the roots forming non-toxic compounds with Al, avoiding the entry of this element into cells.

In a number of crop species, the exudation of organic acids by root apices is a major means of Al tolerance as reported in maize (Piñeros *et al.*, 2002), wheat (Sasaki *et al.*, 2004), and sorghum (Magalhaes *et al.*, 2007). On the other hand, organic acids, especially citrate and malate, are reported to form stable complexes with the Al<sup>3+</sup> in the rhizosphere, reducing the toxic effects in the root system (Kochian *et al.*, 2004a; 2004b).

### **2.3.1 Genes and inheritance for tolerance to aluminium toxicity**

Genes play a significant role in Al tolerance. The first gene identified for Al tolerance isolated in plants was the *ALMT1* gene in wheat which is a malate transporter which is activated by Al (Sasaki *et al.*, 2004). Genes *SbMATE* (Magalhaes *et al.*, 2007) and *HvMATE* (Furukawa *et al.*, 2007) were isolated from sorghum and barley respectively and function as a citrate transporters, also induced by Al. About two years later, advances in research led to the identification of homologous genes of the *ALMT* and *MATE* multigene families which were isolated from several other plant species. In addition, a transcription factor of the zinc finger type called *STOPI*, is related to Al tolerance in Arabidopsis, which

functions in regulating the expression of *AtMATE* and *AtALMT* genes (Liu *et al.*, 2009). Recently, *Nramp aluminium transporter 1 (Nrat1)*, expressed in the plasma membrane and in the tonoplast, was identified to be associated with Al tolerance in rice (Xia *et al.*, 2010) and this suggested a possibility of involvement with the flux of Al and its mobilisation to the cell vacuole.

It is important to note that the genetic control of Al tolerance in crops varies from an inheritance controlled by one or two genes, as observed in wheat, to a quantitative inheritance, where genes with smaller effects act as modifiers, such as in maize (Cançado *et al.*, 2005; Ferreira *et al.*, 2006;). In wheat, tolerance to Al appears to be controlled by one or two major genes, with the main gene located on chromosome 4D (Aniol and Gustafson, 1984; Lagos *et al.*, 1991). Delhaize *et al.* (1993) associated the *Alt1* locus with a large proportion of the variability in tolerance among wheat cultivars. Subsequently, the *ALMT1* gene which encodes a malate transporter activated by Al, was cloned by Sasaki *et al.* (2004) and would be the gene underlying the *Alt1* locus.

Minella and Sorrells (1992) reported that simple inheritance of Al tolerance was observed in barley and identified a gene (*Alp*) which had a major effect in Al tolerance (Minella and Sorrells, 1992; 1997). They concluded that the variations in Al tolerance among barley cultivars were controlled by different alleles at this locus; however, other genes with smaller effects may have an influence on this trait. The *Alp* gene was mapped to chromosome 4H (Tang *et al.*, 2000).

### **2.3.2 Genetic variability in various crops for aluminium tolerance**

Different crop species exhibit different behaviours in soils with high Al saturation (Parentoni *et al.*, 2001). Variations exist within crop species and tribes. For instance rye is considered to be the most Al tolerant species of the Triticale tribe (Miftahudin and Gustafson, 2002) and genes with larger effects on Al tolerance were identified to be located on chromosomes 6RS (*Alt*), 3RL (*Alt2*) and 4RL (*Alt3*).

Parentoni *et al.* (2001) reported some species considered to be extremely tolerant to Al: some of the tropical forage grass species (Gamba, Signal, Jaragua and Capitata grass),

cassava and cowpea. Crops such as rice, coffee, potato, rubber, palm oil, rye and oat are considered to be highly tolerant to Al. However, sweet potato, maize, cabbage, wheat, millet, pea, eggplant, soybean, elephant grass, barley, onions, beet, pumpkin, sorghum and shrubs like leucaena present low to medium tolerance to Al. Carrots, spinach, celery, cotton, common bean and alfalfa are among the species which are extremely sensitive to Al. A large degree of interspecific variability of Al tolerance was reported in several crop species (Parentoni *et al.*, 2001; Samac and Tesfaye, 2003). Also Caniato *et al.* (2007) indicated a wide morphological variability for Al tolerance in a group of 13 sorghum lines, ranging from highly sensitive (20% relative root growth) to highly tolerant (>100% relative root growth), when measured in nutrient solution containing 60  $\mu\text{M}$  of Al activity.

Al tolerance in maize is reported to be of complex inheritance, since progenies derived from crosses between tolerant and sensitive lines show continuous frequency distributions under Al stress (Magnavaca *et al.*, 1987; Sawasaki and Furlani, 1987). Magnavaca *et al.* (1987) reported a predominance of additive effects in the genetic variation linked with Al tolerance in maize. However, Moon *et al.* (1997) identified a gene with partial dominance (*ALM1*) responsible for tolerance to Al toxicity in this species with the favourable allele identified in a line derived from a somaclonal variant of the cateto race (Cat-100-6). Subsequently, Sibov *et al.* (1999) mapped two quantitative trait loci (QTLs), called ALM1 and ALM2 on chromosome 6 and 10, respectively, which are involved in the genetic control of Al tolerance in maize. Ninamango-Cárdenas *et al.* (2003) also mapped five Al tolerance QTLs on maize chromosomes 2, 6 and 8, explaining about 60% of the phenotypic variation of the trait. Of the two QTLs (ALM1 and ALM2), only the one on chromosome 6 was consistent between the two genetic mapping studies in maize, being equivalent to ALM1. Recently, Maron *et al.* (2009) characterised a member of the MATE family in maize, Zm MATE1, co-localised with the major Al tolerance QTL in the same region as chromosome 6. According to the author this candidate gene encodes a protein located in the plasma membrane that activates the citrate release in the root apex.

Al tolerance in rice appears to be quantitatively inherited (Khaliwada *et al.*, 1996; Wu *et al.*, 1997). This observation was confirmed by mapping studies in crosses between different crop species of the genus (Wu *et al.*, 2000; Nguyen *et al.*, 2002). The evaluation of diallel crosses in soybean revealed that additive effects are predominant in Al tolerance (Spehar,

1995; Spehar and Galwey, 1996). Bianchi-Hall *et al.* (2000) reported more than five QTLs with minor effects, and concluded that the control of Al tolerance in this species is quantitative. The diallel crossing in alfalfa, a crop highly Al sensitive, also concluded that non-additive effects were more important than the additive effects in the control of Al tolerance (Campbell *et al.*, 1994).

## **2.4 Types of mechanisms for aluminium tolerance**

Plants have developed different means to overcome Al stress either by precluding Al<sup>3+</sup> from entering the root (extrusion mechanisms) or by being able to deactivate or neutralise toxic Al<sup>3+</sup> absorbed by the root system which is a true tolerance mechanisms. So far the only well documented mechanism of Al resistance is the exclusion of Al from the root tip based on exudation of organic acids, which chelate Al<sup>3+</sup> creating stable, non-toxic complexes (Kochian *et al.*, 2004a; 2004b).

The root apex was identified as the main site of Al-induced root growth inhibition (Bennet and Breen, 1991; Ryan *et al.*, 1993). The most frequently measured effect of Al toxicity is the inhibition of root growth, but it is important to bear in mind that a number of physiological and biochemical processes in the plant cell have been affected before growth inhibition occurs (Rengel, 1996). Many enzymes have been found to be up-regulated upon exposure to Al and these include PODs (Ezaki *et al.*, 1996, Hamel *et al.*, 1998). In transgenic *Arabidopsis*, expression of a POD gene was identified to confer a degree of resistance to Al (Ezaki *et al.*, 2000). PODs were identified to have an important function in plant metabolism and physiology, and are considered to play a role in the responses of plants to infection and abiotic stress stimuli (Gaspar *et al.*, 1985). Many plant defence responses involving PODs have been identified and these include lignification (Walter, 1992), cross-linking or bonding of cell wall compounds (Bradley *et al.*, 1992), suberisation or impregnation of cell walls and wound healing (Sherf *et al.*, 1993). The gene encoding the *Arabidopsis* blue copper binding protein induces Al resistance in yeast cells (Ezaki *et al.*, 2000).

### 2.4.1 Physiological mechanisms of aluminium tolerance

Hartwig *et al.* (2007) reported that Al detoxification can be accomplished by its complexation in the symplast with different organic compounds and/or by compartmentalisation of Al or its complexes in vacuoles. In this case Al would change little or nothing in plant metabolism, enabling growth and development even after Al input into the symplast. This tolerance mechanism is associated with endemic species of regions with acidic soils, where the ability to address Al toxicity is a prerequisite for survival. There are a few crop species that accumulate high concentrations of Al in their shoots without suffering from toxicity (Ryan *et al.*, 2001; Jansen *et al.*, 2002).

According to Kochian *et al.* (2004b) the main tolerance mechanisms that promote Al exclusion or prevent its absorption by the roots include Al immobilisation in the cell wall, Al selective permeability in the plasma membrane, pH increases in the rhizosphere or the root apoplast and release of organic acids such as citrate, oxalate and malate, and phenolic compounds by the roots. The production and release of organic acids is perhaps the major mechanism of Al tolerance. Evidence that supports this statement was discussed and concluded by Kochian *et al.* (2004b) and they include:

- i) A strong correlation exists between Al tolerance and exudation of organic acids in many crop species.
- ii) The addition of organic acids in the nutrient medium reduces Al toxicity.
- iii) Al/organic acid complexes (di and tri-carboxylic) do not cross the membrane and are not significantly absorbed by the roots.
- iv) The exudation of organic acids activated by Al occurs at the root apex, the location of the primary effect of Al toxicity.
- v) The activation of the exudation mechanism is triggered specifically by  $Al^{3+}$ .
- vi) In the plasma membrane there are anionic channels activated by Al that facilitate the efflux of organic acids.

Ma *et al.* (2001) also reported the identification of two temporal patterns of organic acid exudation as follows:

- i) The plants are characterised by having an almost immediate response to the release of organic acids by the roots when exposed to Al. The authors suggested that the process

appears to involve the activation of pre-existing proteins, as found in wheat, tobacco and barley.

ii) An existence of a lag-phase between Al exposure and organic acids release was found and this process is assumed to involve the stimulation of gene expression (Ma *et al.*, 2001; Magalhaes *et al.*, 2007).

It is also indicated that genotypes with more robust antioxidant systems are usually more tolerant to excess Al, but the mechanism by which Al exacerbates the formation of reaction oxygen species (ROS) is still not fully understood (Darko *et al.*, 2004).

#### **2.4.2 Genetic mechanism for aluminium tolerance**

A study using QTL mapping reported five distinct genomic regions which are significant for Al tolerance in maize (Ninamango-Cárdenas *et al.*, 2003). Consequently, maize has been the subject of breeding programmes seeking to increase Al tolerance or understand the basis for it. The use of nutrient solution experiments by various authors showed that the trait is quantitatively inherited with a prevalence of additive genetic effects (Lopes *et al.*, 1987; Sawasaki and Furlani, 1987). Prioli (1987) suggested that due to its high heritability the trait is expected to be controlled by a small number of genes.

Miranda *et al.* (1984) found that the inheritance of two dominant genes is responsible for tolerance to Al toxicity. Rhue *et al.* (1978) and, Garcia and Silva (1979) also found that tolerance to Al toxicity is determined by one dominant locus for sensitivity. In another study using restriction fragment length polymorphism (RFLP) Sibov *et al.* (1999) also identified indicators that were involved with the two loci (or two groups) located on chromosome 6 and 10. Brondani and Paiva (1996) associated Al tolerance with a gene or block of genes located on chromosome 2 while Torres *et al.* (1997) associated chromosome 8 to Al tolerance. Ninamango-Cárdenas *et al.* (2003) also identified five genomic regions presumably linked to maize Al tolerance, suggesting that this trait is quantitatively inherited and controlled by a few genes. Their study identified four QTLs for Al tolerance in maize located on chromosome 2, 6 and 8. Boni *et al.* (2009) suggested that different results on inheritance of Al tolerance could be a function of the germplasm used because there is a possibility that the genotypic constitution of genetic material can generate a differentiated

and apparently inconsistent phenotypic expression. The authors recommended more efforts on the inheritance mechanisms of Al tolerance in maize because the results reported in the literature are considered to be inconsistent and inconclusive.

## **2.5 Use of modern tools in breeding for low pH tolerance: QTLs, marker assisted selection and transgenic's**

In maize, Al tolerance is seemingly a quantitative trait and Guimarães *et al.* (2012) recommended the use of a combination of strategies for QTL introgression, complemented by early phenotyping of lines to increase the chances of success in generating tolerant materials. The existence of a QTL with a major effect co-localised with genes homologous to the *ALT<sub>SB</sub>* gene was reported (Sibov *et al.*, 1999; Ninamango-Cárdenas *et al.*, 2003). Maron *et al.* (2009) recommended that other genomic regions should be monitored on the basis of early phenotypic selection or genome-wide selection (GWS). Parentoni *et al.* (2003) reported a high correlation between performance *per se* in maize inbred lines and its general combining ability (GCA) evaluated in diallel crosses in a study of phenotypic selection in nutrient solution. In another study, three cycles of marker-assisted backcrossing in maize were sufficient to recover approximately 99% of the recurrent genome (Morris *et al.*, 2003).

Molecular markers for assisted introgression are also available for sorghum and were described by Magalhaes *et al.* (2007). Oliveira *et al.* (2010) also reported that markers distributed in the sorghum genome, are available and are being used for introgression of superior alleles of the *ALT<sub>SB</sub>* gene in elite lines from Brazil and Niger. In barley, transgenic plants that overexpress the *ALMT1* gene were created. Wheat genes that showed a high increase in the rate of malate release, lead to an increased tolerance to Al (Delhaize *et al.*, 2004). Magalhaes *et al.* (2007) also demonstrated that *Arabidopsis* plants transformed with the *ALT<sub>SB</sub>* genes showed higher Al tolerance and citrate exudation than non-transgenic plants. These results showed that the overexpression of these heterologous genes confers increased tolerance to Al and suggest an additional strategy for crops which have limited genetic variability for this trait.

Significant advances in the knowledge of the physiological and molecular basis for Al tolerance were obtained through the cloning of genes of major effects, such as *AMLT1* in wheat (Sasaki *et al.*, 2004) and *ALT<sub>SB</sub>* in sorghum (Magalhaes *et al.*, 2007), which are involved in the Al exclusion mechanism. Guimarães *et al.* (2012) indicated that newly identified genes and QTL have provided important support for a broader understanding of other mechanisms involved in Al tolerance in plants and the availability of cultivars with higher levels of Al tolerance would increase in time and efficiency with the broad integration of molecular and physiological knowledge into breeding programmes.

## **2.6 Diallel evaluation**

The Danish animal breeder, Schmidt first coined the diallel crossing concept in 1919 (Pirchiner, 1979), it was later introduced into plant breeding. According to Sughrone and Hallauer (1997) “diallel” refers to making all possible crosses among a group of genotypes. The genotypes could for example, be individuals, clones or homozygous lines. According to Griffing (1956) and, Mather and Jinks (1977) the diallel mating design enable the determination of a magnitude of additive and non-additive components of heritable variation. It is the most popular technique used by plant breeders to get information of value on inbred lines of different parents and to assess the gene action in various traits (Pickett, 1993).

Griffing (1956) came up with a range of diallel analytical procedures. This has permitted plant breeders to come up with the right selection strategies and compare heterotic patterns at an early stage of hybrid production (Gouis, 2002). The four methods used are i) parents, ii) F<sub>1</sub> and reciprocal crosses iii) parents and F<sub>1</sub>, and iv) only the F<sub>1</sub>. Depending on the decision by the plant breeder, the linear analysis model can be for either fixed or random effects. When the genotypes are highly selected and inbred, a fixed model for analysis is commonly engaged for applied breeding programmes (Agrobases, 2010). In this case when testing for combining ability the sampling error becomes the residual and consequently variance components and standard errors can be estimated. When estimating additive and dominance variances, the following is assumed: absence of epistasis, absence of reciprocal differences, normal diploid segregation, absence of linkage and multiple alleles, homozygous parents, independent gene distribution, and zero inbreeding coefficients

(Griffing, 1956). However, it has been noted that these assumptions are rarely observed in practice (Baker, 1978) and since diallel cross analysis guides the selection of parents with additive and non-additive effects for specific traits, it enables the plant breeders to select parents to be used in hybridisation or population breeding programmes (Murtaza *et al.*, 2005).

## **2.7 Combining ability analysis**

Griffing (1956) outlined a general procedure for diallel analysis which permits non-allelic interaction. This technique partitions the average measured performance of a cross into major components apart from the general mean ( $\mu$ ) and environment variance ( $\sigma^2_e$ ) by use of the analysis of variance (ANOVA).

### **2.7.1 General combining ability analysis**

GCA is used to denote the parents/line/hybrid's mean performance/contribution in a cross combination (Sprague and Tatum, 1942). Falconer and Mackay (1996) defined it as the average performance of the parental inbred line in all single crosses, when expressed as a deviation from the average of all crosses. Additive and additive epistatic variances are the primary components of GCA (Matzinger, 1963). Additive, additive x higher order interactions of additive genetic variance have been responsible for the differences in important variation in GCA (Baker, 1978).

### **2.7.2 Specific combining ability analysis**

Specific combining ability (SCA) refers to those cases in which cross combinations perform relatively better or worse than would be anticipated on the basis of the mean performance of the parental inbred lines (Sprague and Tatum, 1942). It is thus the deviation to a greater or lesser extent from the sum of the GCA of the two parents. SCA is due to non-additive gene action (Falconer and Mackay, 1996). In other words, variations or differences in SCA are considered to be attributable to non-additive genetic variance (Baker, 1978).

### **2.7.3 Importance of combining ability analysis**

GCA and SCA effects are significant in identification of parents and crosses which are responsible for the expression of a particular type of gene action (Meredith, 1984). It is important to note that both GCA and SCA are effective genetic parameters used in deciding the next stage of the breeding programme (Dabholkar, 1992). Multiple crossing or composite breeding programmes are facilitated through selection of parents based on GCA for development of synthetics and choice of suitable F<sub>1</sub>, especially where one intends to use appropriate selection techniques like recurrent selection, mass selection and reciprocal selection (Dabholkar, 1992).

### **2.7.4 Research findings on combining ability studies in maize**

Gowda (2013) carried out a study to investigate the GCA effects of parental inbred lines and SCA effects of single-cross hybrids for yield and yield related traits and explore their use in the generation of hybrids. A total of 170 F<sub>1</sub> were developed and tested by crossing 34 parental inbred lines with five testers. The SCA: GCA ratio of variances revealed that there were prevalence of non-additive gene action in the expression of all the traits under investigation. Six inbred lines were identified with good GCA for yield and yield related traits.

El-Badawy (2013) carried out a study involving a half diallel cross with seven inbred lines of maize under two different N levels for six quantitative characters. Results indicated that mean squares for all traits were significant for GCA and SCA. Ratios of GCA:SCA indicated that the additive and additive x additive types of gene action were responsible for the expressions for days to 50% anthesis, number of kernel rows per ear and shelling percentage in both N levels and combined analysis. Significant interaction mean squares between N levels and GCA and SCA were detected for most traits. The results suggested that the crosses may be of great importance in breeding programmes either towards development of maize hybrids or synthetic varieties.

Kurawa (2012) conducted genetic analysis studies of progenies from diallel crosses among eight varieties of different maturity groups of maize and observed significant differences

for GCA and SCA, indicating presence of additive as well as non-additive gene action. In both environments, the GCA mean squares were highly significant and higher than the SCA mean squares for all traits with a few exceptions. The study revealed significant GCA x environment interaction, indicating different parental varieties behaved differently under different environments, hence there was need to select different parental varieties for hybrid production for a specific environment. Significant SCA x environment interaction indicated hybrid performance varied with respect to environments. A suggestion was made to have specific hybrids produced for specific environments.

Vivek *et al.* (2009), in a combining ability study of parental inbred lines for grain yield and resistance to seven diseases, observed significant differences for both GCA and SCA effects for most diseases. Correlations between GCA effects for disease scores were generally non-significant, suggesting the possibility of pyramiding genes for disease resistance in the parental inbred lines. Matthews *et al.* (2008), in a study of a diallel cross among the nine lines observed that both SCA and GCA were significant sources of variation in the inheritance for resistance to ear damage. GCA was also a significant source of variation in the inheritance for resistance to larval growth. GCA effects for reduced larval weight were significant for two lines.

## **2.8 Heritability estimation**

Heritability was defined as the proportion of variance due to heritable differences and genotypic variance to the total phenotypic variance (Meredith, 1984). The higher the proportionate value, the more transmissible is the character. On the other hand, the lower the ratio or proportionate value, the higher the influence of the environment on the phenotypic expression of the character. Thus it defines the proportion of the total variance that is due to the mean effects of genes.

Heritability can be defined in two senses:

- i) Broad sense heritability which is total genetic variance (Meredith, 1984). While Dudley and Moll (1969) defined it as the ratio of total genetic variance to phenotypic variance which expresses the degree to which individuals' phenotypes are determined by their genotype.

ii) Narrow sense heritability which is the ratio of additive genetic variance to phenotypic variance (Dudley and Moll, 1969) and expresses the extent to which phenotypes are determined by the genes transmitted from parents.

### **2.8.1 Importance of narrow sense heritability**

Inbreeding is important mostly when developing inbred lines through selfing and selection, and is needed when heterosis or vigour is desired. To avoid mating related individuals (inbreeding), narrow sense heritability is employed in estimating the degree of relatedness or resemblance between parents and progenies (Meredith, 1984; Chaudhary, 1991). Narrow sense heritability estimates the degree of correspondence between breeding values and phenotypic values and expresses the level of genetic variance in the population, which is mainly responsible for altering the genetic make-up of the population through selection (Falconer, 1989; Dabholkar, 1992).

Narrow sense heritability ( $h^2$ ) can be written as:

$$h^2 = V_A / V_P$$
$$h^2 = V_A / (V_P = V_A + V_D + V_I + V_E)$$
$$V_P = V_A + V_D + V_I + V_E$$

Where:  $V_A$  denotes additive variance,  $V_D$  denotes dominance variance,  $V_I$  denotes interaction variance, and  $V_E$  denotes environmental variance (Falconer, 1989).

It is important to note that heritability is valid not only of the trait under investigation, but also of a population being sampled and the environmental conditions to which individuals have been exposed to (Falconer, 1989; Dabholkar, 1992). Populations which are genetically more uniform are anticipated to express lower heritability than genetically diverse populations, since environmental variances constitute part of the phenotypic variance, which influences the degree of heritability.

### **2.8.2 Research findings on heritability studies in maize**

In a study to investigate broad sense heritability and correlations between the traits and total grain yield, Aminu and Izge (2012) reported heritability estimates for number of stands per plot, anthesis-silking interval, plant height, weight of cobs and grain yield of above 60% within a range of 60.61-67.44%, while days to 50% anthesis, days to 50% silking, ear height and de-husked cobs, recorded heritability estimates of below 60% i.e.

47.91%, 50.03%, 58.45% and 55.06% respectively. Higher and relatively moderate broad sense heritability of the traits suggested that variations were transmissible and had the potential for generating high yielding varieties via selection of promising plants in succeeding generations.

The correlation analysis which indicates associations or some relationships indicated that anthesis-silking interval ( $r = 0.88$ ), number of cobs per plant ( $r = 0.55$  and  $r = 0.32$ ), number of cobs per plot ( $r = 0.83$ ), weight of cobs ( $r = 0.8$  and  $r = 0.86$ ), de-husked cobs ( $r = 0.95$ ,  $r = 0.49$  and  $r = 0.87$ ) and 100-seed weight ( $r = 0.46$  and  $r = 0.32$ ) exhibited positive and significant genotypic (g), phenotypic (p) and environmental (e) correlation with grain yield. The authors, Aminu and Izge (2012) concluded that heritability as well as correlations were good methods for improving yield and selecting genotypes tolerant to drought.

In another study to investigate genetic variation, heritability and genetic advance of grain yield and its component traits Bello *et al.* (2012) reported that the effect of interaction of genotype and genotype by year were significant for ear weight and grain yield, while the effect of year was highly significant for all the traits. Additive gene effects contributed to high levels of phenotypic and genotypic coefficients of variation as well as high heritability accompanied with high genetic advance recorded for grain yield, number of grains per ear, ear weight, as well as plant and ear heights. This indicated that effective selection is possible for improving these traits.

## **2.9 Heterosis**

Heterosis is essential in crop improvement and is considered to be the phenomena of enhanced hybrid performance (Hartl and Clark, 1989). Falconer (1989) defined heterosis as the difference between the crossbred and parental inbred lines or simply the superiority over inbred lines. It is often expressed in two ways: Where interest is primarily in the  $F_1$  performance *per se*, it is expressed as the  $F_1$  minus the highest performing parent, expressed as a percentage of that parent used and is referred to as “high parent heterosis” (Meredith, 1984). Secondly, as the  $F_1$  minus mid-parent expressed as a percentage of the mid-parent, this was also referred to as “mid-parent heterosis” (Meredith, 1984; Lamkey and Edwards, 1999). For heterosis to occur there should be heterozygosity as a fundamental precondition

(Flintham *et al.*, 1997). Other geneticists believe that dominance and epistasis are the essential genetic foundation of heterosis such that loci with no dominance do not cause heterosis. The degree of heterosis following a cross between two particular parental inbred lines or populations is dependent on the square of the difference of rate of gene occurrence or frequency between populations such that heterosis in the  $F_1$  is  $HF_1 = \sum dy^2$ , where “d” denotes the deviation of the heterozygote from the homozygote mid-parent, while “y” denotes gene frequency (Coors, 1999).

### **2.9.1 Research findings on heterosis studies in maize**

Salazar *et al.* (1997) conducted a study to investigate eight segregating populations and their 56 reciprocal crosses, in five acidic-soil locations in order to establish relative importance of nuclear and cytoplasmic factors for yield, days to silking, ear height, ears per plant, and ear rot. Average and specific heterosis represented 65 and 31% of the total sum of squares for heterosis for yield. Population heterosis effects for yield were not significant, suggesting the effects would be of little importance in selecting parental inbred lines for developing superior hybrids. Specific heterosis effects were negative and significantly different for yield and ears per plant only for one cross, suggesting that non-additive gene effects were at play in determining yield of specific cross combinations. According to the author, the unavailability of reciprocal differences for all characters suggested that nuclear genes were responsible for tolerance to soil acidity.

### **2.10 Correlations**

When a change in one variable is associated with change in another variable it is referred to as correlation (Falconer, 1989). The measure of association between two traits is referred to as a correlation coefficient. Correlations are either positive or negative; positive when an increase in one variable leads to an increase in another or when it is negative, an increase in one variable will lead to a decrease in another (Falconer, 1989). Associated traits are considered to be significant for three reasons: (i) gene action leading into association through the pleiotropic action of genes whereby one gene affects more than one phenotypic

trait (ii) linked to alterations brought in by selection and (iii) linked with natural selection (Falconer and Mackay, 1996).

Two types of correlations are considered to be important in plant breeding (Meredith, 1984):

i) Correlation via phenotype which is considered to be the association between two traits that is visible and determined from measurements of the same traits in a number of individuals in a given population. Phenotypic values are estimated by genotypic values and environmental deviations. This type of correlation is composed of correlation due to environmental agents and non-additive gene action (Falconer, 1989; Dabholkar, 1992). In cases where two traits have high heritability, association due to environmental agents will be insignificant (Falconer, 1989).

ii) Genetic correlation is considered to be the correlation of breeding values which is a result of additive gene action (Falconer and Mackay, 1996). Genetic association between two or more traits may result from one gene affecting many traits (pleiotropic effects) or gene linkage that governs inheritance of two or more traits (Falconer, 1989). This depicts the degree to which the two measurements are associated genetically.

### **2.10.1 Research findings on correlation in maize**

Bulent (1996) conducted a study to investigate the associations among several agronomic characteristics in parental inbred lines, hybrids and between parental inbred lines and their offspring of maize in short season areas. Phenotypic correlations of each measured trait between as well as among hybrids and inbred lines were not the same. The highest correlation coefficient ( $r = 0.78$ ) was recorded between days to 50% anthesis and grain yield. The trend showed that the better yielding inbred lines did not necessarily give rise to better yielding offspring. Given that days to 50% pollen shed plays a significant role in two main traits (yield and moisture stress tolerance) for hybrids, suggested that an effort to achieve most favourable anthesis dates during inbred line development could be an important criterion to predict short season hybrid performance.

Alake *et al.* (2008) conducted a study to determine genetic variation and correlation in yield and yield associated traits of tropical maize. There was close similarity between the

genotypic correlation coefficient and phenotypic correlation coefficient for all characters, indicating that selection for these traits would be successful. Heritability estimates were high for all the traits investigated except for days to 50% flowering. Traits investigated showed significant association in the positive direction with grain yield except for days to flowering and silking which showed significant negative genotypic association with grain yield. Principal component analysis (PCA) showed that some characters contributed significantly to variations found in the maize genotypes evaluated and these included seedling emergence, kernel weight, grain yield, number of kernels per row, number of kernel rows per ear and days to anthesis. Also some traits like number kernel rows per ear, grain yield per plant, ear length and number of kernels per row showed high heritability coupled with high genetic advance and could be considered as criteria when selecting maize for grain yield.

## **2.11 Stability analysis**

### **2.11.1 Stability definition and its concept**

Yield stability of a genotype refers to the situation whereby a particular genotype is capable of evading fluctuations in yield over a range of environmental conditions (Heinrich *et al.*, 1983). Related to stability is wide adaptability which refers to a situation whereby a genotype exhibits good performance over a wide geographical region under changeable climatic environmental conditions. Adaptability or stability of a cultivar usually relates to three biological mechanisms. These are: physiological, morphological and phenological. Individual genotypes may react to brief fluctuations in an environment in two different ways. Genotypes that are capable of buffering against environmental changes and develop a replica of phenotype over a range of environments are known to possess a 'biological or static' stability. This is usually not beneficial in crop breeding, since it will not show improved vigour or heterosis under improved growing conditions. On the other hand 'agronomic or dynamic' stability allows a predictable response to environments and stability with respect to the agronomic concept, and has no deviation from this response to environments (Becker and Leon, 1988). With respect to quantitative characters, the many genotypes usually respond similarly to desirable or unfavourable environmental conditions. According to Baker (1988) genotype by environment interactions (GEI) could be classified into two types:

- i) Qualitative interactions. This is sometimes called crossover interactions and it is here that the direction of true treatment differences varies.
- ii) Quantitative interactions. This is sometimes called non-crossover interactions and the real treatment differences vary in the degree, but not in direction (ranking of genotypes does not change from one environment to another).

The crossover or qualitative interaction is significant in agricultural production in comparison to non-crossover or quantitative interactions (Baker, 1988; Crossa, 1990). With respect to quantitative characters, the majority of genotypes respond in a similar manner to desirable or undesirable environmental conditions. If a crossover (qualitative) type of GEI (one that leads to genotype rank changes) is present, selection of genotypes based on mean yield by use of combination of stability and yield would likely be lower than for those genotypes selected based on yield alone (Kang *et al.*, 1991). This could also be clarified by investigating the outcome to growers when investigators commit a Type I error i.e. rejecting the null hypothesis when it is true and Type II error i.e. accepting the null hypothesis when it is false, when selection was based on yield alone and when it was based on both yield and stability.

### **2.11.2 Phenotypic stability analysis techniques**

Phenotype is considered to be an organism's actual visible properties, such as morphology, development, and behaviour. Several procedures for assessing phenotypic stability have been proposed by various authors. However, Lin *et al.* (1986) studied the statistical relationship between nine stability statistics and identified and classified three concepts of stability into the following types:

- I) Type I: a stable genotype is identified with a small variance across all environments. This type of stability is significant when the test locations considered are not very diverse and is identical to the statistical concept of stability as described by Becker and Leon (1988).
- II) Type II: a stable genotype has a reaction to the environment similar to the mean reaction of all genotypes in the experiment. Type II stability is identical to the dynamic concept described by Becker and Leon (1988).

III) Type III: the residual mean square from the regression model on the environmental index is a small value i.e. a smaller deviation from the regression. Type III stability is dynamic and the method of Eberhart and Russel (1966) is employed for its estimation.

#### **2.11.2.1 Cultivar performance technique for estimating cultivar stability**

Cultivar performance measure ( $P_i$ ) is another technique proposed by Lin and Bins (1988). Whereby  $P$  denotes a genotype while  $i$  is the mean squares of the distance between genotype one and the genotype with the maximum performance. A small  $P_i$  value indicates a small difference between the genotype with maximum yield and the best performing genotype. A pairwise GEI mean square between the maximum and each genotype can be estimated as indicated by Crossa (1990).

#### **2.11.2.2 Wricke's ecovalence technique for estimating cultivar stability**

Ecovalence is another technique of Wricke (1962) who proposed the procedure for measuring stability by employing each genotype's contribution to the GEI sum of squares as a stability measure. Ecovalence ( $W_i$ ) was the name given to this concept or statistic. Genotypes associated with  $W_i$  values that are low or close to zero tend to have smaller deviations from the mean across environments and are more stable and possess a high ecovalence i.e. low value of  $W_i$  = high ecovalence. Becker and Leon (1988) demonstrated ecovalence by plotting yield numerical values of genotypes in several environments against the respective environmental means.

#### **2.11.2.3 Shukla's stability variance parameter for estimating cultivar stability**

Stability variance technique is another statistic procedure developed by Shukla (1972). The technique is dependent on the residuals from the additive model. The variance of a cultivar is defined as the variance of the cultivar across locations. For the purpose of ranking, stability variance ( $\sigma^2_i$ ) is equivalent to ecovalence ( $W_i$ ) (Wricke, 1962). Shukla's (1972) stability variance ( $\sigma^2_i$ ) is also equivalent to Type II stability (Lin *et al.*, 1986). According to Shukla (1972) there was an indication that genotypes could not be described if there was

a small proportion of GEI due to heterogeneity among regression coefficients. In addition, there is absence of independence between means of sites and performance as well as between intercepts and slopes. Instead he suggested the use of the GEI sums of squares which is partitioned into variance components corresponding to each of the genotypes. The identification of a stable genotype according to Shukla (1972) is as follows: stability variance ( $\sigma^2_i$ ) must be equal to the environmental variance ( $\sigma^2_e$ ), for a variety to be classified as stable thus  $\sigma^2 = 0$ . A relatively large value of  $\sigma^2_i$  implies greater instability of genotype  $i$  since the stability variance is the difference between two sums of squares, the value can be negative, but negative estimates of variances are rare in variance components computations and are assumed as  $v$  equivalent to zero in most cases.

#### **2.11.2.4 Regression coefficient and deviation mean squares**

Yield is considered as the most important trait for variety selection. To this effect the most widely used criteria for selecting high yield and stable performance is average yield performance, site mean yield regression response and deviations from regression (Eberhart and Russel, 1966; Freeman, 1973). The first estimated parameter is the slope  $bi$  from the regression of the yields of genotype  $I$  on an environmental index (Finlay and Wilkinson, 1963). Where  $b$  is equal or close to 1.0, it implies that a genotype responds to changeable environmental conditions in a similar manner as the sample mean. Finlay and Wilkinson (1963) proposed that regression coefficient approaching zero imply stable performance. The joint linear regression has been employed as a technique for analysing and interpreting the non-additive GEI of two way-classification data.

Finlay and Wilkinson (1963) proposed that regression coefficient close to 1.0 imply average stability. When that is accompanied with high mean yield, varieties have good general adaptation. Conversely, when accompanied with low mean yield, genotypes have poor adaptation to all test environments. Regression values increasing above 1.0 describe genotypes with increasing sensitivity to environmental change, for example below average stability and specifically adapted to high yielding environments. Regression coefficients decreasing below 1.0 provide a measure of greater buffering ability to changeable environmental conditions (above average stability) and therefore specifically adapted to

low yielding environmental conditions. Stability analysis provides a method to establish the reaction of a genotype to changeable environmental conditions.

### **2.11.3 Multivariate techniques for stability analysis**

Multivariate approaches are employed in stability analysis in order to obtain additional information on multivariate reaction to environments by genotypes. These analyses are suited for computing two-way matrices of genotypes and environments (Crossa, 1990). According to Becker and Leon (1988) multivariate analysis has three major objectives: i) to remove noise from data patterns, ii) to summarise the data and iii) to show the structure in the data.

Genotypes with similar reaction can be clustered, hypothesised and later evaluated and data can be easily summarised and analysed. The aim of the several multivariate analysis techniques is to allocate test varieties into qualitatively homogeneous stability subsets (Becker and Leon, 1988). Within subsets, no significant GEI occurs, while differences among subsets are due to GEI. However, there are some setbacks in multivariate analysis techniques which include: i) many dissimilarity techniques and clustering strategies exist and selecting between them can consequently depict different cluster groups and ii) non-existent structure could find its way into the data.

#### **2.11.3.1 Additive main effects and multiplicative interaction analysis technique**

This model is observed to constitute ANOVA for genotypes and environment main effects with PCA of the GEI into one model with additive and multiplicative parameters. It has been considered important in getting insight of complicated GEI (Kang, 1996). Results are plotted in a very enlightening biplot that depicts both major and interaction effects for both test varieties and test locations. The additive main effects and multiplicative interaction (AMMI) model is employed to split estimated interaction components and adjust mean yield for the interaction. This model partitions data into pattern rich models and eliminates noise-rich residual to increase efficiency. The main advantage for using AMMI is that it gives information for a large component of variability in its first few components with

successive readings indicating decreasing percentage pattern and decreasing percentage of noise (Purchase, 1997).

The model AMMI is important in gaining insight of complicated interactions, gaining efficiency, improving selection efficiency and increasing experimental efficiency (Gauch, 1990). It has become an important statistical tool in identification of morphological and physiological characters associated with tolerance to stress and reveals the relative significance of several environmental factors or stresses (Gauch, 1993). The other merit of this model is that it can be employed in modelling and gaining insight about interactions. According to Crossa (1990) the AMMI model is particularly useful in organising patterns and associations for genotypes and environments. The analysis procedure is that in the first ANOVA the total variation is partitioned into three orthogonal sources, test varieties as genotypes, test locations as environments and GEI. As a guide, Ramagosa and Fox (1993), reported that in most yield trials the proportion of sum of squares due to variations among environments ranges from 80-90% and that the variation due to GEI are often larger than the genotypic differences. The other uniqueness of AMMI analysis is that the interaction principal component analysis (IPCA) sum of squares alone is often larger than that for genotype. Generally as genotypes and environments become more diverse, GEI tends to increase and may reach 40-60% of the total variation. Usually the environmental main effect, which contributes up to 90% of the total variation, is not much relevant, especially in selection techniques.

The AMMI technique produces graphs (biplots) which focus on the data structure relevant to selection, in other words on the genotype and GEI sources (Ramagosa and Fox, 1993). PCA partitions GEI into various orthogonal axes. There is some controversy on the number of axes included in the AMMI model and how judgements and indications of genetic stability can be made if too many axes are considered. Gauch and Zobel (1996) pointed out that generally AMMI 1 and AMMI 2 models with IPCA 1 and IPCA 2 respectively, are often chosen and that the graphical outlay of axes, either as IPCA 1 or IPCA 2 against main effects, or IPCA 1 against IPCA 2, is not an issue and it gives adequate information. With AMMI 3 and higher models, IPCA 3 and higher axes are mostly dominated by noise, have little or no predictive value and no biological explanation and can thus be ignored. Another major importance of the AMMI model is that it gives a cost-effective means for obtaining

efficiency in research experiments and maximising benefits on investments (Gauch and Zobel, 1996). Gains in efficiency of yield estimations have been observed to be equivalent to subsequent additional number of replication by a factor of two to five (Crossa and Cornelius, 1993).

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## CHAPTER 3

### **A hydroponic nutrient solution experiment for testing low pH tolerance in tropical and sub-tropical maize genotypes**

#### **3.1 Abstract**

Low pH soil is a major constraint to maize production by resource-poor farmers due to toxic levels of  $Al^{3+}$  and P fixation. Selecting tolerant maize genotypes under low pH soil in the field is confounded by associated external factors like diseases. In this study, 70 maize genotypes were evaluated in a hydroponic nutrient solution experiment at Chitedze Research Station in a glasshouse. The objective was to identify tolerant genotypes to the presence of  $KAl(SO_4)_2$  in a nutrient solution as a proxy to low pH soil conditions. A completely randomised design (CRD) with four replicates was used. Significant differences ( $p < 0.05$ ) were observed for initial seminal root length (ISRL), final seminal root length (FSRL), root tolerance index (Rti) and net seminal root length (NSRL). In terms of FSRL the genotype sum of squares contributed 91.1% of variation and this was supported by a heritability value of 92%. The environmental influence contribution was low (7.6%). The NSRL, which represented the effective root growth or elongation during the experimental period varied significantly ( $p < 0.05$ ) among the maize genotypes tested. The sum of squares for genotypes contributed 58.3% to the total variation. The heritability was 74% implying that the phenotypic differences among the maize genotypes in terms of NSRL were due to genetic differences. In terms of NSRL, genotypes IWD C3 SYN F2-B, VPO52, and LPHpop4 were considered tolerant, and DT-YSTR SYNTHETIC-B, TZE-W POP DTC2 STR-B, TZE-YDT STR C4-B, LPHpop3, LPHpop13 and LPHpop14 were sensitive to Al toxicity. The tolerant genotypes identified in this study will be used in the National Maize Breeding Programme as source populations to develop new low pH tolerant parental inbred lines.

#### **3.2 Introduction**

Acid soils generally have low pH, contain toxic levels of Al and Mn and are deficient in Ca, Mg, P, potassium (K), and Mo. These characteristics are known to limit the fertility of

acid soils (Duque-Vargas *et al.*, 1994). Al is a major soil constituent and its toxicity is observed in most acid soils where the pH levels are below 5.5. Low pH is a major constraint to production of maize and other crops on tropical soils. Al toxicity is reported to inhibit the growth of crop plants on approximately 40% of the earth's potentially arable soils (Manyowa *et al.*, 1988). At low pH (pH<5) toxic Al<sup>3+</sup> ions are released into the soil, and hinder root growth, this greatly affects the development of the entire plant (Kochian, 1995; Kidd and Proctor, 2000). Al toxicity causes short, thick and under developed roots and plants. This significantly reduces nutrient uptake and increases susceptibility to drought (Sasaki *et al.*, 1996). More than eight million ha of acid soils are planted to maize in the tropics (Pandey *et al.*, 1994) and soil acidity reduces yield by about 10% of the maize produced in the developing countries (Borrero *et al.*, 1995).

Soil acidity is becoming common in most parts of Malawi and is limiting crop production. Continuous cultivation and burning of crop residues during land preparation has contributed to the problem. More acid soils occur in the high rainfall areas (>1000 mm per year) with moderate to high leaching, whereas the alkaline soils occur in low rainfall areas (< 500 mm per year). The bulk of very acid soils are located in Lilongwe, Mzuzu and Blantyre ADDs. Chilimba (1994) reported higher Al saturation percentages in Nkhatabay, Mulanje, Bembeke and Lunyangwa. The soils database prepared by the Soils Commodity Team indicated that over 40% of the country has soil pH less than 5.5 (Munthali, 2007).

Considerable genetic tolerance to soil acidity is expressed in maize (Piñeros and Kochian, 2001). In addition, extensive genetic variability with respect to Al tolerance exists in plants both at inter and intraspecific levels (Ishikawa and Wagatsuma, 1998). In maize, the majority of commercial genotypes are sensitive to Al toxicity, such that breeding for more adapted cultivars seems to be the best strategy to improve farming of this crop in regions with acid soils.

### **3.2.1 Hydroponic nutrient solution**

Trejo-Téllez and Gómez-Merino (2012) defined a nutrient solution for hydroponic systems as an aqueous solution containing mainly inorganic ions from soluble salts of essential elements for higher plants. A hydroponic system of cultivation refers to growing plants in

water containing dissolved nutrients (therefore without soil). Currently 17 elements are considered essential for most plants, these are carbon (C), hydrogen (H), oxygen (O<sub>2</sub>), sulphur (S), copper (Cu), zinc (Zn), boron (B), chlorine (Cl), nickel (Ni), N, K, P, Ca, Mg, Fe, Mn, and Mo (Salisbury and Ross, 1992). With the exception of C and O<sub>2</sub>, which are supplied from the atmosphere, the essential elements are obtained from the growth medium.

### **3.2.2 Justification for use of hydroponic nutrient solution experiment**

Conventional breeding methods take time and are influenced by environmental conditions and sometimes the expression of genes is masked by epistatic interactions, whereby the expression of a particular gene depends on another gene. Testing of maize for Al tolerance can be effectively done in the field if the environment (such as diseases and weather) can be manipulated and this could be expensive and time consuming considering the number of genotypes that need to be evaluated. Reports indicate that there are three efficient and less time consuming methods used: screening in nutrient solutions (Magnavaca *et al.*, 1987; Cançado *et al.*, 1999), potted soils (Ahlrichs *et al.*, 1990) and root staining with haematoxylin (Ruiz-Torres *et al.*, 1992). Among these three methods, nutrient solution screening seems to be attractive since it is less expensive and provides adequate Al stress, thereby allowing preliminary screening of a large number of genotypes in a small area and consequently reduces the number of promising genotypes to be analysed in the field (Magnavaca *et al.*, 1987). In addition the results obtained with the solution culture screening method correlate positively with those obtained using field screening (Gomez-Urea *et al.*, 1996) showing that this method could be representative of what happens in the field. The effect of Al tolerance in nutrient solution culture could be quantified in terms of root length measurements. Early symptoms of Al toxicity occur in the root for obvious reasons as roots are in direct contact with toxic Al<sup>3+</sup> ions. Sensitive genotypes tend to accumulate higher amounts of Al in their root tissues (Carver *et al.*, 1988).

Al solution tests improve efficiency in selection for tolerance because there is effective control of environmental variations, which is difficult to achieve under field conditions. In the present study, KAl(SO<sub>4</sub>)<sub>2</sub> was used as a proxy to low pH soil conditions and the nutrient composition was according to Magnavaca *et al.* (1987). The cost of liming low pH soils

and applying adequate P to a maize crop is beyond the reach of most small-scale farmers. Maize breeding for low pH tolerance could offer a solution. However, conventional breeding methods take time and are influenced by environmental conditions. A more suitable solution would be to select Al tolerant maize genotypes for use in acid soils which, in the long run, is less expensive, sustainable and more environmentally friendly. The Al solution test improves efficiency of selection for tolerance because there is effective control of environmental variations, which is difficult to achieve under field conditions. The objective of this study was to identify maize genotypes that are tolerant to low pH or Al toxicity.

### **3.3 Materials and methods**

#### **3.3.1 Experimental materials**

Two hundred and ninety OPV maize genotypes were sourced in October 2010 from CIMMYT-Zimbabwe and CIMMYT-Colombia (267 and 23 genotypes, respectively). The genotypes which had adequate seed for both hydroponic and field trials were selected. Further selections were carried out based on grain texture (a preferred trait in Malawi) and important genetic variation based on their pedigrees which have pre-characterised attributes. This included QPM, maize tolerance to: drought, DM, MSV, striga as well as yellow and orange genotypes. Finally, a total of 70 OPVs including those released and commonly grown in Malawi were used in the hydroponic nutrient solution experiment to determine their response to Al toxicity in a hydroponic nutrient solution experiment (Table 3.1).

**Table 3.1 Description of the tropical and sub-tropical maize genotypes used in the study**

	<b>Pedigree</b>	<b>Origin</b>	<b>Pre-characterised traits</b>
1	99TZEFY-STR QPM CO-B	IITA	QPM, STR, yellow
2	DT-WSTR SYNTHETIC-B	IITA	DT, STR, white
3	DT-YSTR SYNTHETIC-B	IITA	DT, STR, yellow
4	EVDY-Y2000 STR QPM CO-B	IITA	DT, STR, yellow QPM
5	EVD-W 99 STR QPM CO-B	IITA	QPM, STR, white
6	IAR-FLINT-Q-B	IITA	Flint
7	IWD C3 SYN F2-B	IITA	White colour
8	LOW N POOL C3-B	IITA	Low N
9	MULTICOB EARLY DT -B	IITA	DT, multiple cobbing
10	OBA SUPER1(9021-18(IITA))-B	IITA	QPM
11	OBATANPA/IWDC2SYNF2/IWDC2SY	IITA	QPM
12	OBATANPA/IWDC2SYNF2/IWDC2SYNF2-B	IITA	QPM
13	OBATANPA/TZLCOMP4C3F2/TZLC	IITA	QPM
14	OBATANPA/TZLCOMP4C3F2/TZLCOMP4C3F2-B	IITA	QPM
15	POP66 SR/DMR-LSRY/DMR-LSRY	IITA	MSV, DM
16	POP66 SR/TZUTSR-WSGY/T	IITA	MSV, DM, white
17	SYN DTE STR-Y-B	IITA	DT, MSV, yellow
18	SYN DTE STY-W-B	IITA	DT, MSV, white
19	TZE COPM3 DTV C2 F2-B	IITA	DT
20	TZE E-WPOP X LD(SET2)-B	IITA	White
21	TZE-W POP DTC2 STR-B	IITA	DT, MSV, STR
22	TZE-WDT STR QPM-CO-B	IITA	DT, MSV, QPM, STR
23	TZE-YDT STR C4-B	IITA	MSV, DT, STR
24	TZE-YPOP DTC2 STR-B	IITA	DT, yellow, MSV, STR
25	VPO0721	CMMYT Zimbabwe	GLS, LB, MSV
26	VPO5148	CMMYT Zimbabwe	GLS, LB, MSV
27	VPO5173	CMMYT Zimbabwe	GLS, LB, MSV
28	VPO5179	CMMYT Zimbabwe	GLS, LB, MSV
29	VPO5187	CMMYT Zimbabwe	GLS, LB, MSV
30	VPO52	CMMYT Zimbabwe	GLS, LB, MSV
31	VPO627	CMMYT Zimbabwe	GLS, LB, MSV
32	VPO630	CMMYT Zimbabwe	GLS, LB, MSV
33	VPO710	CMMYT Zimbabwe	GLS, LB, MSV
34	VPO712	CMMYT Zimbabwe	GLS, LB, MSV
35	VPO716	CMMYT Zimbabwe	GLS, LB, MSV
36	VPO717	CMMYT Zimbabwe	GLS, LB, MSV

37	VPO738	CMMYT Zimbabwe	GLS, LB, MSV
38	VPO739	CMMYT Zimbabwe	GLS, LB, MSV
39	VPO741	CMMYT Zimbabwe	GLS, LB, MSV
40	VPO743	CMMYT Zimbabwe	GLS, LB, MSV
41	VPO744	CMMYT Zimbabwe	GLS, LB, MSV
42	VPO76	CMMYT Zimbabwe	GLS, LB, MSV
43	VPO86	CMMYT Zimbabwe	GLS, LB, MSV
44	VPO96	CMMYT Zimbabwe	GLS, LB, MSV
45	VPO97	CMMYT Zimbabwe	GLS, LB, MSV
46	LPHpop4 = Cimcali 05B ROYA1	CMMYT Colombia	low pH
47	LPHpop3 = Cerrito98SCMV2B(SA7)-#-B	CMMYT Colombia	low pH
48	LPHpop6 = Cimcali03HCG1A	CMMYT Colombia	low pH
49	LPHpop8 = GLSI01HG"A"	CMMYT Colombia	low pH
50	LPHpop9 = Granada 01Phaeo1AS2	CMMYT Colombia	low pH
51	LPHpop10 = GRANADA01PHAE1AS1COGSCMV	CMMYT Colombia	low pH
52	LPHpop11 = ICAV-305	CMMYT Colombia	low pH
53	LPHpop13 = PSA3	CMMYT Colombia	low pH
54	LPHpop14 = S03TLYQAB05	CMMYT Colombia	low pH
55	LPHpop15 = S03TLYQAB05	CMMYT Colombia	low pH
56	LPHpop16 = VILLAVICENCIO01PHAEIOIACLA	CMMYT Colombia	low pH
57	LPHpop17 = Villavicencio03Asp1C(LET-EARLY)	CMMYT Colombia	low pH
58	LPHpop18 = Menegua03Gloeo1C(S3)	CMMYT Colombia	low pH
59	LPHpop19 = Villavicencio03Phaeo1A	CMMYT Colombia	low pH
60	LPHpop20 = MENEGUA01PHAEO	CMMYT Colombia	low pH
61	LPHpop21 = Caicedonia00Phaeo1A	CMMYT Colombia	low pH
62	LPHpop23 = POB SIKUANI	CMMYT Colombia	low pH
63	LPHpop1 = Cap. Miranda 99Bact1F-1	CMMYT Colombia	low pH
64	LPHpop2 = Cerrito98Achap2B-#-B	CMMYT Colombia	low pH
65	LPHpop7 = Cimcali99BSCMVSA7Ac-#-B	CMMYT Colombia	low pH
66	LPHpop12 = menegua01cog1c(pob cog)	CMMYT Colombia	low pH
67	ZM309 (Msungabanja)	Malawi	DT, GLS MSV, LB
68	ZM523 (Mwayi OPV)	Malawi	DT, GLS MSV, LB
69	ZM623	Malawi	GT, LB, MSV
70	ZM721	Malawi	GT, LB, MSV

**QPM = Quality protein maize, DT = drought, MSV = Maize Streak Virus, LB = Leaf Blight, GLS = Gray leaf spot, STR = Striga, SR = Streak, TZ = Tropical Zea, TZE = Tropical Zea Early, TZL = Tropical Zea Late, IWD = Intermediate White Dent, IAR = Institute of Agriculture Research, COMP = composite, VPO = Populations from Dr. Viveki's stock ID, CIMMYT-Zimbabwe, LPHpop = Low pH populations (a code to designate long pedigrees for populations) from CIMMYT-Colombia, and ZM = Popular OPVs released in Malawi, breeding stock originated from CIMMYT-Zimbabwe**

### 3.3.2 Experimental procedure and design

The study was carried out during the off season in October 2011 at the Chitedze Research Station. Hundred seeds were randomly counted out per genotype and these were washed with distilled water. The seeds were then sterilized in 1% sodium hypochlorite solution for 5 minutes and rinsed twice with distilled water. These seeds were germinated in groups of 20 between paper-sheets moistened with distilled water for 7 days, incubated at 27°C for the first 3 days to allow good germination. After the 7 days roots and shoots were visible and ready for measurement (Figure 3.1A). The ISRL was measured from a random sample of 10 seedlings per genotype from the 20 seedlings per paper sheet. After measurements of the ISRL, all 20 seedlings were transplanted into plastic containers (Figure 3.1B). Four of the containers per genotype which represented replications were placed in a glasshouse and seeds were grown for 7 days in 5 L aerated nutrient solution containing 6 ppm of Al in the form of  $KAl(SO_4)_2$  (Magnavaca, 1982). The remaining container per genotype was used as a control in which only the nutrient solution was used i.e. no  $KAl(SO_4)_2$ . After 7 days from sowing the seedlings were transplanted on plastic gauze with a fine mesh so that only the roots of the seedlings were immersed in the nutrient solution. The procedure was to open the plastic gauze with a tweezer and insert the roots until they are in contact with the solution.



A: Germination in moistened paper after 7 days

B: 7 days after transplanting in  $KAl(SO_4)_2$

**Figure 3.1 Germination of maize genotypes in news prints paper and appearance 7 days after transplanting**

### 3.3.3 Nutrient solution preparation

The composition of nutrient solutions used for growth of maize genotypes was as described by Magnavaca (1987) ( $\mu\text{mol element l}^{-1}$ ): 10 900  $\text{NO}_3\text{-N}$ ; 3500 Ca; 2300 K; 1300  $\text{NH}_4\text{-N}$ ; 850 Mg; 590 S; 590 Cl; 25 B; 9.1 Mn; 2.29 Zn; 0.88 Mo; 0.63 Cu; 77 Fe as ferric hydroxylethylene diamine triacetate (FeHEDTA). Al was added to the nutrient solution as  $\text{KAl}(\text{SO}_4)_2$  and P as  $\text{KH}_2\text{PO}_4$ . The pH was checked and adjusted to 4.0 using 0.1 M HCl.

### 3.3.4 Data collection, measurements and calculations of derived data

After 7 days, FSRL was measured in centimetres from a random sample of 10 seedlings per genotype per replication. The measurements were averaged and the mean ISRL and FSRL were used to calculate the relative seminal root length (RSRL) as derived data using the following equations:

$$\text{i) RSRL} = \frac{\text{FSRL} - \text{ISRL}}{\text{ISRL}}$$

$$\text{ii) RTi} = \frac{\text{RSRL (Al}^+\text{ treated plants)}}{\text{RSRL (Al}^+\text{ control plants)}}$$

$$\text{iii) \% Response} = \frac{\text{RSRL (Al}^+\text{ treated plants)}}{\text{RSRL (Al}^+\text{ control plants)}} \times 100$$

$$\text{iv) NSRL} = \frac{\text{FSRL}}{\text{ISRL}}$$

### 3.3.5 Statistical analysis

#### 3.3.5.1 Analysis of variance

The data was subjected to ANOVA using Agrobase (2010). ANOVAs for each measured parameter and derived data was carried out. A graph for NSRL with standard errors was plotted in Excel.

### 3.4 Results

#### 3.4.1 Observed symptoms of aluminium toxicity

The presence of Al in the nutrient solution caused a delay in the vegetative growth of some maize genotypes with reduced development of new leaves and decreased development of shoots. Al toxicity symptoms were evident after 3 days from transplanting, such as lateral root shortening, darkening and stunting. The maize genotypes DT-YSTR SYNTHETIC-B, TZE-W POP DTC2 STR-B, TZE-YDT STR C4-B, LPHpop3, LPHpop13, and LPHpop14 were sensitive to Al with a minimum NSRL of 0.6 cm each. Purple colour and interveinal leaf chlorosis was observed in shoots of Al-stressed maize plants in the susceptible genotypes after 7 days from transplanting (Figure 3.2).



**Figure 3.2 Partial view of purple colouration and shortened roots observed in susceptible genotypes**

In some susceptible genotypes purple-green colour (depicting P deficiency) and chlorosis were observed. On the other hand the maize genotypes IWD C3 SYN F2-B (3.0 cm), VPO52 (3.5 cm) and LPHpop4 (3.0 cm), did not present severe symptoms of Al toxicity in their shoots (Figure 3.3).



**Figure 3.3 Partial view of new roots emerged from tolerant genotypes 7 days after transplanting**

### 3.4.2 Analysis of variance

Significant differences ( $p < 0.05$ ) were observed for FSRL, RTi and NSRL (Table 3.2). In terms of FSRL the results indicated that the contribution of genotype estimated using sum of squares was high (91.1%) and this was supported by a high heritability value of 96.0% (Table 3.2). The environmental influence was low (7.6%). The repeatability, which is derived from the coefficient of determination ( $R^2$ ) was high (92.0%). This meant that the phenotypic differences between the genotypes in the trial were due to genotypic differences, hence little of the phenotypic differences were due to environmental effects. Genotypes VPO76, TZE-WDT STR QPM-CO-B, EVD-W 99 STR QPM CO-B, SYN DTE STY-W-B and LPHpop10 had long root lengths, values of 10.0, 9.4, 8.5, 8.5 and 8.1 cm respectively, while LPHpop7 and DT-YSTR SYNTHETIC-B had the shortest root length, values of 1.5 and 1.7 cm, respectively (Table 3.2; Appendix 1).

RTi results indicated that the influence of the genetic component estimated using sum of squares (53.2%) was moderate and this was supported by a moderate heritability value of 56.0% (Table 3.2). Entries SYN DTE STY-W-B, LPHpop10, VPO717, VPO76 and

EVDT-Y2000 STR QPM CO-B had high root tolerance index values of 1.1, 1.0, 1.0, 0.9 and 0.8, respectively (Appendix 1). With respect to percent response as described in Section 3.3.5, results indicated that the influence of the genetic component estimated using sum of squares (54.3%) was moderate and this was supported by moderate heritability value of 58.0% (Table 3.2). Entries SYN DTE STY-W-B, LPHpop10, VPO717, VPO76 and EVDT-Y2000 STR QPM CO-B had a high percent response of 5.4, 4.0, 0.5, -9.0 and -15.7% respectively (Table 3.2; Appendix 1). Compared to the control plants, entry SYN DTE STY-W-B had the highest percent response and Rti of 5.4 and 1.1 respectively.

NSRL results indicated that the influence of the genetic component estimated using sum of squares (59.0%) was moderately high and this was supported by high heritability value of 74.0%. Entries VPO52, IWD C3 SYN F2-B, LPHpop4, ZM309, had the highest NSRL of 3.5, 3.0, 3.0, and 2.5 respectively (Table 3.2). NSRL which represented the effective root growth or elongation during the experimental period varied significantly ( $P < 0.05$ ) among the maize genotypes tested. The sum of squares for genotypes contributed 58.3% to the total variation. The heritability value from the ANOVA was 74% (Table 3.2) implying that the phenotypic differences among the maize genotypes in terms of NSRL were due to genetic differences. On average, the roots grew 1.3 cm in Al nutrient solution and the maximum was 3.5 cm. In the control, the roots grew 5.6 cm on average with a maximum of 11.5 cm. Thus there was a clear effect of Al toxicity on the maize genotypes (Appendix 1). VPO52 (3.5 cm) had the highest NSRL (Figure 3.4) followed by IWDC3SYNF2-B and LPHpop4 with 3.0 cm each.

No significant correlation was observed between NSRL and FSRL and Zero Al (control) treatments, as well as for percentage response versus Rti. The rest were all significantly ( $P < 0.01$ ) and positively correlated except NSRL vs ISRL which were significantly ( $P < 0.01$ ) and negatively correlated (Table 3.4).

**Table 3.2 Root length measurements and derived data before and 7 days after transplanting the glasshouse hydroponic experiment**

	<b>G</b>	<b>Pedigree</b>	<b>ISRL (cm)</b>	<b>FSRL (cm)</b>	<b>Zero Al (cm)</b>	<b>Rti</b>	<b>% response</b>	<b>RSRL Trtd (cm)</b>	<b>RSRL Zero Al (cm)</b>	<b>NSRL (cm)</b>	<b>Rank</b>
Top 10 genotypes	30	VPO52	1.37	3.45	4.37	0.79	-21.13	2.47	3.30	3.47	1
	7	IWD C3 SYN F2-B	1.40	3.46	5.07	0.68	-32.09	2.02	3.20	3.02	2
	46	LPHpop 4	1.43	4.01	5.70	0.72	-27.84	1.99	3.31	2.99	3
	67	ZM309	0.78	1.84	3.40	0.61	-38.53	1.50	3.82	2.50	4
	18	SYN DTE STY-W-B	3.43	8.49	8.20	1.05	5.37	1.49	1.39	2.49	5
	20	TZE E-WPOP X LD(SET2)-B	1.27	2.73	3.70	0.74	-26.37	1.21	1.97	2.21	6
	1	99TZE FY-STR QPM CO-B	2.85	4.99	6.73	0.74	-25.87	1.19	2.03	2.19	7
	58	LPHpop 18	1.37	2.36	3.97	0.62	-38.38	1.10	2.74	2.10	8
	11	OBATANPA/IWDC2SYNF2/IWDC2SY	1.40	2.89	3.97	0.72	-27.79	1.06	1.83	2.06	9
	37	VPO738	1.87	3.48	4.17	0.84	-16.62	1.03	1.43	2.03	10
Bottom 10 genotypes	68	ZM523	3.27	2.45	4.53	0.54	-46.28	-0.25	0.41	0.75	61
	14	OBATANPA/TZLCOMP4C3F2/TZLCOMP4C3F2-B	3.83	2.56	4.77	0.56	-43.75	-0.26	0.36	0.74	62
	12	OBATANPA/IWDC2SYNF2/IWDC2SYNF2-B	3.93	2.81	4.47	0.64	-36.22	-0.27	0.19	0.73	63
	8	LOW N POOL C3-B	6.13	4.29	6.13	0.71	-29.39	-0.28	0.06	0.72	64
	54	LPHpop 14	6.23	4.05	6.43	0.65	-35.51	-0.36	0.03	0.64	65
	23	TZE-YDT STR C4-B	5.23	3.21	4.67	0.80	-20.35	-0.38	-0.07	0.62	66
	3	DT-YSTR SYNTHETIC-B	2.87	1.67	4.27	0.41	-59.28	-0.39	0.26	0.61	67
	21	TZE-W POP DTC2 STR-B	4.47	2.72	5.60	0.49	-50.96	-0.39	0.50	0.61	67
	47	LPHpop 3	5.73	3.36	6.07	0.57	-42.74	-0.41	0.09	0.59	69
	53	LPHpop 13	4.33	2.31	4.37	0.53	-46.80	-0.44	0.06	0.56	70
		<b>Mean</b>	<b>3.73</b>	<b>4.08</b>	<b>5.64</b>	<b>0.71</b>	<b>-29.09</b>	<b>0.32</b>	<b>0.89</b>	<b>1.32</b>	
		<b>LSD</b>	<b>0.95</b>	<b>1.20</b>	<b>1.87</b>	<b>0.22</b>	<b>22.01</b>	<b>0.88</b>	<b>1.27</b>	<b>0.88</b>	
		<b>Prob</b>	<b>0.001</b>	<b>0.00</b>	<b>0.001</b>	<b>0.00</b>	<b>0.001</b>	<b>0.001</b>	<b>0.001</b>	<b>0.00</b>	
	<b>CV (%)</b>	<b>15.8</b>	<b>18.3</b>	<b>20.5</b>	<b>19</b>	<b>46.9</b>	<b>171.5</b>	<b>88.3</b>	<b>42</b>		
	<b>Min</b>	<b>0.78</b>	<b>1.47</b>	<b>2.67</b>	<b>0.41</b>	<b>-59.28</b>	<b>-0.44</b>	<b>-0.07</b>	<b>0.56</b>		
	<b>Max</b>	<b>10.10</b>	<b>9.99</b>	<b>11.50</b>	<b>1.05</b>	<b>5.37</b>	<b>2.47</b>	<b>3.82</b>	<b>3.47</b>		
	<b>R-Squared</b>	<b>0.96</b>	<b>0.92</b>	<b>0.85</b>	<b>0.53</b>	<b>0.54</b>	<b>-</b>	<b>-</b>	<b>0.69</b>		
	<b>Heritability</b>	<b>0.98</b>	<b>0.96</b>	<b>0.91</b>	<b>0.56</b>	<b>0.58</b>	<b>-</b>	<b>-</b>	<b>0.74</b>		
	<b>MSE</b>	<b>0.35</b>	<b>0.56</b>	<b>1.34</b>	<b>0.02</b>	<b>185.90</b>	<b>0.30</b>	<b>0.61</b>	<b>0.30</b>		

LSD = Least significant difference, CV = coefficient of variation, Min = minimum, Max = maximum, MSE = Mean square error, G = genotype, ISRL = initial seminal root length; FSRL = final seminal root length; Zero Al = Zero Aluminium or control; RTi = root tolerance index; RSRL/TRTD = relative seminal root length treated with aluminium; RSRLzero Al = relative seminal root length with zero aluminium; NSRL = net seminal root length.

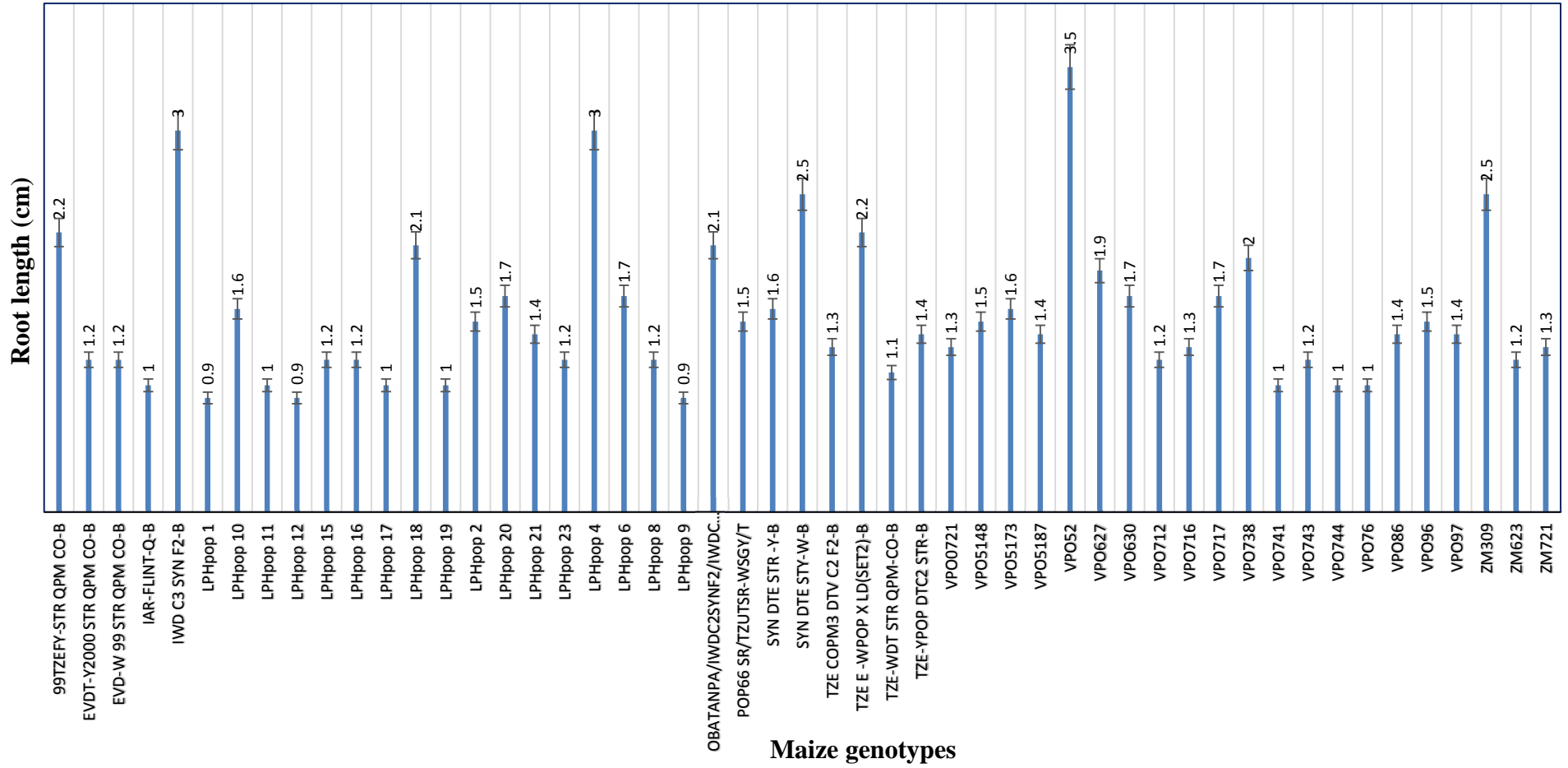


Figure 3.4 Graph of nett seminal root length for genotypes

**Table 3.3 Genetic and phenotypic variances and heritability estimates from ANOVA for the measured and derived data**

	Variable	Genetic variance	Phenotypic variance	Broad-sense heritability	R-Squared	CV (%)	P for entry
1	ISRL	4.78	5.18	0.90	0.95	17.2	***
2	FSRL	4.28	4.80	0.89	0.92	18.3	***
3	NSRL	0.27	0.57	0.47	0.69	41.5	***
4	Rti	0.0086	0.027	0.32	0.54	19.2	***
5	% Response	85.68	271.595	0.32	0.54	46.9	***
6	Al Control	4.40	5.78	0.76	0.85	20.6	***

CV = coefficient of variation, ISRL= Initial seminal root length; FSRL = Final seminal Root length; NSRL = Net seminal root length; RTi = Root tolerance index; Al Control = Aluminium control, \*\*\* P for entry from ANOVA

**Table 3.4 Pearson's coefficient of correlation among the measured and derived data**

Variable	ISRL	FSRL	Zero Al(control)	Rti	% response
FSRL	0.72**				
Zero Al(control)	0.76**	0.91**			
Rti	0.22**	0.56**	0.19**		
%Response	0.22**	0.56	0.19**	1.00	
NSRL	-0.49**	0.08	-0.03	0.27**	0.27**

\*\*P<sub>≤</sub>0.01; ISRL = initial seminal root length; FSRL = final seminal root length; NSRL = net seminal root length; RTi = root tolerance index; Al control = aluminium control

### 3.5 Discussion

The results indicated that after 7 days from transplanting into the hydroponic nutrient solution, there was a general reduction in root growth of all the tested genotypes at low pH of 4.0 and  $KAl(SO_4)_2$ . The change in colour from green to purple in susceptible genotypes could be attributed to low P stress as it is fixed under low pH conditions. Purple discolouration is among the key symptoms for P stress in maize plants. Al toxicity is reported to be responsible for significant changes in biochemical and structural patterns of plant cells reflecting on reduction of cell multiplication (Minocha *et al.*, 1992) and cell growth altering auxin action in cell wall (Ma *et al.*, 1999). The root is the plant organ most affected by Al toxicity and more specifically, its tip is considered to be the main site for Al toxicity (Archambault *et al.*, 1997). As a result, root elongation is considered to be the most sensitive parameter in a short period of time and thus may represent the whole plant reaction to Al. Noble *et al.* (1988) demonstrated that it was possible to observe damage within 24 hr caused by Al in roots of soybean plants directly proportional to Al concentration in the nutrient solution.

In susceptible genotypes the common symptom is poor plant growth as a result of root injury (Meda and Furlani, 2005; Juan-Ping *et al.*, 2006). This is because root development plays a major role in a plant's response to water and nutrient availability. Poorly developed roots negatively affect exploration of bulk soil reducing nutrient and water uptake (Okiyo *et al.*, 2010). The observation of Al toxicity symptoms, like root darkening and root-tip stunting in this hydroponic experiment could be related to the rapid entry of Al into the root cells. In the susceptible genotypes the roots were thick and shortened and this is in agreement with Blancaflor *et al.* (1998) and Horst *et al.* (1999). They reported that the root morphological alterations like shrinking and tip curling, observed in roots of some of the plant materials, could be attributed to alterations in the root's cytoskeleton. The authors also demonstrated that changes in the organisation and stability of microtubules and microfilaments in root cells of maize were correlated to Al toxicity, besides the rapid inhibition of root elongation. Braccini *et al.* (2000) also reported that in the presence of Al, root elongation of coffee genotypes were more affected than root dry matter.

In the present study, root elongation was the best parameter to make comparisons among maize genotypes with respect to Al tolerance. It also appeared to be a cheaper and better technique compared to other methods reported like root staining with haematoxylin (Ruiz-Torres *et al.*, 1992). According to Concado *et al.* (1999) root apices are excised and photographed both stereoscopic and light microscopes after staining with 0.2% haematoxylin (Merck) and to observe the presence of haematoxylin – Al complexes in internal tissues you need to carryout transversal sectioning of the apices. This suggest more labour costs and time for this method considering the large number of genotypes evaluated in breeding programmes. The root is the first plant organ to be affected as it is directly in contact with the toxic Al<sup>3+</sup> ions. The shoot can be difficult to measure as it may require processing dry weight and fresh weight biomass using destructive sampling. Reports also indicate that shoots do not provide a true picture on Al tolerance (Mascarenhas *et al.*, 1984; Bernai and Clark, 1998). Bernai and Clark (1998) observed that differences in dry matter production in sorghum genotypes were not as significant as root elongation, the latter being the best discriminative parameter. Root elongation has been considered the most sensitive characteristic to quantify Al tolerance due to the fact that the elongation zone is the site where Al toxicity is primarily detected (Blancaflor *et al.*, 1998). The most sensitive site for Al action in the root is the distal transition zone (DTZ) (Kollmeier *et al.*, 2000). These authors showed that application of Al solution exclusively in the DTZ inhibited root elongation in a similar pattern to whole-root application. The differential tolerance to Al by the genotypes tested could be related to Al exclusion mechanisms (Silva *et al.*, 2000; 2002) and/or symplast tolerance (Watanabe *et al.*, 2001). Other research findings indicated that exclusion mechanisms are based on the reduction of Al<sup>3+</sup> activity in root tips, like the exudation of low molecular weight organic compounds, which may form stable complexes with Al, reducing its toxicity to roots, such as citrate (Miyasaka *et al.*, 1991), malate (Delhaize *et al.*, 1993), polypeptides (Basu *et al.*, 1994) and flavonoids (Kidd *et al.*, 2001). More than one type of organic acid may be released by Al stressed roots (Larsen *et al.*, 1998; Ma *et al.*, 2000).

### **3.6 Conclusions and recommendations**

It was possible to demonstrate that nutrient solution is a practical and efficient tool for evaluating maize genotypes for low pH tolerance. Root elongation appears to be the best

parameter to compare for Al tolerance among genotypes. In this experiment genotypes IWD C3 SYN F2-B, VPO52 and LPHpop4 were considered highly tolerant, SYN DTE STY-W-B, ZM309 were considered tolerant and 99TZEFY-STR QPM CO-B and OBATANPA/IWDC2SYNF2/IWDC2SY moderately tolerant and DT-YSTR SYNTHETIC-B, TZE-W POP DTC2 STR-B, TZE-YDT STR C4-B, LPHpop3, LPHpop13, and LPHpop14 were sensitive to Al toxicity. Conventional breeding methods will continue to be used until Al-tolerant genes from various plants and/or microbes are isolated and transgenic plants with significantly increased Al tolerance are produced. The highly tolerant genotypes identified in this study namely IWD C3 SYN F2-B, VPO52 and LPHpop4 will be used in the National Maize Breeding Programme in Malawi as source populations to develop new low pH tolerant parental inbred lines using the pedigree method.

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## CHAPTER 4

### Phenotypic evaluation for tolerance to low pH in tropical and sub-tropical maize germplasm

#### 4.1 Abstract

Low-soil pH is one of the major abiotic factors contributing to low yields in Malawi. In this study 45 maize genotypes were evaluated using a (0, 1) alpha lattice design with three replications, using optimal and low pH sites for two consecutive seasons. The objective was to evaluate genetically diverse maize genotypes for tolerance to low pH soils by using phenotypic traits. A mean reduction in grain yield and yield components (100 seed weight, plant vigour etc.) of 69.9% was recorded under low pH conditions. Genotypes LPHpop16, LPHpop3, VPO739, VPO5173 and LOW N POOL C3-B performed relatively better under low pH conditions. Comparison between the glasshouse hydroponic trial and low pH field trial, indicated that among the top 10 yielding genotypes, SYN DTE –STY-W-B performed well and ranked first in terms of root tolerance index (RTi) with a net seminal root length (NSRL) of 2.5 cm followed by VPO717 with RTi of 1.0 cm and NSRL of 1.7 cm. Phenotypic traits associated with grain yield, such as plant vigour, 100 seed weight, shelling percentage, number of ears per plant, ear height and plant height can be used alongside grain yield when selecting germplasm for tolerance to low pH stress. Clustering of the genotypes based on morphological data across low pH environments showed that genotypes had similar chances of being grouped in any of four clusters. The five top yielding genotypes across low pH and optimal environments were comprised of two genotypes from CIMMYT-Colombia and three from CIMMYT-Zimbabwe. Under low pH conditions the top five yielding genotypes consisted of four from CIMMYT-Zimbabwe and one from CIMMYT-Colombia. Selection for low pH tolerance could be effectively carried out *in situ*.

#### 4.2 Introduction

Malawi is located to the south east of Africa and is a land locked country, extending from 9°45' to 17°5' south of the equator and is about 900 km in length from north to south and

varying in width from 80 to 160 km. Its population is estimated at about 14 million people and is increasing with an average growth rate estimated at 3.3% per year (Saka *et al.*, 2004). Malawi is endowed with many natural resources. Its main resource base is agriculture, which plays a key role in the country's economy. The agriculture sector remains the engine of economic growth in Malawi as it contributes over 40% to GDP of which over 70% is generated by the smallholder sector; 90% of export earnings, provides employment to over 85% of the country's population and is a source of income for over 60% of the rural poor (Kumwenda and Kachule, 2003).

Maize is the most important staple food in Malawi such that in certain districts other foods like rice, sweet potato and cassava are considered as snacks and households will look for resources to buy maize. Approximately 70% of the cultivated area of the customary land is planted to maize each year. About 1.2 million ha of land in the country are planted to maize, producing an average grain yield of 1.1 MT ha<sup>-1</sup> (MOA, 2003; 2005) against a potential of 6 MT ha<sup>-1</sup> and 8 MT ha<sup>-1</sup> for OPVs and hybrid maize, respectively (Zambezi *et al.*, 1993). There clearly exists a yield gap between potential yields achievable and what the smallholder farmers are currently producing. The challenge for the country to attain sustainable maize productivity growth is to reduce the existing yield gap.

The introduction of targeted input subsidy in 2007 improved national yield performance, but still at household level some yield gaps exist against potential yields achievable due to inadequate availability of stress tolerant varieties for some ecologies. FAOSTAT (2013) reported that performance of maize in Malawi remained nearly static through the mid-2000s but it seems to have picked up in recent years. Farmers are being encouraged to combine proper use of subsidised fertiliser and compost manure to improve the maize yields. Unfortunately the quality of compost manure is compromised by the organic materials used due to poverty levels. Reducing the yield gap at household level and obtaining potential yields achievable requires combined efforts. These include breeding for stress tolerance in maize and use of effective agronomic practices, bearing in mind that the country's population is increasing while land is static.

Malawi upland soils are highly weathered and low in pH and available P. About 40% of the country's soils are oxisols and ultisols (US taxonomy), which are low in pH and P

(Munthali, 2007). Apart from low pH, low N soils are also a big problem in highly populated areas. Crop rotation with fallow is rarely practiced. Most farmers are practicing mono-cropping and in some places it is associated with removal of crop residues after harvest which could be depleting soil nutrients and organic matter. This has led to low-soil fertility, especially in the mid-altitude ecology [600-1300 metre above sea level (masl)] of the country.

### **4.3 Materials and methods**

#### **4.3.1 Description of sites**

The study was conducted at Research station sites from 2011 to 2013. The sites included four low pH sites and five optimal sites. In 2013 the genotypes were also evaluated at a managed low N site. The sites were Tsangano Experimental Research Station (1524 masl, 1071 mm annual rainfall, sandy clay loam soil, annual maximum (max) temperature of 22.9°C). Bembeke Research Station - a low pH site (1524 masl, 1053 mm annual rainfall, sandy clay loam soil, 23.4°C annual max temperature). Bvumbwe Research Station - a low pH site (1174 to 1228 masl, 1219 mm annual rainfall 25.2°C annual max temperature), Lunyangwa Research Station - a low pH site (1342 masl, 1270 mm annual rainfall, 32°C annual max temperatures). Baka Research Station, low-land (786 masl, 800 mm annual rainfall, sandy loam soil, 28.4°C annual max temperatures). Chitedze Research Station - low N managed site (1219 masl, 954 mm annual rainfall, sandy clay loam, 26.2°C annual max temperature). Chitala Research Station, low-land (610 masl, 800 mm annual rainfall, 28.3°C max annual temperature).

#### **4.3.2 Experimental materials**

A total of 202 genotypes were obtained from CIMMYT-Colombia and CIMMYT-Zimbabwe. These were used in preliminary screening in four sets of field trials in the 2010/11 season to come up with a workable set of genotypes (results not shown). In the 2011/12 and 2012/13 seasons a total of 45 genotypes were planted at 10 sites. Data from Bvumbwe sites for 2011/12 and 2012/13 was not included in the combined analyses due to gaps in the trial for 2011/12 as a result of cut worm and wire worm damage. The managed

low N site data for 2011/12 at Chitedze was also not included due to vandalism before harvest but the results for the 2012/13 site were reported separately. The genotypes were selected from the list provided in Chapter 3 (Table 3.1).

### **4.3.3 Experimental design**

The experimental design used was a (0, 1) alpha lattice with three replications. Plot sizes were: two rows of 5.1 m per plot with 17 planting stations per row. Two seeds were planted per station and thinned to one while leaving two at both ends of the row. The phenotypic and agronomic traits measured in the study are listed in Table 4.1.

### **4.3.4 Salient management activities**

#### **4.3.4.1 Fertilizer application**

The Malawi government recommended rate of 92 kg N and 60 kg P<sub>2</sub>O<sub>5</sub> was applied as split application as follows: 131 g of 23:21:0 + 4S was applied per row or 8 g per station if using the dollop method as basal dressing. This was applied within 14 days after emergence. This was followed by an application of 54 g of urea per row or 3 g per station if using the dollop method. This top dressing fertilizer was applied 21 days from planting as recommended (MOA, 1994).

### **4.3.5 Soil characterisation for low pH sites**

#### **4.3.5.1 Soil sampling and laboratory analysis**

Soil samples were collected from four low pH sites in 2010 using the grid sampling method at 15 cm depth as top soil and at 30 cm as sub-soil. Five samples of the same depth were thoroughly mixed to form a composite sample from which three sub-samples were prepared for analyses. The samples were analysed at the Soils and Agriculture Engineering Laboratory at Chitedze Research Station. Parameters analysed included pH, % organic carbon, % organic matter, % N, P, K, Ca, Mg and Al.

**Table 4.1 List of phenotypic and agronomic traits and measuring procedure**

No	Abbreviation	Trait	Units	Trait description
1	AD	Days to anthesis	Days	The number of days from planting to 50% pollen shedding
2	DS	Days to silking	Days	The number of days from planting to 50% silking
3	PH	Plant height	cm	Measured from the ground surface to the flag leaf using a calibrated stick
4	EH	Ear height	cm	Measured from the ground surface to the node bearing the main ear
5	EPP	Ears per plot	Number	Number of cobs harvested per plot divided by number of plants per plot
6	SL	Stem lodging	Number	Number of plants with stalks broken below the ear per plot
7	RL	Root lodging	Number	Number of plants fallen with exposed roots per plot
8	SWT	100 seed weight	g	Weight of 100 seeds per plot
9	SH	Shelling percentage	%	The ratio of grain weight to cob weight expressed as a percentage
10	GT	Grain texture	Score (1-5)	A scale of 1-5 was used where 1 = very flint (no depression at the top of the kernel), 3 = intermediate, 5 = very dent (the kernel has a depression)
11	GY	Grain yield	Kg/ha	Total grain yield from all the ears of each plot with moisture level adjusted to 12.5% and converted to kg/ha
12	GLS	Gray leaf spot	Score (1-5)	Scored using a scale of 1-5, where 1= no disease symptoms, 2 = presence of the disease, 3 = moderate infection, 4 = heavily infested, 5 = severe infection
13	LB	Leaf blight disease	Score (1-5)	Scored using a scale of 1-5, where 1= no disease symptoms, 2 = presence of the disease, 3 = moderate infection, 4 = heavily infested, 5 = severe infection
14	MSV	Maize streak virus disease	Score (1-5)	Scored using a scale of 1-5, where 1= no disease symptoms, 2 = presence of the disease, 3 = moderate infection, 4 = heavily infested, 5 = severe infection
15	Rust	Rust disease	Score (1-5)	Scored using a scale of 1-5, where 1= no disease symptoms, 2 = presence of the disease, 3 = moderate infection, 4 = heavily infested, 5 = severe infection
16	VIG	Plant vigour	Score (1,3,5)	Scored using a scale, where 1= healthy, vigorous plant, upright; 3 = moderate, 5 = weak, slender or thin plants
17	ASI	Anthesis-silking interval	Days	The difference between days from planting to 50% pollen shedding (AD) and days to 50% silking (DS) i.e. ASI = DS-AD

#### 4.4 Data analyses

The collected data and derived data were analysed using GenStat 16<sup>th</sup> Edition (2013), and Agrobase (2010). Dendrogram construction was carried out in the Number Cruncher Statistical System (NCSS) (Hintze, 2007) using unweighted pair group method with arithmetic mean (UPGMA). Morphological data were used and construction was based on Euclidean distance and standard deviation as scale type. The genotypic and phenotypic variances were computed from expected mean squares of ANOVA using the formula according to Hallauer and Miranda (1988) as follows:

Phenotypic variance denoted as  $\sigma^2_p$

$$\sigma^2_p = \sigma^2_e + \sigma^2_g$$

Where:  $\sigma^2_e$  = error variance and  $\sigma^2_g$  = genotypic variance

Genotypic variance is denoted as  $\sigma^2_g$

$$\sigma^2_g = (\text{MSg} - \text{MSe})/r$$

Where: MSg = Mean square of genotypes, MSe = mean square error, and r = number of replicates in the experiment.

Genotypic coefficient of variation (GCV)

$$\text{GCV} = (\sqrt{\sigma^2_g} / X) 100$$

Where,  $\sigma^2_g$  = genotypic variance and X = mean of the measured trait

Phenotypic coefficient of variation (PCV)

$$\text{PCV} = (\sqrt{\sigma^2_p} / X) 100$$

Where:  $\sigma^2_p$  = Phenotypic variance, X = mean of the trait.

Broad sense heritability was computed using the following equation:

$$H^2_B = \sigma^2_g / \sigma^2_p$$

Where  $H^2_B$  is the broad sense heritability,  $\sigma^2_g$  = genotypic variance and  $\sigma^2_p$  = phenotypic variance.

Genetic advance is denoted as GA.

$$GA = k \sigma_p H^2$$

Where:  $k$  = the standardised selection differential at 5% selection (2.063),  $\sigma_p$  = phenotypic standard deviation of the character and  $H^2$  heritability estimate

The across sites correlation coefficients ( $r$ ) between the means for grain yield and other agronomic traits were computed in Agrobase (2010). The PCA was carried out using both GenStat Version 16 for latent loadings (Eigen vectors) and Agrobase (2010) for Eigenvalues and percent cumulative variation to give an insight of the explained variation in the genotypes and traits measured.

## 4.5 Results

All results were reported at a significance level of  $P \leq 0.05$  unless otherwise indicated.

### 4.5.1 Soil analytical results

The pH of the soils and soil nutrients for the sites are presented in Table 4.2. Lunyangwa had highly acidic soils (pH 4.50 sub-soil and pH 4.69 top soils). Tsangano and Bembeke had acidic soils at pH 5.35 and 5.38, 5.13 and 5.01 for top soil and sub-soil, respectively. Bvumbwe was classified as having moderately acidic soils at pH 5.67 and 5.66 top soil and sub-soil, respectively. The classification was based on Soil Test Interpretation Guide (Horneck *et al.*, 2011) as well as according to Mehlich (1984).

At classification of < 0.88 low, 0.88-2.35 medium and 2.35 high (Mehlich III - Mehlich, 1984) all the test sites had medium to low organic carbon. With respect to organic matter (% OM), at classification of 1.5 low, 1.5-4.0 medium and > 4.0 high all the sites had medium to low OM. In terms of percent N at classification of 0.08 very low 0.08-0.12 low, 0.12-0.2 medium, 0.20-0.30, it was only Bembeke site which was medium in sub-soil (0.16). The rest of the sites were low N. Tsangano had low P in top soil, medium in sub-soil, Bvumbwe had very high P and Lunyangwa had medium in top soil and low in sub-soil. Bembeke had low P in both sections of the soil profile at classification of P

(8  $\mu\text{g g}^{-1}$  very low, 9-18  $\mu\text{g g}^{-1}$  low, 19-25  $\mu\text{g g}^{-1}$  medium (adequate range) > 34  $\mu\text{g g}^{-1}$  very high) (Mehlich III - Mehlich, 1984).

**Table 4.2 Soil characterization for the low pH sites**

Low pH site	Depth (cm)	pH	%OC	%OM	%N	P ( $\mu\text{g g}^{-1}$ )	Ca ( $\mu\text{g g}^{-1}$ )	Mg ( $\mu\text{g g}^{-1}$ )	Al%
Tsangano	0-15	5.35	1.23	2.13	0.11	16.75	2.02	0.37	0.40
	15-30	5.38	1.97	3.40	0.10	19.27	2.18	0.37	1.33
Bvumbwe	0-15	5.67	1.37	2.36	0.12	64.58	3.38	0.95	0.60
	15-30	5.66	1.16	1.99	0.10	62.51	2.81	0.74	0.33
Lunyangwa	0-15	4.69	1.50	2.59	0.13	24.30	1.15	0.21	0.87
	15-30	4.51	1.05	1.81	0.09	10.46	0.58	0.11	0.60
Bembeke	0-15	5.13	1.82	3.14	0.16	16.30	2.05	0.42	0.60
	15-30	5.01	1.33	2.28	0.11	13.64	1.32	0.25	0.80

OC = organic carbon; OM = organic matter; N = nitrogen; P = phosphorus; Ca = calcium; Mg = magnesium; Al = aluminium

#### 4.5.2 Combined ANOVA for grain yield and agronomic traits at four low pH environments across two seasons 2011/12 and 2012/3

Mean squares from the combined ANOVA across four sites and two seasons are given for the top 10 and bottom 10 maize genotypes in terms of grain yield and agronomic traits (Table 4.3). Results for all entries under low pH soil environments are presented in Appendix 7. Genotype mean squares were significant for days to anthesis, anthesis-silking interval, days to 50% silking, plant and ear height, number of ears per plant, LB disease, husk cover, GLS disease, grain texture, 100 seed weight and plant vigour. Location mean squares were significant for all the measured and derived traits. Season mean squares were significant for all characteristics except for ear height, husk cover, and stem lodging. The GEI (Table 4.3 - GxE) was significant for anthesis date, days to 50% silking, ear height, husk cover, grain texture, rotten ears, 100 seed weight, and plant vigour. Interaction of genotype by season (GxY) mean squares were significant for days to anthesis, days to 50% silking, plant height, number of ears per plant, LB disease, GLS disease, MSV disease, rust disease, grain texture, rotten ears and 100 seed weight.

The interaction of environment by season (ExY) was not significant for husk cover, plant height and rotten ears but was significant for the rest of the traits. Interaction of genotype by environment by season (GxExY) was significant for anthesis date, anthesis-silking interval, days to 50% silking, LB disease and root lodging.

#### **4.5.3 Estimated contributions to total sum of squares across four low pH soil environments for the 2011/12 and 2012/13 seasons**

Contribution to total sum of squares for genotype was the highest for husk cover (14.5%) and grain texture (8.4%, Table 4.4). Contribution due to environment was high for plant vigour (47.1%), grain yield (63.4%), ear height (41.7%) and days to 50% silking (40.6%). The contribution of season was high for rust disease (18.1%). GxE contribution was significant for grain texture (23.5%) and ear rots (27.4%). The interaction of genotype by season (GxY) sum of squares made the highest contribution to gray leaf spot and MSV (8.8%) and husk cover variation (7.9%). The interaction of ExY made the highest contribution to 100 seed weight (22.8%), root lodging (19.4%) and ear height (14.1%). The interaction of GxExY made the highest contribution to shelling percentage (11.8%) and anthesis date (11.2%) (Table 4.4).

#### **4.5.4. Estimated percent reduction for grain yield and other salient phenotypic traits at four low pH soil environments versus four optimal environments across 2011/12 and 2012/13 seasons**

The reduction in traits was estimated as the difference between the trait's trial mean for optimal and low pH and subtracted from 100% expressed as a percentage of the optimal i.e.  $\text{Reduction \%} = (X_{\text{optimal}} - X_{\text{lowph}}) / X_{\text{optimal}} * 100\%$ . The results are presented in Table 4.5. The combined mean reduction was 69.9%. The highest reduction was recorded for shelling percentage (87.5%) followed by number of ears per plant (78.0%) and then by plant height (76.6%).

**Table 4.3 Mean squares for combined ANOVA for grain yield and agronomic traits at four low pH environments across 2011/12 and 2012/13 seasons**

Source	Genotypes	Environment	Year	GxE	GxY	ExY	GxExY	MSE
Df	44	3	1	132	44	3	132	714
GY	4.04E+05	310600000**	65600000**	4.11E+05	5.01E+05	6821000**	4.59E+05	3.99E+05
AD	60.55**	5708.84**	9122.61**	35.8**	49.45**	1501.4**	50.84**	18.05
ASI	5.62**	922.76**	28.34**	3.83	3.67	207.43**	4.52**	3.2
DS	62.99***	9903.85**	8172.76**	33.67**	53.27**	2245.89**	45.59**	18.3
EH	292.1*	59649.2**	164.8	310.1**	219.5	30332.6**	131.2	185.6
EPP	0.33*	8.71**	35.07**	0.19	0.36*	7.81**	0.24	0.22
LB	0.88*	36.22**	48.87**	0.71	1.2**	42.24**	0.81*	0.59
HC	4.99**	36.2**	0.08	3.58**	2.73	1.64	2.72	2.02
GLS	2.47*	20.29**	20.14**	1.94	3.09**	38.11**	1.67	1.59
GT	1.75**	64.85**	47.70**	1.65**	0.85*	3.25**	0.48	0.49
MSV	0.17	17.84**	20.01**	0.14	0.41**	6.63**	0.28	0.2
PH	16886**	230489**	274015**	7926	15505*	17503	11701	10306
RE	1.36	13.14**	45.22**	2.61**	2.12*	1.53	2.63	1.43
RL	3.28	268.03**	325.41**	3.02	3.77	342.43**	3.65*	2.73
Rust	0.4	53.38**	80.96**	0.36	0.59**	25.82**	0.36	0.32
SL	3.06	48.77**	1.63	2.73	2.55	135.27**	2.89	2.5
SWT	60.58**	2419.84**	1117.69**	29.63**	42.7**	3397.1**	31.84	20.97
SH	139.2	12739**	1131*	188.4	148.2	908**	209.6	176.6
VIG	1.81**	184.46**	9.42**	0.92**	0.75	49.95**	0.66	0.56

\*\*\*P≤0.001; \*\*P≤0.01; \*P≤0.05; G = genotype, E = environment, Y = year, MSE = mean square error, Df = degree of freedom, GY = grain yield (kg ha<sup>-1</sup>), AD = days to anthesis (days), ASI = anthesis-silking interval (days), DS = days to silking (days), EH= ear height (cm), EPP = ears per plant (#), LB = leaf blight disease (1-5), HC = husk cover, GLS = gray leaf spot disease (1-5), GT = grain texture (1-5), MSV = maize streak virus disease (1-5), PH = plant height (cm), RE = rotten ears (#), RL = root lodging (#), Rust = rust disease (1-5), SL= stem lodging (#), SWT = 100 seed weight (g), SH = shelling percentage, VIG = vigour (1-5), # = number

**Table 4.4 Estimated percentage contributions to total sum of squares for traits at four low pH environments combined for 2011/12 and 2012/13 seasons**

<b>Variation</b>	<b>GY</b>	<b>AD</b>	<b>ASI</b>	<b>DS</b>	<b>EH</b>	<b>EPP</b>	<b>LB</b>	<b>GLS</b>	<b>GT</b>	<b>HC</b>	<b>MSV</b>	<b>PH</b>	<b>RE</b>	<b>RL</b>	<b>Rust</b>	<b>SL</b>	<b>SWT</b>	<b>SH</b>	<b>VIG</b>
Genotype	1.2	4.5	3.4	3.8	3.0	5.1	5.2	7.0	8.4	14.5	3.8	6.8	4.8	2.7	3.9	4.7	6.0	2.6	6.8
Environment	63.4	28.6	38.3	40.6	41.7	9.1	9.6	2.6	21.4	4.8	17.6	6.4	3.1	15.2	23.8	5.1	16.2	16.4	47.1
Year	4.5	15.3	0.4	11.2	0.0	12.2	6.5	1.3	5.2	0.0	9.9	2.5	3.6	6.1	18.1	0.1	2.5	0.5	0.8
GxE	3.7	7.9	7.0	6.1	9.5	8.7	8.3	11.1	23.5	20.8	6.2	9.6	27.4	7.5	7.1	12.6	8.7	10.6	10.4
GxY	1.5	3.6	2.2	3.2	2.3	5.5	7.2	8.8	4.1	7.9	8.8	6.3	7.4	3.1	5.9	3.9	4.2	2.8	2.8
ExY	1.4	7.5	8.6	9.2	14.1	5.4	11.2	4.9	0.7	0.0	3.3	0.3	1.2	19.4	5.8	9.5	22.7	1.2	4.3
GxExY	4.1	11.2	8.3	8.2	2.7	7.2	9.4	9.5	4.7	0.3	6.0	9.5	1.6	9.1	3.5	8.9	9.1	11.8	2.5
Residual	19.4	21.4	31.6	17.7	26.6	46.7	41.8	54.8	31.7	47.6	44.0	58.3	50.9	36.8	31.6	54.7	29.7	53.7	25.5
Rep	0.8	0.0	0.2	0.0	0.0	0.3	0.8	0.6	0.2	4.2	0.5	0.3	0.0	0.1	0.3	0.4	1.0	0.4	0.1
<b>CV (%)</b>	<b>42.4</b>	<b>5.1</b>	<b>67.1</b>	<b>5.0</b>	<b>28.1</b>	<b>54.2</b>	<b>35.2</b>	<b>82.9</b>	<b>58.9</b>	<b>27.8</b>	<b>36.5</b>	<b>77.0</b>	<b>102.5</b>	<b>92.1</b>	<b>44.6</b>	<b>127.6</b>	<b>20.3</b>	<b>19.2</b>	<b>27.4</b>
<b>Min</b>	<b>0.8</b>	<b>0.0</b>	<b>0.2</b>	<b>0.0</b>	<b>0.0</b>	<b>0.3</b>	<b>0.8</b>	<b>0.6</b>	<b>0.2</b>	<b>0.0</b>	<b>0.5</b>	<b>0.3</b>	<b>0.0</b>	<b>0.1</b>	<b>0.3</b>	<b>0.1</b>	<b>1.0</b>	<b>0.4</b>	<b>0.1</b>
<b>Max</b>	<b>63.4</b>	<b>28.6</b>	<b>38.3</b>	<b>40.6</b>	<b>41.7</b>	<b>46.7</b>	<b>41.8</b>	<b>54.7</b>	<b>31.7</b>	<b>47.6</b>	<b>44.0</b>	<b>58.3</b>	<b>50.9</b>	<b>36.8</b>	<b>31.6</b>	<b>54.7</b>	<b>29.7</b>	<b>53.7</b>	<b>47.1</b>

G = Genotype, E = environment, Y = year, CV = coefficient of variation, Min = minimum, Max = maximum, GY = grain yield (kg ha<sup>-1</sup>), AD = days to anthesis (days), ASI = anthesis-silking interval (days), DS = days to silking (days), EH= ear height (cm), EPP = ears per plant (#), LB = leaf blight disease (1-5), GLS = gray leaf spot disease (1-5), GT = grain texture (1-5), HC = husk cover, MSV = maize streak virus disease (1-5), PH = plant height (cm), RE = rotten ears (#), RL = root lodging (#), Rust = rust disease (1-5), SL= stem lodging (#), SWT = 100 seed weight (g), SH = shelling percentage, VIG = vigour (1-5), # = number

**Table 4.5 Estimated reduction of grain yield and other salient traits under low pH versus optimal conditions across the 2011/12 and 2012/13 seasons**

<b>Trait</b>	<b>Optimal</b>	<b>Low pH</b>	<b>% Reduction</b>
GY	3372.9	1490.3	44.2
EH	77.6	46.8	60.3
EPP	1.1	0.9	78.0
PH	172.3	131.9	76.6
SWT	29.9	22.6	75.5
SH	79.2	69.3	87.5
VIG	4.0	2.7	67.5
<b>Mean</b>			<b>69.9</b>

GY = grain yield (kg ha<sup>-1</sup>), EH = ear height (cm), EPP = ears per plant (#), PH = plant height (cm), SWT = 100 seed weight (g), SH = shelling percentage, VIG = vigour (1-5), # = number

#### **4.5.5 Genotypic and phenotypic variance components, genetic advance and broad sense heritability estimates across four low pH soil environments combined for 2011/12 and 2012/13 seasons**

The results in Table 4.6 indicated that phenotypic variances were higher than genotypic variances. Grain texture (0.56), days to 50% silking (0.55), anthesis date (0.54) and plant vigour (0.53) had relatively high broad sense heritability estimates. The genetic coefficients of variations were lower than phenotypic coefficients of variation except for plant height. The expected genetic advance was highest for plant height followed by ear height and these were followed by anthesis date and 100 seed weight. Grain yield had the lowest expected genetic advance with high phenotypic coefficient of variation (Table 4.7).

**Table 4.6 Genotypic variances, phenotypic variances and heritability estimates at low pH sites across two seasons 2011/12 and 2012/13**

<b>Trait</b>	<b><math>\sigma^2_g</math></b>	<b><math>\sigma^2_p</math></b>	<b>H<sup>2</sup>b</b>	<b>H<sup>2</sup>b %</b>
GY	2.300	401.400	0.006	0.623
AD	21.250	39.300	0.541	54.071
ASI	1.200	4.400	0.273	27.273
DS	22.345	40.645	0.550	54.976
EH	53.250	238.850	0.223	22.294
EPP	0.055	0.275	0.200	20.000
LB	0.145	0.735	0.197	19.728
HC	0.450	2.050	0.220	21.951
GLS	0.630	1.120	0.563	56.250
GT	1.485	3.505	0.424	42.368
MSV	0.015	0.185	0.081	8.108
PH	3290.000	13596.000	0.242	24.198
RE	0.035	1.395	0.025	2.509
RL	0.540	2.740	0.197	19.708
Rust	0.040	0.360	0.111	11.111
SL	0.280	2.780	0.101	10.072
SWT	19.815	40.785	0.486	48.584
SH	18.700	157.900	0.118	11.843
VIG	0.620	1.180	0.525	52.542
<b>Min</b>	<b>0.015</b>	<b>0.185</b>	<b>0.081</b>	<b>0.623</b>
<b>Max</b>	<b>3290.000</b>	<b>13596.000</b>	<b>0.563</b>	<b>56.250</b>

Min = minimum, Max = maximum, GY = grain yield (kg ha<sup>-1</sup>), AD = days to anthesis (days), ASI = anthesis-silking interval (days), DS = days to silking (days), EH= ear height (cm), EPP = ears per plant (#), LB = leaf blight disease (1-5), HC = husk cover, GLS = gray leaf spot disease (1-5), GT = grain texture (1-5), MSV = maize streak virus disease (1-5), PH = plant height (cm), RE = rotten ears (#), RL = root lodging (#), Rust = rust disease (1-5), SL= stem lodging (#), SWT = 100 seed weight (g), SH = shelling percentage, VIG = vigour (1-5), # = number

**Table 4.7 Genotypic coefficient of variation, phenotypic coefficient of variation and expected genetic advance at low pH sites across two seasons 2011/12 and 2012/13**

Trait	GCV%	PCV%	GA	GA (% of mean)
GY	12.4	164.1	0.5	32.7
AD	5.1	6.9	2.6	3.2
ASI	6.7	12.9	1.1	41.0
DS	5.1	6.9	2.7	3.1
EH	10.7	22.6	2.7	5.7
EPP	2.6	5.6	0.5	54.8
LB	2.6	5.8	0.6	26.9
HC	9.3	14.3	1.3	74.6
GLS	4.5	9.7	0.8	37.4
GT	5.0	6.7	1.1	43.7
MSV	1.0	4.2	0.2	19.8
PH	49.9	101.5	7.6	5.8
RE	1.8	11.2	0.2	21.3
RL	3.9	12.9	0.6	31.9
Rust	1.8	5.3	0.4	29.4
SL	4.8	15.0	0.6	47.7
SWT	9.4	13.4	2.5	11.2
SH	5.2	16.8	1.7	2.4
VIG	4.8	6.6	1.1	39.7
<b>Min</b>	<b>1.0</b>	<b>4.2</b>	<b>0.2</b>	<b>2.4</b>
<b>Max</b>	<b>49.9</b>	<b>164.1</b>	<b>7.6</b>	<b>74.6</b>

GCV = genotypic coefficient of variation, PCV = phenotypic coefficient of variation, GA = genetic advance, GY = grain yield (kg ha<sup>-1</sup>), AD = days to anthesis (days), ASI = anthesis-silking interval (days), DS = days to silking (days), EH= ear height (cm), EPP = ears per plant (#), LB = leaf blight disease (1-5), HC = husk cover, GLS = gray leaf spot disease (1-5), GT = grain texture (1-5), MSV = maize streak virus disease (1-5), PH = plant height (cm), RE = rotten ears (#), RL = root lodging (#), Rust = rust disease (1-5), SL= stem lodging (#), SWT = 100 seed weight (g), SH = shelling percentage, VIG = vigour (1-5), # = number, Min = minimum, Max = maximum.

#### **4.5.6 Mean performance for grain yield and other traits across four low pH soil environments combined for 2011/12 and 2012/13 seasons**

The grain yield trial mean was 1490 kg ha<sup>-1</sup> (Table 4.8) and the top five performing genotypes were G24 (LPHpop16) G30 (LPHpop3) G22 (VPO739), G15 (VPO5173) and G27 (LOW N POOL C3-B). The latest maturing genotype was G35 (86 AD = 172 days

**Table 4.8 Mean performance for grain yield and other agronomic traits across four low pH environments combined for 2011/12 and 2012/13 seasons**

	Entry	GY	AD	ASI	DS	EH	EPP	LB	HC	GLS	GT	MSV	PH	RE	RL	Rust	SL	SWT	SH%	VIG
Top 10 genotypes																				
	G24	1792	79.7	3.0	83.0	51.2	0.8	2.4	1.6	2.3	2.4	1.1	134	1.4	2.3	1.5	1.2	24	73	2.7
	G30	1765	82.5	2.9	85.4	44.0	0.9	1.9	2.4	2.1	2.3	1.3	119	1.1	2.0	1.3	0.6	24	74	2.1
	G22	1753	81.6	2.5	84.8	48.2	0.9	2.2	2.1	2.1	2.4	1.2	126	1.2	1.7	1.2	1.1	23	70	2.7
	G15	1746	82.5	3.5	86.0	48.6	0.9	2.3	1.7	2.4	2.7	1.1	132	1.2	2.2	1.5	1.4	22	70	2.8
	G27	1701	84.7	2.0	86.7	49.8	0.9	2.1	1.0	1.9	2.8	1.2	129	1.2	1.3	1.1	1.7	27	69	2.4
	G6	1655	82.0	2.0	83.1	44.4	0.9	2.4	1.3	2.7	2.8	1.2	129	1.6	1.4	1.2	0.7	22	68	2.7
	G16	1650	80.8	3.1	82.9	38.9	0.8	1.9	1.9	2.1	2.6	1.3	116	1.0	1.3	1.4	1.6	24	71	2.7
	G21	1602	83.8	2.8	86.6	42.6	0.9	2.3	1.7	2.0	2.9	1.1	126	1.0	1.8	1.4	1.0	22	71	2.6
	G34	1599	81.6	3.0	84.0	44.7	0.8	2.0	1.1	1.8	1.8	1.3	123	0.9	1.7	1.3	1.1	22	70	2.7
	G26	1575	84.5	3.2	87.7	47.5	0.8	2.6	2.2	2.1	1.9	1.3	125	1.2	1.3	1.2	0.9	23	68	2.7
Bottom 10 genotypes																				
	G25	1401	84.5	2.7	86.4	50.8	0.9	2.2	1.5	1.9	2.5	1.2	141	1.1	1.5	1.3	1.1	23	69	2.5
	G35	1396	86.3	3.0	89.3	45.2	0.8	2.1	2.3	2.3	3.2	1.1	126	1.4	2.0	1.2	1.1	26	69	2.8
	G33	1385	83.0	3.0	86.5	46.6	0.8	2.0	1.2	2.1	2.5	1.2	127	1.0	2.4	1.3	1.3	22	70	2.5
	G41	1376	80.3	2.8	83.5	40.6	0.8	2.0	1.9	3.7	2.6	1.3	118	1.0	1.8	1.2	1.0	21	67	2.8
	G4	1372	83.5	3.4	87.3	48.6	0.8	2.3	2.2	2.2	2.7	1.1	127	1.7	1.8	1.4	0.9	23	68	2.4
	G14	1362	83.6	2.7	86.3	46.5	0.8	2.2	2.5	2.3	2.3	1.2	148	0.9	2.0	1.0	1.3	22	68	2.7
	G44	1359	83.9	2.4	86.7	48.1	0.9	2.1	1.2	2.1	2.4	1.3	135	0.9	1.8	1.4	1.0	23	67	3.3
	G10	1314	84.6	2.8	87.5	46.2	1.0	2.3	1.4	2.1	2.9	1.3	123	0.9	2.3	1.4	1.3	19	68	2.5
	G2	1282	82.4	2.3	84.8	48.2	0.8	2.4	1.6	1.8	2.4	1.2	127	0.9	2.5	1.4	1.7	22	65	3.0
	G31	1195	80.3	2.6	82.9	45.4	0.9	2.0	2.3	2.1	2.6	1.3	123	1.3	2.2	1.3	0.9	22	64	3.1
	<b>Mean</b>	<b>1490.6</b>	<b>83.1</b>	<b>2.7</b>	<b>85.8</b>	<b>46.8</b>	<b>0.9</b>	<b>2.2</b>	<b>1.7</b>	<b>2.1</b>	<b>2.5</b>	<b>1.2</b>	<b>131.9</b>	<b>1.2</b>	<b>1.8</b>	<b>1.3</b>	<b>1.2</b>	<b>22.6</b>	<b>69.3</b>	<b>2.7</b>
	<b>LSD</b>	<b>358</b>	<b>2.4</b>	<b>1</b>	<b>2.4</b>	<b>7.7</b>	<b>0.3</b>	<b>0.4</b>	<b>0.8</b>	<b>0.7</b>	<b>0.4</b>	<b>0.3</b>	<b>58</b>	<b>0.7</b>	<b>0.9</b>	<b>0.3</b>	<b>0.9</b>	<b>3</b>	<b>8</b>	<b>0.4</b>
	<b>CV (%)</b>	<b>42.4</b>	<b>5.1</b>	<b>67</b>	<b>5</b>	<b>28</b>	<b>54</b>	<b>35</b>	<b>83</b>	<b>59</b>	<b>28</b>	<b>37</b>	<b>77</b>	<b>103</b>	<b>92.1</b>	<b>44.6</b>	<b>127.6</b>	<b>20.3</b>	<b>19</b>	<b>27.4</b>
	<b>SE</b>	<b>631</b>	<b>4.2</b>	<b>1.8</b>	<b>4.3</b>	<b>13.6</b>	<b>0.5</b>	<b>0.8</b>	<b>1.4</b>	<b>1.3</b>	<b>0.7</b>	<b>0.5</b>	<b>101.5</b>	<b>1.2</b>	<b>1.7</b>	<b>0.6</b>	<b>1.6</b>	<b>4.6</b>	<b>13</b>	<b>0.8</b>
	<b>Min</b>	<b>1195.0</b>	<b>79.7</b>	<b>1.6</b>	<b>82.9</b>	<b>38.0</b>	<b>0.7</b>	<b>1.8</b>	<b>0.8</b>	<b>1.6</b>	<b>1.8</b>	<b>1.1</b>	<b>116.0</b>	<b>0.8</b>	<b>1.2</b>	<b>1.0</b>	<b>0.6</b>	<b>19.0</b>	<b>64.0</b>	<b>2.1</b>
	<b>Max</b>	<b>1792.0</b>	<b>86.3</b>	<b>3.5</b>	<b>89.3</b>	<b>53.9</b>	<b>1.0</b>	<b>2.7</b>	<b>2.6</b>	<b>3.7</b>	<b>3.2</b>	<b>1.5</b>	<b>148</b>	<b>1.7</b>	<b>2.7</b>	<b>1.5</b>	<b>2.2</b>	<b>27.0</b>	<b>75.0</b>	<b>3.3</b>

LSD = Least significant difference, CV = coefficient of variation, SE = Min = minimum, Max = maximum, GY = grain yield (kg ha<sup>-1</sup>), AD = days to anthesis (days), ASI = anthesis-silking interval (days), DS = days to silking (days), EH= ear height (cm), EPP = ears per plant (#), LB = leaf blight disease (1-5), HC = husk cover, GLS = gray leaf spot disease (1-5), GT = grain texture (1-5), MSV = maize streak virus disease (1-5), PH = plant height (cm), RE = rotten ears (#), RL = root lodging (#), Rust = rust disease (1-5), SL= stem lodging (#), SWT = 100 seed weight (g), SH = shelling percentage, VIG = vigour (1-5), # = number

physiological maturity period) and the earliest of the group was G24 (80 AD = 160 days physiological maturity period). Maturity period for any maize genotype is twice the number of days to flowering. The genotype most susceptible to gray leaf spot disease was G 41. The shortest genotype as a result of low pH effect was G 16 (116 cm). Genotype G27 recorded the highest 100 seed weight (27 g) followed by G35 (26 g). The best shelling percentage was recorded for G30 (74%). The genotype with the best plant vigour was G30 followed by G27 and G4 (Table 4.8).

#### **4.5.7 Pearson's correlation coefficients between grain yield and agronomic traits across four low pH soil environments combined for 2011/12 and 2012/13 seasons**

Grain yield was positively and highly significantly correlated with anthesis date ( $r = 0.12$ ), anthesis-silking interval ( $r = 0.35$ ), days to silking ( $r = 0.21$ ), number of ears per plant ( $r = 0.17$ ) and shelling percentage ( $r = 0.19$ ). Days to anthesis were significantly correlated with most traits excluding stem lodging and it was negatively correlated with plant and ear height as well as root lodging. Anthesis-silking interval also correlated with traits like: days to silking, shelling percentage and ears per plant. Days to silking correlated negatively with plant and ear height ( $-0.20$  and  $-0.40$ ) as well as with root lodging ( $-0.10$ ). Correlation for plant height was positively with ear height, husk cover and 100 seed weight,  $0.40$ ,  $0.10$  and  $0.2$ , respectively. Ear height correlated with traits like, root lodging, 100 seed weight, ears per plant, shelling percentage, vigor and diseases such as leaf blight and rust. Number of ears per plant furthermore correlates with among others, grain texture, 100 seed weight and vigor (Table 4.9).

#### **4.5.8 Principal component analysis results, eigenvalues and eigenvectors for the traits across four low pH soil environments combined for 2011/12 and 2012/13 seasons**

Results indicated that eight principal components were generated (Table 4.10) which accounted for 100% variability present in the maize genotypes evaluated. The first five PC had eigenvalues higher than 1 and their cumulative percentages accounted for 71.1% of the total variation present among the genotypes.

**Table 4.9 Pearson's correlation coefficients for grain yield and agronomic traits across four low pH environments for two seasons**

	GY	AD	ASI	DS	PH	EH	RL	SL	HC	RE	GT	LB	GLS	Rust	MSV	SWT	VIG	SH	
<b>AD</b>	0.12**																		
<b>ASI</b>	0.35**	0.30**																	
<b>DS</b>	0.12**	0.90**	0.30**																
<b>PH</b>	-0.02	-0.10**	-0.02	-0.20**															
<b>EH</b>	-0.03	-0.30**	-0.01	-0.40**	0.40**														
<b>RL</b>	0.02	-0.10**	0.03	-0.10**	0.07	0.34**													
<b>SL</b>	0.03	0.04	0.01	0.03	-0.03	-0.01	0.04												
<b>HC</b>	-0.02	0.10**	-0.01	0.04	0.10**	0.08	-0.20**	0.02											
<b>RE</b>	-0.06	0.20**	-0.03	0.20**	-0.03	-0.20**	-0.20**	0.10**	0.30**										
<b>GT</b>	0.03	0.09**	0.02	0.12**	0.01	-0.02	0.00	-0.04	-0.10**	0.04									
<b>LB</b>	0.01	0.12**	0.02	0.09**	0.06	0.09**	0.12**	0.17**	0.08	0.22**	0.30**								
<b>GLS</b>	-0.02	0.14**	0.00	0.14**	0.04	0.03	0.13**	0.03	0.00	0.16**	0.30**	0.50**							
<b>Rust</b>	-0.08	0.08*	-0.01	0.01	0.16	0.11**	-0.08	0.15**	0.30**	0.34**	0.30**	0.60**	0.40**						
<b>MSV</b>	-0.02	0.08*	-0.01	0.02	0.06	0.04	-0.03	0.20**	0.13**	0.29**	0.30**	0.70**	0.40**	0.60**					
<b>SWT</b>	0.05	0.06*	0.04	0.05	0.20**	0.40**	0.38**	0.05	-0.03	0.01	-0.03	0.20**	0.20**	0.04	0.00				
<b>VIG</b>	0.03	0.13**	0.02	0.16**	0.01	0.09**	0.29**	0.12**	-0.07	0.14**	0.10**	0.30**	0.20**	0.20**	0.10	0.40**			
<b>SH</b>	0.19**	0.27**	0.30**	0.24**	0.05	0.09**	0.07	0.05	0.05	0.06	0.10**	0.10**	0.10**	0.10	0.10	0.20**	0.10**		
<b>EPP</b>	0.17**	0.67**	0.30**	0.65**	0.07	0.12**	0.07	0.05	0.05	0.06	0.10**	0.10**	0.10**	0.10**	0.10**	0.20**	0.10**	0.40*	

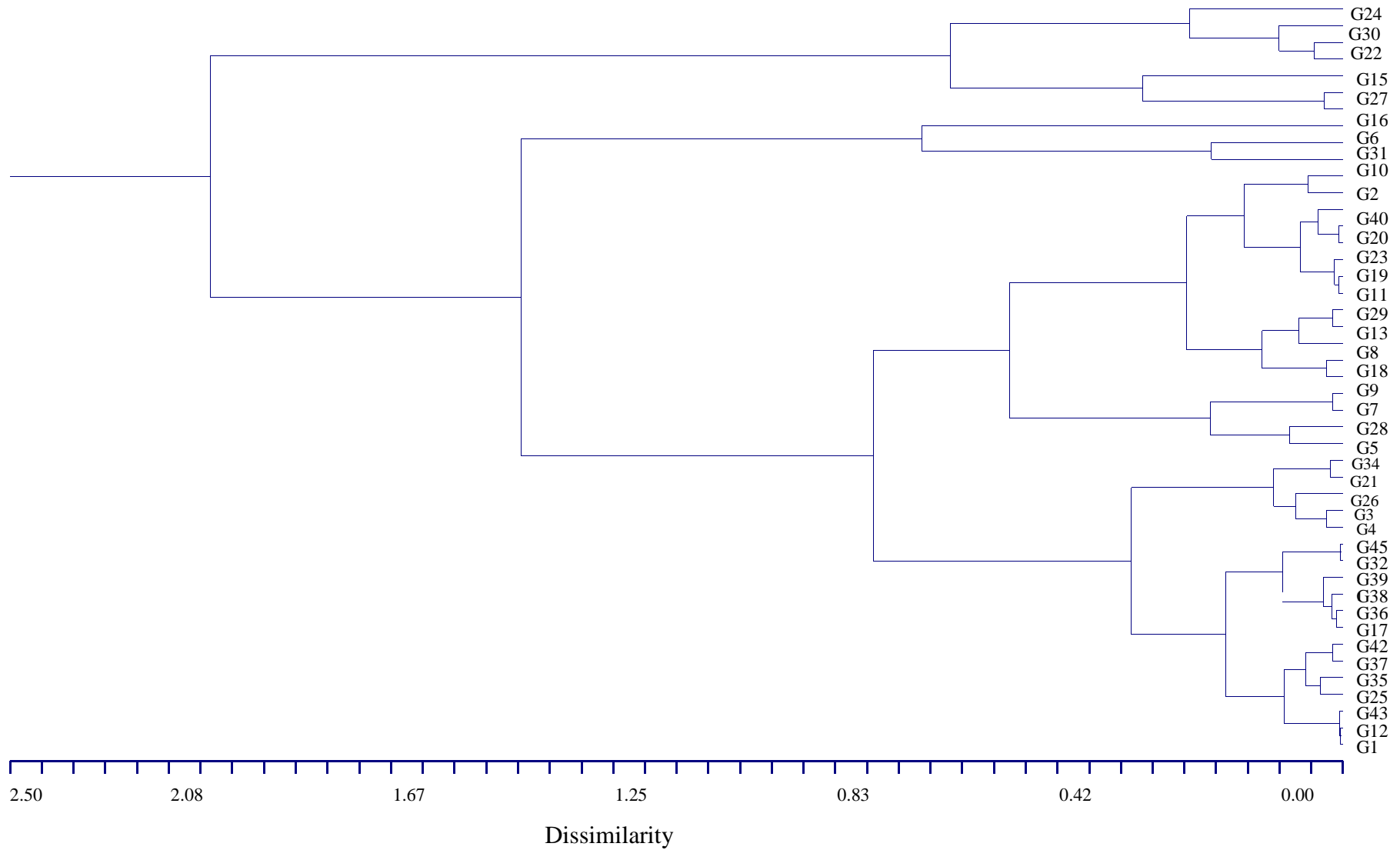
\*\*P<0.01, \* P<0.05, GY = grain yield (kg ha<sup>-1</sup>), AD = days to anthesis (days), ASI = anthesis-silking interval (days), DS = days to silking (days), PH = plant height (cm), EH= ear height (cm), RL = root lodging (#), SL= stem lodging (#), HC = husk cover, RE = rotten ears (#), GT = grain texture (1-5), LB = leaf blight disease (1-5), GLS = gray leaf spot disease (1-5), Rust = rust disease (1-5), MSV = maize streak virus disease (1-5), SWT = 100 seed weight (g), VIG = vigour (1-5), SH = shelling percentage, EPP = ears per plant (#), (#) = number.

**Table 4.10 Eigenvalues, percentages and cumulative percentages for the measured and derived data across four low pH soil environments combined for 2011/12 and 2012/13 seasons**

Principal component	Eigenvalues	As percentages	Cumulative percentages
1	2.2	18.5	18.5
2	1.8	15.7	34.2
3	1.5	13.1	47.4
4	1.5	12.5	59.8
5	1.3	11.3	71.1
6	1.2	10.0	81.1
7	1.1	9.7	90.8
8	1.1	9.2	100.0

#### **4.5.9 Clustering of maize genotypes evaluated at four low pH soil environments combined across 2011/12 and 2012/13 seasons**

The dendrogram was constructed using the UPGMA cluster analysis method. Forty five maize genotypes were clustered based on means for grain yield. At cut off point 1.0, three main clusters were observed (Figure 4.1) with a high cophenetic correlation of  $r_{cop} = 0.82$ . Thirteen genotypes from CIMMYT-Zimbabwe were grouped in cluster two with only two from CIMMYT-Colombia. The top performing genotypes were grouped in cluster three which comprised of 24 genotypes from both Colombia and Zimbabwe. Cluster one was comprised of six genotypes which were also from both CIMMYT research centres.



**Figure 4.1 Dendrogram based on Euclidean distance and UPGMA clustering using morphological data for genotypes at four low pH environments combined for 2011/12 and 2012/13 seasons**

#### **4.5.10 Performance of maize genotypes across four optimal soil environments combined for 2011/12 and 2012/13 seasons**

Mean squares from the combined ANOVA across four sites and across two years presented are for the top10 and bottom 10 maize genotypes in terms of grain yield (Table 4.11). Results for all entries under optimal environments are presented in Appendix 7. Significant differences due to genotypes were observed for 16 measured traits except for anthesis-silking interval, husk cover and root lodging. Environment mean squares were significant for all traits except for 100 seed weight. Season mean squares were significant for all traits excluding MSV and 100 seed weight. G x E mean squares were significant for most traits except for grain yield, anthesis-silking interval, number of ears per plant, leaf blight and gray leaf spot disease as well as shelling percentage. G x Y interaction mean squares were not significant for anthesis-silking interval, maize streak virus, rotten ears and rust disease. The interaction of E x Y mean squares was significant for traits except for diseases: leaf blight, MSV and rust, and the following traits: husk cover, stem and root lodging, rotten ears, plant vigour and 100 seed weight. The interaction G x E x Y was only significant for grain yield, anthesis date, days to 50% silking, gray leaf spot, grain texture and plant vigour (Table 4.11).

#### **4.5.11. Estimated contributions to total sum of squares across four optimal environments combined for 2011/12 and 2012/13 seasons**

Contribution due to genotype was the highest for 100 seed weight (14.1%), MSV (13.4%) and stem lodging (12.0%) (Table 4.12). Contribution due to environment was high for anthesis date (67.0%), plant vigour (53.7%), Rust (48.1%), ear height (48.2%). The contribution due to the effect of season was high for root lodging (24.1%), rust disease (18.5%) and rotten ears (16.5%). G x E contribution to variation was high for MSV (19.1%), husk cover (21.1%) and stem lodging (12.5%). The interaction of genotype by season sum of squares made the highest contribution to stem lodging (22.8%), root lodging (14.2%) and 100 seed weight (65.7%). The interaction of E x Y made the highest contribution to gray leaf spot (12.5%) anthesis date (14.1%) and days to 50% silking

**Table 4.11 Mean squares for combined ANOVA for grain yield and agronomic traits at four optimal environments for 2011/12 and 2012/13 seasons**

Source	Genotypes	Environment	Year	GxE	GxY	ExY	GxExY	MSE
Df	44	7	1	307	44	6	264	1334
GY	5753000***	1.1E+08**	4E+07**	1.33E+06	4978000**	21230000**	1597000**	1.15E+06
AD	117.10**	22428.24**	3497.57**	22.12**	48.65**	7204.79**	18.78**	8.70
ASI	3.30	442.03**	437.178**	5.00	6.20	207.96**	5.30	4.90
DS	111.87**	20256.23**	6094.80**	20.60**	49.30**	6081.36**	20.66**	9.30
EH	690.00**	77831**	3466.30**	270.1**	457.30**	8028.30**	236.10	199.10
EPP	0.07**	0.40**	1.22**	0.03	0.0812**	0.65**	0.03	0.03
LB	0.80**	79.38**	50.09**	0.32	1.01**	6.44	43.10	0.27
HC	2.70	326.10**	9.44*	4.55**	4.10**	4.40	23.10	2.10
GLS	0.93**	41.80**	1.85*	0.30	0.96**	63.96**	0.55*	0.34
GT	2.40**	55.50**	45.90**	1.10*	1.60**	8.67**	1.03*	0.80
MSV	0.60**	35.28**	0.00	0.40**	0.00	5.41	32.10	0.20
PH	1277.50**	101791.30**	29780.1**	557.8*	933.30**	21985.9**	490.80	432.80
RE	1.18**	85.10**	80.96**	0.67**	0.30	5.20	22.10	0.40
RL	4.20	1571.03**	2323.97**	10.51**	31.02**	6.41	42.10	4.30
Rust	0.80**	118.80**	137.14**	0.45**	0.20	3.40	21.10	0.30
SL	3.94**	128.45**	8.80**	2.05**	7.48**	2.40	29.20	1.30
SWT	80.30**	145.60	50.80	29.20**	89.86**	2157.90	30.30	25.40
SH	241.80**	3012.40**	2294.20**	119.90	174.20**	1538.10**	100.60	108.00
VIG	0.70**	138.75**	6.65**	0.53**	1.45**	0.60	0.53**	0.30

\*\*\*P<0.01; \*\*P<0.05; G = genotype, E = environment, Y = year, MSE = mean square error, Df = degree of freedom, GY = grain yield (kg ha<sup>-1</sup>), AD = days to anthesis (days), ASI = anthesis-silking interval (days), DS = days to silking (days), EH= ear height (cm), EPP = ears per plant (#), LB = leaf blight disease (1-5), HC = husk cover, GLS = gray leaf spot disease (1-5), GT = grain texture (1-5), MSV = maize streak virus disease (1-5), PH = plant height (cm), RE = rotten ears (#), RL = root lodging (#), Rust = rust disease (1-5), SL= stem lodging (#), SWT = 100 seed weight (g), SH = shelling percentage, VIG = vigour (1-5), # = number

**Table 4.12 Estimated percent contributions to total sum of squares at four optimal environments across 2011/12 and 2012/13 seasons**

Source	GY	AD	ASI	DS	EH	EPP	LB	GLS	GT	HC	MSV	PH	RE	RL	Rust	SL	SWT	SH	VIG
Genotypes	11.9	5.0	2.2	5.1	6.3	7.5	6.6	7.8	9.3	4.3	13.4	6.5	10.6	1.9	4.8	12.0	14.1	7.6	4.0
Environments	15.6	65.7	19.5	63.0	48.2	3.1	44.6	24.2	14.7	34.6	38.5	35.2	34.7	32.6	48.1	17.8	1.2	6.4	53.7
Year	1.8	3.4	6.4	6.3	0.7	3.1	9.4	0.4	4.0	0.3	0.0	3.4	16.5	24.1	18.5	0.6	0.2	1.6	0.9
GxE	8.3	2.8	9.7	2.8	7.3	10.9	7.9	9.8	12.4	21.1	19.1	8.4	12.1	9.6	7.9	12.5	10.2	11.3	9.0
GxY	10.3	2.1	4.0	2.3	4.2	9.2	8.3	8.1	6.3	6.4	0.9	4.7	2.8	14.2	1.5	22.8	15.7	5.5	8.2
ExY	3.0	14.1	6.1	12.6	3.3	5.0	0.0	12.3	1.5	0.0	0.0	5.1	0.1	1.0	1.8	0.1	8.6	3.3	0.1
GxExY	9.9	1.6	6.8	1.9	4.3	9.3	0.7	4.7	8.0	1.0	0.0	5.0	0.0	0.3	0.2	0.5	5.3	9.4	3.0
Rep	0.6	0.0	0.2	0.0	0.0	0.7	0.3	0.5	0.1	0.0	0.1	0.6	0.3	0.2	1.0	0.5	0.4	0.0	0.1
Residual	38.7	5.3	45.1	6.0	25.7	51.3	22.2	32.2	43.7	32.4	28.0	31.1	22.9	16.1	16.2	33.1	44.4	54.9	21.1
<b>CV (%)</b>	<b>31.8</b>	<b>4.6</b>	<b>194.2</b>	<b>4.6</b>	<b>18.2</b>	<b>15.2</b>	<b>27.1</b>	<b>103.1</b>	<b>36.0</b>	<b>103</b>	<b>35.9</b>	<b>12.1</b>	<b>80.3</b>	<b>301.5</b>	<b>31.3</b>	<b>169.4</b>	<b>16.8</b>	<b>13.1</b>	<b>27.3</b>
<b>Min</b>	<b>0.6</b>	<b>0.0</b>	<b>0.2</b>	<b>0.0</b>	<b>0.0</b>	<b>0.7</b>	<b>0.0</b>	<b>0.4</b>	<b>0.1</b>	<b>0.0</b>	<b>0.0</b>	<b>0.6</b>	<b>0.0</b>	<b>0.2</b>	<b>0.2</b>	<b>0.1</b>	<b>0.2</b>	<b>0.0</b>	<b>0.1</b>
<b>Max</b>	<b>38.7</b>	<b>65.7</b>	<b>45.1</b>	<b>63.0</b>	<b>48.2</b>	<b>51.3</b>	<b>44.6</b>	<b>32.2</b>	<b>43.7</b>	<b>34.6</b>	<b>38.5</b>	<b>35.2</b>	<b>34.7</b>	<b>32.6</b>	<b>48.1</b>	<b>33.1</b>	<b>44.4</b>	<b>54.9</b>	<b>53.7</b>

G = genotype, E = environment, Y = year, CV = coefficient of variation, Min = minimum, Max = maximum, GY = grain yield (kg ha<sup>-1</sup>), AD = days to anthesis (days), ASI = anthesis-silking interval (days), DS = days to silking (days), EH = ear height (cm), EPP = ears per plant (#), LB = leaf blight disease (1-5), GLS = gray leaf spot disease (1-5), GT = grain texture (1-5), HC = husk cover, MSV = maize streak virus disease (1-5), PH = plant height (cm), RE = rotten ears (#), RL = root lodging (#), Rust = rust disease (1-5), SL = stem lodging#, SWT = 100 seed weight (g), SH = shelling percentage, VIG = vigour (1-5), # = number

(12.6%). The interaction of G x E x Y made the highest contribution to grain yield (9.9%), shelling percentage (9.4%) and number of ears per plant (9.3%)

#### 4.5.12 Genotypic and phenotypic variance components, genetic advance and broad sense heritability estimates across four optimal environments combined for 2011/12 and 2012/13 seasons

The results in Table 4.13 indicated that phenotypic variances were higher than genotypic variances. Anthesis date (0.86), days to 50% silking (0.85) and grain yield (0.67) had relatively high broad sense heritability estimates. Root lodging had the lowest heritability estimate (0.02). The genetic coefficient of variation was lower than phenotypic coefficient of variation. The expected genetic advance (GA) was the highest for grain yield (68.9) followed by plant height (29.5) and ear height (22.5). It was low for number of ears per plant (0.2) and root lodging (0.4) (Table 4.14).

**Table 4.13 Genotypic ( $\sigma^2_g$ ) and phenotypic ( $\sigma^2_p$ ) variances and broad sense ( $H^2b$ ) heritability estimates at four optimal environments across 2011/12 and 2012/13 seasons**

Trait	$\sigma^2_g$	$\sigma^2_p$	$H^2b$	$H^2b$ %
GY	230.20	345.00	0.67	66.71
AD	54.19	62.90	0.86	86.14
ASI	0.77	4.11	0.19	18.82
DS	51.29	60.58	0.85	84.67
EH	245.45	444.60	0.55	55.21
EPP	0.02	0.05	0.41	40.70
LB	0.27	0.53	0.50	49.98
HC	0.34	2.40	0.14	13.98
GLS	0.29	0.64	0.46	46.20
GT	0.79	1.61	0.50	49.51
MSV	0.20	0.40	0.52	51.63
PH	422.40	855.20	0.49	49.39
RE	0.38	0.80	0.48	47.68
RL	0.08	4.24	0.02	1.97
Rust	0.26	0.54	0.49	49.05
SL	1.30	2.64	0.49	49.44
SWT	27.47	52.80	0.52	52.00
SH	66.90	174.90	0.38	38.25
VIG	0.20	0.50	0.39	39.28

$\sigma^2_g$  = genotypic variance,  $\sigma^2_p$  = phenotypic variance,  $H^2b$  = broad sense heritability,  $H^2b$  % = broad sense heritability as percentage, GY = grain yield ( $\text{kg ha}^{-1}$ ), AD = days to anthesis (days), ASI = anthesis-silking interval (days), DS = days to silking (days), EH = ear height (cm), EPP = ears per plant (#), LB = leaf blight disease (1-5), HC = husk cover, GLS = gray leaf spot disease (1-5), GT = grain texture (1-5), MSV = maize streak virus disease (1-5), PH = plant height (cm), RE = rotten ears (#), RL = root lodging (#), Rust = rust disease (1-5), SL = stem lodging (#), SWT = 100 seed weight (g), SH = shelling percentage, VIG = vigour (1-5), # = number.

**Table 4.14 Genotypic coefficient of variation, phenotypic coefficient of variation and expected genetic advance at optimal combined for 2011/12 and 2012/13 seasons**

Trait	GCV%	PCV%	GA	GA % of mean
GY	8.3	10.1	68.9	2.4
AD	9.2	9.9	10.6	21.8
ASI	8.2	19.0	1.3	69.2
DS	8.8	9.6	10.3	20.5
EH	17.8	23.9	22.5	31.0
EPP	1.3	2.1	0.2	16.6
LB	3.7	5.3	0.7	39.5
HC	4.9	13.1	0.8	32.1
GLS	4.3	6.3	0.8	46.8
GT	6.5	9.2	1.3	68.4
MSV	4.1	5.7	0.6	55.0
PH	15.7	22.3	29.5	17.3
RE	6.9	10.0	0.9	109.2
RL	3.5	24.8	0.4	12.1
Rust	4.0	5.7	0.7	44.4
SL	13.8	19.7	1.6	243.0
SWT	9.6	13.3	7.5	26.1
SH	9.2	14.9	11.7	13.2
VIG	3.1	5.0	0.6	28.4

GCV = genotypic coefficient of variation, PCV = phenotypic coefficient of variation, GA = genetic advance, GY = grain yield (kg ha<sup>-1</sup>), AD = days to anthesis, ASI = anthesis-silking interval (days), DS = days to silking (days), EH = ear height (cm), EPP = ears per plant (#), LB = leaf blight disease (1-5), HC = husk cover, GLS = gray leaf spot disease (1-5), GT = grain texture (1-5), MSV = maize streak virus disease (1-5), PH = plant height (cm), RE = rotten ears (#), RL = root lodging (#), Rust = rust disease (1-5), SL = stem lodging (#), SWT = 100 seed weight (g), SH = shelling percentage, VIG = vigour (1-5), # = number.

#### **4.5.13 Mean performance for grain yield and other traits across four optimal environments combined for 2011/12 and 2012/13 seasons**

The grain yield trial mean was 3.3 t ha<sup>-1</sup> and the top seven performing genotypes were G20 (4.5 t ha<sup>-1</sup>), G33 (4.0 t ha<sup>-1</sup>), G8 (4.0 t ha<sup>-1</sup>), G27 (4.0 t ha<sup>-1</sup>), G5 (3.9 t ha<sup>-1</sup>), G31 (3.8 t ha<sup>-1</sup>) and G10 (3.8 t ha<sup>-1</sup>) (Table 4.15). The lowest yielding were G40 (2.3 t ha<sup>-1</sup>) and G2 (2.3 t ha<sup>-1</sup>). The earliest maturing genotype was G24 (59 AD i.e. 118 days maturity period) and the shortest genotype was G2 (156 cm). The tallest genotype was G5 (189 cm). The best shelling percentage was recorded for G20 (83%) while G2 had the lowest shelling percentage. The genotype with the best plant vigour was G20 (1.5). High 100 seed weight was recorded for G40 (33.2 g).

**Table 4.15 Mean performance for grain yield across four optimal environments combined for 2011/12 and 2012/13 seasons**

	Entry	GY	AD	ASI	DS	EH	EPP	LB	HC	GLS	GT	MSV	PH	RE	RL	Rust	SL	SWT	SH	VIG
Top ten																				
genotypes	G20	4489	65.0	1.0	66.7	81	1.1	1.9	1.3	1.5	1.8	1.2	173	1.1	1.4	1.3	0.4	32.0	83.0	1.5
	G33	4003	61.3	1.2	63.4	75	1.2	1.8	1.5	1.4	1.9	1.6	168	0.7	1.0	1.7	0.9	29.5	82.1	1.9
	G8	3965	65.9	1.1	67.6	85	1.2	1.9	1.6	1.9	1.7	1.2	182	0.7	1.4	2.0	1.2	30.1	80.9	1.9
	G27	3965	66.4	1.4	68.4	82	1.2	1.9	1.6	1.7	2.1	1.2	179	0.8	0.3	1.6	-0.1	30.7	79.8	1.9
	G5	3921	68.3	1.0	70.0	87	1.1	1.9	1.9	1.5	1.9	1.1	189	0.7	0.8	1.4	1.0	32.1	79.6	1.9
	G39	3902	64.1	0.6	65.6	70	1.1	2.0	1.2	1.5	1.9	1.1	161	0.9	0.6	1.8	0.8	31.4	79.3	2.1
	G31	3820	64.8	1.0	66.6	82	1.1	2.2	1.3	1.8	1.8	1.2	176	0.8	0.3	1.7	1.0	27.6	80.4	2.0
	G10	3817	67.7	2.0	70.2	86	1.1	2.0	0.9	1.9	2.0	1.2	183	0.8	0.6	1.7	0.2	31.1	81.8	2.0
	G36	3757	62.9	1.1	64.9	83	1.1	1.9	1.3	1.6	2.0	1.3	179	0.8	0.8	1.9	0.7	28.6	79.5	2.0
	G43	3750	61.9	0.9	63.8	69	1.1	2.0	1.3	1.6	1.9	1.2	162	0.9	0.2	1.7	1.7	31.0	77.4	1.9
Bottom ten																				
genotypes	G3	3014	64.1	0.8	65.7	75	1.2	1.4	1.3	1.7	1.6	1.3	163	0.7	0.5	1.5	1.0	30.1	76.4	2.0
	G42	2996	63.2	1.0	64.9	71	1.0	1.8	0.9	1.6	1.7	1.2	168	0.5	1.6	1.7	0.5	31.3	78.7	2.2
	G24	2983	59.1	1.3	61.1	72	1.1	2.2	1.8	1.3	2.0	1.1	167	0.7	0.7	1.7	0.8	29.1	75.9	2.2
	G16	2901	63.2	0.8	64.9	80	1.0	1.8	2.1	1.8	1.8	1.1	173	0.8	0.5	1.8	0.6	32.3	77.7	2.0
	G7	2663	65.6	1.4	67.6	78	1.1	1.9	0.9	1.4	2.0	1.3	171	0.8	0.7	1.5	0.6	30.9	80.0	2.0
	G37	2594	65.3	1.3	67.2	66	1.1	1.7	1.0	1.5	2.1	1.2	162	0.6	0.2	1.9	0.9	30.4	74.1	2.0
	G11	2517	67.8	1.0	69.4	76	1.1	2.0	1.3	1.8	1.8	1.4	169	0.7	0.6	1.6	0.5	26.7	77.5	2.1
	G38	2413	69.0	1.1	70.3	78	1.0	1.9	1.0	1.4	1.9	1.4	175	0.5	0.8	1.4	0.8	30.5	72.8	2.0
	G2	2326	62.1	1.2	64.5	69	1.0	2.0	1.3	1.9	1.8	1.3	156	1.0	0.5	1.5	1.5	29.3	67.4	2.1
	G40	2295	66.5	1.1	68.0	73	1.1	1.9	1.2	1.4	1.9	1.2	169	0.8	0.6	1.4	0.5	33.2	77.8	2.0
	<b>Mean</b>	<b>3304</b>	<b>64.7</b>	<b>1.1</b>	<b>66.5</b>	<b>76.9</b>	<b>1.1</b>	<b>1.9</b>	<b>1.3</b>	<b>1.6</b>	<b>1.9</b>	<b>1.3</b>	<b>171.3</b>	<b>0.8</b>	<b>0.7</b>	<b>1.6</b>	<b>0.8</b>	<b>30.4</b>	<b>78.1</b>	<b>2.0</b>
	<b>LSD</b>	<b>608</b>	<b>1.7</b>	<b>1.3</b>	<b>1.7</b>	<b>8</b>	<b>0.1</b>	<b>0.3</b>	<b>0.8</b>	<b>0.3</b>	<b>0.5</b>	<b>0.3</b>	<b>12</b>	<b>0.4</b>	<b>1.2</b>	<b>0.3</b>	<b>0.7</b>	<b>2.9</b>	<b>5.9</b>	<b>0.3</b>
	<b>CV</b>																			
	<b>(%)</b>	<b>31.8</b>	<b>4.6</b>	<b>194</b>	<b>4.6</b>	<b>18.2</b>	<b>15.2</b>	<b>27.1</b>	<b>103</b>	<b>36</b>	<b>40</b>	<b>35.9</b>	<b>12.1</b>	<b>80.3</b>	<b>302</b>	<b>31.3</b>	<b>169</b>	<b>16.8</b>	<b>13.1</b>	<b>27.3</b>
	<b>Min</b>	<b>2295</b>	<b>59.1</b>	<b>0.6</b>	<b>61.1</b>	<b>66.0</b>	<b>1.0</b>	<b>1.4</b>	<b>0.9</b>	<b>1.3</b>	<b>1.5</b>	<b>1.0</b>	<b>156.0</b>	<b>0.5</b>	<b>0.1</b>	<b>1.3</b>	<b>0.0</b>	<b>25.9</b>	<b>67.4</b>	<b>1.5</b>
	<b>Max</b>	<b>4489</b>	<b>69.0</b>	<b>2.9</b>	<b>70.3</b>	<b>89.0</b>	<b>1.2</b>	<b>2.4</b>	<b>2.6</b>	<b>2.1</b>	<b>2.7</b>	<b>1.7</b>	<b>189.0</b>	<b>1.5</b>	<b>1.8</b>	<b>2.1</b>	<b>1.8</b>	<b>33.7</b>	<b>86.5</b>	<b>2.5</b>

LSD = Least significant difference, CV = coefficient of variation, Min = minimum, Max = maximum, GY = grain yield (kg ha<sup>-1</sup>), AD = days to anthesis (days), ASI = anthesis-silking interval (days), DS = days to silking (days), EH = ear height (cm), EPP = ears per plant (#), LB = leaf blight disease (1-5), HC = husk cover, GLS = gray leaf spot disease (1-5), GT = grain texture (1-5), MSV = maize streak virus disease (1-5), PH = plant height (cm), RE = rotten ears (#), RL = root lodging (#), Rust = rust disease (1-5), SL = stem lodging (#), SWT = 100 seed weight (g), SH = shelling percentage, VIG = vigour (1-5), # = number.

#### **4.5.14 Pearson's correlation coefficients between grain yield and other agronomic traits across optimal environments combined for 2011/12 and 2012/13 seasons**

Days to anthesis were positively and significantly correlated with all traits apart from anthesis-silking interval (Table 4.16). Grain yield was positively and significantly correlated with days to anthesis days ( $r = 0.16$ ), days to silking ( $r = 0.16$ ), plant and ear height ( $r = 0.16$  and  $r = 0.14$ , respectively) as well as with number of ears per plant ( $r = 0.80$ ) and shelling percentage ( $r = 0.91$ ). Negative correlations among other traits were recorded for example anthesis-silking interval was negatively correlated with root lodging ( $r = -0.10$ ), husk cover ( $r = -0.18$ ), leaf blight ( $r = -0.20$ ) and gray leaf spot ( $r = -0.10$ ) disease as well as with plant vigor ( $r = -0.33$ ). Anthesis-silking interval also correlated positively with traits such as days to silking ( $r = 0.19$ ), plant and ear height ( $r = 0.27$  and  $0.25$ , respectively), MSV ( $r = 0.09$ ) and 100 seed weight ( $r = 0.18$ ). Days to silking correlated significantly with all traits except for plant vigor. Ear rots were positively and significantly correlated with stem lodging ( $r = 0.30$ ), husk cover ( $r = 0.20$ ), grain texture ( $r = 0.20$ ), leaf blight ( $r = 0.50$ ), gray leaf spot ( $r = 0.40$ ), rust ( $r = 0.30$ ), MSV ( $r = 0.60$ ), 100 seed weight ( $r = 0.30$ ) and plant vigor ( $r = 0.30$ ). Plant ( $r = -0.33$ ), and ear height ( $r = -0.40$ ), were negatively correlated with plant vigour. This implies that tall plants (higher values) were associated with good vigour as lower scores of plant vigour (below 3 in the scale of 1-5) indicates good plant vigour and higher scores indicate poor vigour and short plants. Shelling percentage furthermore correlated positively and significantly with number of ears per plant ( $r = 0.90$ ), plant height ( $r = 0.17$ ) and ear height ( $r = 0.20$ ) (Table 4.16).

**Table 4.16 Pearson's correlation coefficients for grain yield and agronomic traits across all optimal environments for two seasons 2011/12 and 2012/13**

	GY	AD	ASI	DS	PH	EH	RL	SL	HC	RE	GT	LB	GLS	Rust	MSV	SWT	VIG	SH
AD	0.16**																	
ASI	0.04	0.09																
DS	0.16**	1.00**	0.19**															
PH	0.16**	0.76**	0.27**	0.80**														
EH	0.14**	0.60**	0.25**	0.60**	0.93**													
RL	0.03	0.25**	-0.10**	0.20**	-0.04	-0.10												
SL	0.03	0.31**	-0.08	0.30**	0.00	-0.04	0.40**											
HC	0.04	0.29**	-0.18**	0.30**	0.10**	0.02	0.20**	0.20**										
RE	0.03	0.39**	-0.03	0.40**	0.06	-0.10	0.06	0.30**	0.20*									
GT	0.08	0.12**	-0.06	0.10**	-0.20**	-0.20	0.10**	0.10**	0.10**	0.20**								
LB	0.07	0.60**	-0.20**	0.60**	0.20**	0.10	0.40**	0.40**	0.50*	0.50**	0.30**							
GLS	0.08	0.60**	-0.10**	0.60**	0.30**	0.20**	0.20**	0.30**	0.40*	0.40**	0.30**	0.70**						
Rust	0.07	0.46**	-0.06	0.50**	0.32**	0.18	-0.20	0.03	0.30*	0.30**	0.30**	0.50**	0.60**					
MSV	0.05	0.45**	0.09**	0.50**	0.25**	0.18	-0.04	0.20**	0.00	0.60**	0.30**	0.50**	0.40**	0.40**				
SWT	0.08	0.58**	0.18**	0.60**	0.36**	0.30**	0.20**	0.30**	0.00	0.30**	0.40**	0.50**	0.50**	0.30**	0.60**			
VIG	0.04	0.12**	-0.33**	0.09	-0.33**	-0.40*	0.40**	0.40**	0.30*	0.40**	0.30**	0.70**	0.50**	0.20**	0.30**	0.20**		
SH	0.91**	0.18**	0.04	0.20**	0.17**	0.20**	0.03	0.03	0.00	0.03	0.10**	0.08	0.10**	0.08	0.06	0.10**	0.10**	
EPP	0.80**	0.16**	0.04	0.20**	0.16**	0.10**	0.03	0.03	0.00	0.03	0.10**	0.07	0.08	0.07	0.05	0.08	0.08	0.90**

\*\*P≤0.01; GY = grain yield (kg ha<sup>-1</sup>), AD = days to anthesis (days), ASI = anthesis-silking interval (days), DS = days to silking (days), PH = plant height (cm), EH = ear height (cm), RL = root lodging (#), SL = stem lodging (#), HC = husk cover, RE = rotten ears (#), GT = grain texture (1-5), LB = leaf blight disease (1-5), GLS = gray leaf spot disease (1-5), Rust = rust disease (1-5), MSV = maize streak virus disease (1-5), SWT = 100 seed weight (g), VIG = vigour (1-5), SH = shelling percentage, EPP = ears per plant (#), # = number.

#### 4.5.15 Principal component analysis results, eigenvalues and eigenvectors for the traits across four optimal environments combined for 2011/12 and 2012/13 seasons

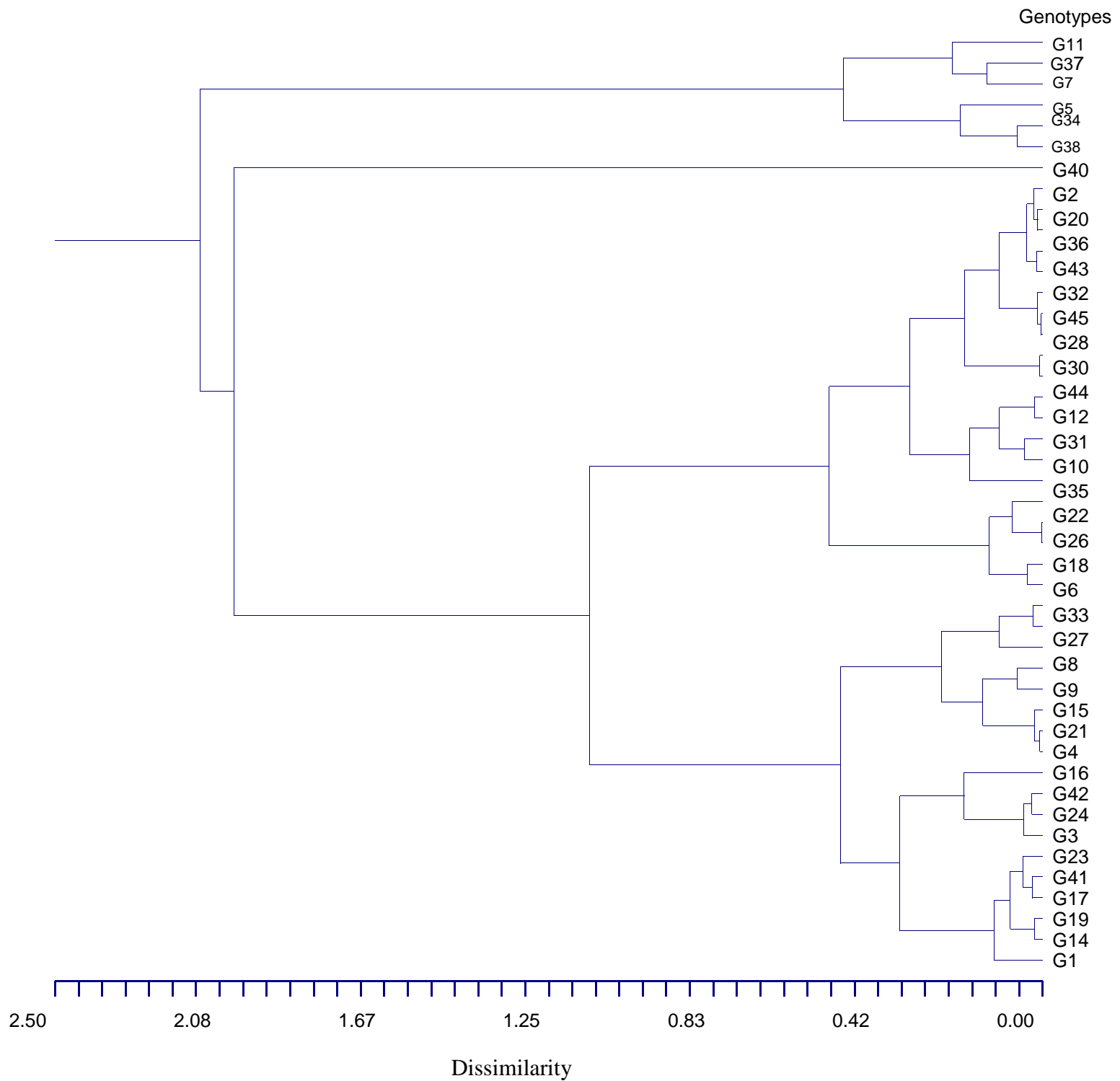
PCA generated 16 principal components (Table 4.17) which accounted for 100% of variability present in the maize genotypes evaluated. The first eight PCs had eigenvalues within a range of 1.0 – 7.1. Their cumulative percentages accounted for 85.9% of the total variation present among the genotypes.

**Table 4.17 Eigenvalues and eigenvectors for the traits across four optimal environments combined for 2011/12 and 2012/13 seasons**

PC	Eigenvalues	As percentages	Cumulative percentages
1	7.16	39.75	39.75
2	1.62	8.98	48.74
3	1.43	7.97	56.70
4	1.30	7.22	63.93
5	1.00	5.56	69.48
6	0.96	5.31	74.79
7	1.00	5.56	80.35
8	1.00	5.56	85.91
9	0.62	3.45	89.36
10	0.48	2.65	92.01
11	0.43	2.40	94.41
12	0.36	2.00	96.41
13	0.29	1.60	98.01
14	0.18	0.99	99.00
15	0.17	0.92	99.92
16	0.01	0.08	100.00

#### 4.5.16 Clustering of the maize genotypes evaluated at four optimal environments combined for the 2011/12 and 2012/13 seasons

Forty five maize genotypes were clustered based on their means for grain yield. At cut off point 1.0, three main clusters were observed (Figure 4.2) with a high cophenetic correlation of  $r_{cop} = 0.82$ . Thirteen genotypes from CIMMYT-Zimbabwe were grouped in cluster II with only two from CIMMYT-Colombia. The top performing genotypes were grouped in cluster III which comprised of 24 genotypes from both Colombia and Zimbabwe. Cluster I was comprised of six genotypes which were also from both research centers.



**Figure 4.2 Dendrogram based on Euclidean distance and UPGMA clustering using morphological data for genotypes at four optimal environments combined for 2011/12 and 2012/13 seasons**

#### **4.5.17 Combined ANOVA for grain yield and agronomic traits for all environments, optimal and low pH for two seasons 2011/12 and 2012/13**

Mean squares from the combined ANOVA across all environments and two seasons are given in Table 4.18. Genotype mean squares were highly significant ( $P \leq 0.01$ ) for grain yield, days to anthesis, days to 50 % silking, ear height, leaf blight disease, husk cover, grain texture, maize streak virus, rust disease, root lodging, 100 seed weight, shelling percentage and plant vigour and significantly ( $P \leq 0.05$ ) with number of ears per plant. Environment mean squares were highly significant for all the traits measured. Season mean squares were highly significant for days to anthesis, anthesis-silking interval, days to 50 % silking, number of ears per plant, plant height, rotten ears and all the diseases, 100 seed weight as well as shelling percentage. GxE was highly significant for grain yield, anthesis date, days to 50% silking, number of ears per plant, husk cover, grain texture, rotten ears, 100 seed weight, and plant vigour as well as MSV and rust. Interaction of GxY was not significant for anthesis-silking interval, number of ears per plant and stem lodging. ExY mean squares were significant for most traits but not for husk cover and rotten ears. Interaction of GxExY was highly significant for grain yield, anthesis date, days to 50% silking, grain texture, root and stem lodging, leaf blight disease and 100 seed weight as well as plant vigor.

#### **4.5.18 Estimated contributions to total sum of squares across all environments for two seasons 2011/12 and 2012/13**

Contribution to total sum of squares for genotype was the highest for 100 seed weight (4.88%) and MSV (4.85%, Table 4.19). Contribution due to environment was highest for days to 50% silking (80.45%) and for season the highest was rust disease (17.0%). GxE contribution was significant for husk cover (26.3%). The interaction of GxY made the highest contribution to gray leaf spot (5.7%). The interaction of ExY made the highest contribution to root lodging (21.9%) and the interaction of GxExY made the highest contribution to shelling percentage (11.03%) (Table 4.19).

**Table 4.18 Mean squares for combined ANOVA for grain yield and agronomic traits for all environments, optimal and low pH for two seasons 2011/12 and 2012/13**

Source	Genotypes	Environment	Year	GxE	GxY	ExY	GxExY	MSE
Df	44	7	1	307	44	6	264	1334
GY	3E+06**	4.5E+08**	1.81E+06	1E+06**	3E+06**	3E+07**	1E+06**	764200
AD	129.46**	41689.99**	1709.56**	32.2**	59.5**	4870.22**	37.4**	13.54
ASI	4.99	712.359**	285.673**	4.24	3.73	191.02**	4.865*	3.99
DS	114.3**	45472.23**	666.96**	33.53**	59.48**	5184.86**	36.23**	13.87
EH	518.4**	132261.7**	1176.5*	340.3*	458.9**	15750.1**	182.2	191.3
EPP	0.16*	9.09**	20.16**	0.12**	0.15	4.58**	0.13	0.12
LB	0.88**	83.57**	12.37**	0.51	1.14**	42.15**	0.83**	0.44
HC	4.12**	192.39**	4.14	4.43**	3.16*	0.20	2.55	2.04
GLS	1.02	55.86**	9.04**	1.21*	2.86**	37.23**	1.20*	0.99
GT	1.52**	55.77**	0.01	1.51**	1.52**	18.64**	0.78**	0.65
MSV	0.41**	16.45**	20.68**	0.28**	0.41**	5.35**	0.19	0.20
PH	5820	261853**	61886**	5203	8806**	52208**	6018	5349
RE	1.20	97.71**	14.17**	2.02**	2.32**	4.31	3.40	1.00
RL	6.33**	395.1**	9.30	3.94*	8.66**	595.95**	5.71**	3.17
Rust	0.83**	89.76**	213.61**	0.42**	0.56**	21.43**	0.27	0.30
SL	3.14*	89.014**	5.55	2.07	2.85	91.38**	2.897**	2.09
SWT	108.32**	5805.43**	740.77**	34.33**	56.64**	2610.86**	36.15**	22.66
SH%	213.40**	14288.60**	3331.90**	154.30	205.40*	1050.30**	152.30	142.20
VIG	1.72**	190.8**	0.24	0.72**	1.23**	19.75**	0.59**	0.43

\*\*P<0.01; \*P<0.05; G = Genotype, E = environment, Y = year, MSE = mean square error, GY = grain yield (kg ha<sup>-1</sup>), AD = days to anthesis (days), ASI = anthesis-silking interval (days), DS = days to 50% silking (days), EH = ear height (cm), EPP = ears per plant (#), LB = leaf blight disease (1-5), HC = husk cover, GLS = gray leaf spot disease (1-5), GT = grain texture (1-5), MSV = maize streak virus diseases (1-5), PH = plant height (cm), RE = rotten ears (#), RL = root lodging (#), Rust = rust disease (1-5), SL = stem lodging (#), SWT = 100 seed weight (g), SH = shelling percentage (%), VIG = vigour (1-5), # = number

**Table 4.19 Relative percent contribution to total sum of squares across two years at eight environments (optimal and low pH)**

Source	GY	AD	ASI	DS	EH	EPP	LB	GLS	GT	HC	MSV	PH	RE	RL	Rust	SL	SWT	SH	VIG
Genotypes	2.55	1.54	1.49	1.27	1.59	2.06	2.73	2.02	3.29	4.10	4.85	2.07	2.52	2.56	2.90	3.40	4.88	2.21	3.31
Environment	57.80	79.10	33.79	80.35	64.70	18.13	35.52	15.12	19.25	26.11	22.31	14.81	27.90	21.81	42.71	13.14	35.66	23.59	58.56
Year	0.03	0.46	1.94	0.17	0.08	5.74	0.88	0.41	0.00	0.09	5.61	0.50	0.67	0.09	16.94	0.14	0.76	0.79	0.01
GxE	6.73	2.68	8.84	2.60	7.30	10.26	9.49	14.39	22.74	26.32	16.57	12.90	25.38	9.56	8.83	13.46	9.28	11.21	9.73
GxY	2.38	0.71	1.11	0.66	1.41	1.87	3.54	5.68	3.30	3.14	4.89	3.13	4.85	3.50	1.96	3.08	2.55	2.13	2.35
ExY	3.38	7.92	7.77	7.85	5.50	7.83	8.96	6.72	4.60	0.00	2.90	2.11	1.57	21.93	3.40	6.75	13.37	1.73	2.60
GxExY	7.24	2.68	8.70	2.41	2.80	9.70	7.74	9.49	8.46	2.54	4.46	10.70	1.31	9.24	1.91	9.41	7.99	11.03	3.42
Rep	0.17	0.00	0.00	0.00	0.00	0.20	0.30	0.40	0.00	0.70	0.30	0.10	0.10	0.10	0.60	0.10	0.50	0.11	0.00
Residual	19.9	4.90	36.34	4.67	16.61	44.18	30.87	45.77	38.35	37.02	38.13	53.67	35.73	31.25	20.79	50.54	24.96	47.8	19.99
CV (%)	36.00	5.00	103.10	4.90	22.20	35.10	34.90	94.00	54.80	34.40	36.50	48.90	101.00	106.00	36.40	142.80	18.40	11.90	27.50
Min	0.03	0.004	0.03	0.009	0.003	0.22	0.28	0.41	0.00	0.005	0.28	0.12	0.07	0.05	0.56	0.09	0.54	0.11	0.01
Max	57.80	79.10	36.30	80.40	64.70	44.20	35.50	45.80	38.30	37.00	38.10	53.70	35.70	31.30	42.70	50.50	35.70	47.80	58.60

**G = Genotype, E = environment, Y = year, CV = coefficient of variation, Min = minimum, Max = maximum, GY = grain yield (kg ha<sup>-1</sup>), AD = days to anthesis (days), ASI = anthesis-silking interval (days), DS = days to silking (days), EH = ear height (cm), EPP = ears per plant (#), LB = leaf blight disease (1-5), GLS = gray leaf spot disease (1-5), GT = grain texture (1-5), HC = husk cover, MSV = maize streak virus disease (1-5), PH = plant height (cm), RE = rotten ears (#), RL = root lodging (#), Rust = rust disease (1-5), SL = stem lodging (#), SWT = 100 seed weight (g), SH = shelling percentage, VIG = vigour (1-5), # = number.**

#### 4.5.19 Genotypic and phenotypic variance components, broad sense heritability and genetic advance estimates across the combined environments for both 2011/12 and 2012/13 seasons

The results in Table 4.20 indicated that phenotypic variances were higher than genotypic variances. Grain texture (0.6), days to 50% silking (0.5), anthesis date (0.5) and plant vigour (0.5) had relatively high broad sense heritability estimates. The genetic coefficients of variations were lower than phenotypic coefficients of variation (Table 4.21). The expected genetic advance was highest for plant height followed by grain texture. Shelling percentage had the lowest genetic advance value with a high phenotypic coefficient of variation.

**Table 4.20 Genotypic variances, phenotypic variances and heritability estimates across optimal and low pH environments for 2011/12 and 2012/13 seasons**

Trait	$\sigma^2_g$	$\sigma^2_p$	H <sup>2</sup> b	H <sup>2</sup> b %
GY	2500.00	401500.000	0.006	0.623
AD	21.250	39.300	0.541	54.071
ASI	1.200	4.400	0.273	27.273
DS	22.345	40.645	0.550	54.976
EH	53.250	238.850	0.223	22.294
EPP	0.055	0.275	0.200	20.000
LB	0.145	0.735	0.197	19.728
GLS	0.450	2.050	0.220	21.951
GT	0.630	1.120	0.563	56.250
HC	1.485	3.505	0.424	42.368
MSV	0.015	0.185	0.081	8.108
PH	3290.00	13596.000	0.242	24.198
RE	0.035	1.395	0.025	2.509
RL	0.540	2.740	0.197	19.708
Rust	0.040	0.360	0.111	11.111
SL	0.280	2.780	0.101	10.072
SWT	19.815	40.785	0.486	48.584
SH	18.700	157.900	0.118	11.843
VIG	0.620	1.180	0.525	52.542

$\sigma^2_g$  = genotypic variance,  $\sigma^2_p$  = phenotypic variance, H<sup>2</sup>b = broad sense heritability, H<sup>2</sup>b % = broad sense heritability as percentage, GY = grain yield (kg ha<sup>-1</sup>), AD = days to anthesis (days), ASI = anthesis-silking interval (days), DS = days to silking (days), EH = ear height (cm), EPP = ears per plant (#), LB = leaf blight disease (1-5), GLS = gray leaf spot disease (1-5), GT = grain texture (1-5), HC = husk cover, MSV = maize streak virus disease (1-5), PH = plant height (cm), RE = rotten ears (#), RL = root lodging (#), Rust = rust disease (1-5), SL = stem lodging (#), SWT = 100 seed weight (g), SH = shelling percentage, VIG = vigour (1-5), # = number.

**Table 4.21 Genotypic coefficient of variation, phenotypic coefficient of variation and genetic advance across all eight environments for two years**

Trait	GCV%	PCV%	GA	GA (% of mean)
GY	10.1	128.5	0.3	10.6
AD	5.4	7.3	2.1	2.9
ASI	7.9	15.1	1.2	63.6
DS	5.4	7.3	2.1	2.8
EH	9.2	19.6	1.1	1.7
EPP	2.4	5.3	0.7	70.8
LB	2.8	6.2	0.7	36.8
GLS	5.0	10.6	0.9	49.6
GT	5.2	6.9	2.2	92.6
MSV	1.1	3.9	0.1	9.0
PH	46.9	95.4	1.7	108.0
RE	1.9	11.7	0.1	5.6
RL	5.7	12.8	0.5	31.2
Rust	1.6	4.9	0.2	11.9
SL	5.3	16.6	0.3	26.5
SWT	8.8	12.6	2.5	9.8
SH	5.0	14.6	0.9	1.2
VIG	5.1	7.0	2.0	85.2

GCV = genotypic coefficient of variation, PCV = phenotypic coefficient of variation, GA = genetic advance, GY = grain yield ( $\text{kg ha}^{-1}$ ), AD = days to anthesis (days), ASI = anthesis-silking interval (days), DS = days to silking (days), EH = ear height (cm), EPP = ears per plant (#), LB = leaf blight disease (1-5), GLS = gray leaf spot disease (1-5), GT = grain texture (1-5), MSV = maize streak virus (1-5), PH = plant height (cm), RE = rotten ears (#), RL = root lodging (#), Rust = rust disease (1-5), SL = stem lodging (#), SWT = 100 seed weight (g), SH = shelling percentage, VIG = vigour (1-5), # = number.

#### **4.5.20 Mean performance for grain yield and other traits across all environments for 2011/12 and 2012/13 seasons**

The grain yield trial mean was  $2.4 \text{ t ha}^{-1}$  (Table 4.22) and the top performing genotype was G20. None of the top ten genotypes were prolific with a mean number of ears per plant of 1. The genotype most susceptible to gray leaf spot disease was again G 41. The tallest genotype was G7 (213 cm), although it ranked with the bottom ten genotypes. Genotype G27 recorded the highest 100 seed weight (27 g) followed by G35 (26 g). In terms of shelling percentage, genotype G30 (74%) recorded the highest.

**Table 4.22 Mean performance combined across two years and across optimal and low pH environments for 2011/12 and 2012/13 seasons**

	Entry	GY	AD	ASI	DS	EH	EPP	LB	HC	GLS	GT	MSV	PH	RE	RL	Rust	SL	SWT	SH	VIG
Top 10	G20	2977	74.4	1.8	76.7	63.8	1.0	1.7	1.5	1.9	2.3	1.2	146	1.0	1.6	1.3	0.9	27.5	77.3	1.9
Genotypes	G27	2850	75.3	1.8	77.3	67.1	1.0	1.7	1.3	1.7	2.6	1.2	153	1.0	1.2	1.4	1.1	29.3	74.2	2.1
	G8	2727	74.3	1.8	76.4	65.6	1.0	2.0	1.4	2.0	2.2	1.2	153	0.8	1.6	1.6	1.4	26.3	73.0	2.2
	G33	2725	72.1	2.1	74.9	60.9	1.0	1.7	1.3	1.7	2.4	1.3	145	0.8	1.9	1.5	1.2	24.8	76.0	2.2
	G5	2719	76.7	1.5	78.5	65.4	1.0	1.9	2.0	1.7	2.3	1.1	156	1.0	1.3	1.4	1.2	27.6	75.4	2.0
	G30	2704	73.6	2.0	76.0	63.5	1.0	1.8	1.8	1.9	2.2	1.3	147	1.0	1.6	1.5	0.6	27.2	76.3	2.1
	G39	2680	72.8	2.0	75.4	59.9	1.0	1.9	1.2	1.8	2.3	1.2	145	1.1	2.4	1.5	1.2	26.7	73.6	2.5
	G45	2678	74.8	1.9	77.1	67.4	0.9	1.7	1.5	1.6	2.8	1.4	157	0.9	1.2	1.5	0.8	28.7	72.5	2.1
	G22	2663	71.8	2.0	74.3	62.3	1.0	1.9	1.6	1.7	2.3	1.3	146	1.0	1.8	1.5	1.0	26.1	75.1	2.6
	G28	2635	72.9	1.7	75.0	62.1	1.0	2.0	1.4	1.9	2.7	1.2	147	0.8	1.8	1.6	0.9	25.2	75.0	2.3
Bottom 10	G16	2275	71.9	2.0	73.9	60.0	0.9	1.7	2.0	1.9	2.3	1.2	143	0.9	1.2	1.6	1.2	27.8	74.2	2.3
Genotypes	G41	2264	69.8	1.7	72.1	54.4	0.9	1.7	1.5	2.1	2.1	1.1	137	0.9	1.4	1.6	0.8	23.9	75.1	2.5
	G1	2259	74.9	3.1	77.1	63.9	0.9	2.0	1.2	1.9	2.1	1.2	151	1.1	1.6	1.5	1.0	26.6	72.2	2.5
	G42	2185	72.7	1.9	75.1	63.7	0.9	2.0	1.3	1.6	2.1	1.2	152	1.1	1.8	1.5	0.9	26.0	73.3	2.7
	G7	2095	72.8	2.3	75.1	61.7	0.9	1.9	1.0	1.6	2.6	1.3	213	1.1	1.4	1.3	0.8	26.7	76.3	2.4
	G11	2009	74.7	1.6	76.6	56.2	0.9	2.0	1.4	2.0	2.3	1.3	140	0.8	1.5	1.5	1.2	22.4	74.6	2.4
	G37	2001	73.6	1.7	75.9	56.3	0.9	1.7	1.7	1.7	2.6	1.2	142	0.9	1.1	1.7	1.1	24.6	72.3	2.3
	G40	1884	75.2	1.8	77.2	60.8	0.9	1.8	1.5	1.6	2.3	1.2	149	0.9	1.7	1.4	0.7	26.5	73.2	2.2
	G38	1883	77.1	1.3	78.5	62.8	0.9	1.8	1.3	1.7	2.3	1.4	147	1.1	1.0	1.3	0.9	25.8	71.1	2.3
	G2	1804	71.8	1.7	74.3	57.6	0.9	2.1	1.4	1.9	2.1	1.2	137	0.9	2.4	1.4	1.5	24.8	66.4	2.6
	<b>Mean</b>	<b>2401</b>	<b>73.7</b>	<b>1.9</b>	<b>75.9</b>	<b>61.8</b>	<b>1.0</b>	<b>1.9</b>	<b>1.5</b>	<b>1.8</b>	<b>2.3</b>	<b>1.2</b>	<b>150</b>	<b>1.0</b>	<b>1.6</b>	<b>1.5</b>	<b>1.0</b>	<b>26.2</b>	<b>73.8</b>	<b>2.3</b>
<b>LSD</b>	<b>506</b>	<b>2.4</b>	<b>1.01</b>	<b>2.4</b>	<b>3.9</b>	<b>0.3</b>	<b>0.44</b>	<b>0.8</b>	<b>0.7</b>	<b>0.56</b>	<b>0.36</b>	<b>58</b>	<b>0.68</b>	<b>0.94</b>	<b>0.32</b>	<b>0.9</b>	<b>2.6</b>	<b>7.5</b>	<b>0.4</b>	
<b>CV</b>	<b>36</b>	<b>5</b>	<b>103</b>	<b>4.9</b>	<b>22.2</b>	<b>35</b>	<b>34.9</b>	<b>94</b>	<b>55</b>	<b>34.4</b>	<b>36.5</b>	<b>49</b>	<b>101</b>	<b>106</b>	<b>36.4</b>	<b>143</b>	<b>18</b>	<b>11.9</b>	<b>28</b>	
<b>Min</b>	<b>1804</b>	<b>69.8</b>	<b>1.3</b>	<b>72.1</b>	<b>54.4</b>	<b>0.9</b>	<b>1.7</b>	<b>1.0</b>	<b>1.6</b>	<b>2.1</b>	<b>1.1</b>	<b>137</b>	<b>0.8</b>	<b>1.0</b>	<b>1.3</b>	<b>0.6</b>	<b>22.4</b>	<b>66.4</b>	<b>1.9</b>	
<b>Max</b>	<b>2977</b>	<b>77.1</b>	<b>3.1</b>	<b>78.5</b>	<b>67.4</b>	<b>1.0</b>	<b>2.1</b>	<b>2.0</b>	<b>2.1</b>	<b>2.8</b>	<b>1.4</b>	<b>213</b>	<b>1.1</b>	<b>2.4</b>	<b>1.7</b>	<b>1.5</b>	<b>29.3</b>	<b>77.3</b>	<b>2.7</b>	

LSD = Least significant difference, CV = coefficient of variation, Min = minimum, Max = maximum, GY = grain yield (kg ha<sup>-1</sup>), AD = days to anthesis (days), ASI = anthesis-silking interval (days), DS = days to silking (days), EH = ear height (cm), EPP = ears per plant (#), LB = leaf blight disease (1-5), HC = husk cover, GLS = gray leaf spot disease (1-5), GT = grain texture (1-5), MSV = maize streak virus disease (1-5), PH = plant height (cm), RE = rotten ears (#), RL = root lodging (#), Rust = rust disease (1-5), SL = stem lodging (#), SWT = 100 seed weight (g), SH = shelling percentage, VIG = vigour (1-5), # = number.

**Table 4.23 Pearson's correlation coefficients for grain yield and agronomic traits across optimal and low pH environments for 2011/12 and 2012/13 seasons**

	GY	AD	ASI	DS	PH	EH	RL	SL	HC	RE	GT	LB	GLS	RUST	MSV	SWT	VIG	SH
AD	0.12**																	
ASI	0.23**	0.09**																
DS	0.12**	0.99**	0.09**															
PH	0.05	0.14**	-0.01	0.11**														
EH	0.06**	0.02	0.01	0.01	0.62**													
RL	0.03	0.20**	0.01	0.20**	-0.01	0.02												
SL	0.03	0.25**	0.01	0.24**	-0.01	-0.1**	0.21**											
HC	0.01	0.18**	0.00	0.15**	0.11**	0.05	0.03	0.09**										
RE	-0.02	0.35**	-0.03	0.34**	-0.06**	-0.2**	-0.02	0.19**	0.20**									
GT	0.06	0.09	0.01	0.09	-0.06	-0.14	0.05	0.03	-0.02	0.10								
LB	0.04	0.40**	0.01	0.40**	0.06	-0.01	0.28**	0.29**	0.25**	0.34**	0.3**							
GLS	0.03	0.38**	0.00	0.37**	0.08**	0.00	0.18**	0.14**	0.15**	0.26**	0.27**	0.58**						
RUST	0.01	0.22**	0.00	0.20**	0.23**	0.18**	-0.2**	0.07**	0.30**	0.27**	0.28**	0.51**	0.45**					
MSV	0.02	0.35**	-0.02	0.33**	0.08**	0.03	0.00	0.22**	0.08**	0.41**	0.26**	0.58**	0.39**	0.48**				
SWT	0.07**	0.45**	0.02	0.44**	0.21**	0.25**	0.30**	0.18**	-0.01	0.16**	0.25**	0.32**	0.30**	0.17**	0.4**			
VIG	0.04	0.20**	0.01	0.19**	-0.1**	-0.2**	0.38**	0.24**	0.12**	0.25**	0.22**	0.48**	0.34**	0.17**	0.18**	0.3**		
SH	0.55**	0.15**	0.19**	0.15**	0.09**	0.11**	0.05	0.04	0.05	0.04	0.12**	0.09**	0.08**	0.08**	0.07**	0.12**	0.09**	
EPP	0.46**	0.24**	0.19**	0.24**	0.10**	0.10**	0.05	0.04	0.05	0.04	0.13**	0.09**	0.08**	0.08**	0.07**	0.12**	0.10**	0.6**

**\*\*P<0.01; GY = grain yield (kg ha<sup>-1</sup>), AD = days to anthesis (days), ASI = anthesis-silking interval (days), DS = days to silking (days), PH = plant height (cm), EH= ear height (cm), RL = root lodging (#), SL= stem lodging (#), HC = husk cover, RE = rotten ears (#), GT = grain texture (1-5), LB = leaf blight disease (1-5), GLS = gray leaf spot disease (1-5), Rust = rust disease (1-5), MSV = maize streak virus disease (1-5), SWT = 100 seed weight (g), VIG = vigour (1-5), SH = shelling percentage, EPP = ears per plant (#), # = number.**

#### **4.5.21 Pearson's correlation coefficients between grain yield and other agronomic traits across optimal and low pH environments combined for 2011/12 and 2012/13 seasons**

Grain yield was positively and significantly correlated with number of ears per plant ( $r = 0.46$ ), anthesis date ( $r = 0.12$ ), anthesis-silking interval ( $r = 0.23$ ), day to 50% silking ( $r = 0.12$ ), ear height ( $r = 0.06$ ), 100 seed weight ( $r = 0.07$ ) and shelling percentage ( $r = 0.55$ ) (Table 4.23). Correlation among other traits indicated that shelling percentage was positively and significantly correlated with anthesis date ( $r = 0.15$ ), anthesis-silking interval ( $r = 0.19$ ), days to 50% silking ( $r = 0.15$ ) and number of ears per plant ( $r = 0.06$ ) as well as plant height and ear height ( $r = 0.09$  and  $0.11$ ). Ear rots were positively and significantly correlated with traits such as days to anthesis ( $r = 0.35$ ), day to 50% silking ( $r = 0.34$ ), stem lodging ( $r = 0.19$ ), husk cover ( $r = 0.20$ ), leaf blight ( $r = 0.34$ ), gray leaf spot ( $r = 0.26$ ), rust ( $r = 0.27$ ), MSV ( $r = 0.41$ ). Ear height ( $r = -0.2$ ) was negatively correlated with plant vigour and seed size (100 seed weight) was positively correlated with grain texture ( $r = 0.25$ ) and plant vigour ( $r = 0.3$ ).

#### **4.5.22 Principal component analysis results, eigenvalues and eigenvectors for the traits across all environments combined for 2011/12 and 2012/13 seasons**

Results indicated that eight principal components were generated across all environments and these accounted for 100% variability present in the maize genotypes evaluated (Table 4.24). The first five PC had higher Eigenvalues and their cumulative percentages accounted for 71.1% of the total variation present among the genotypes.

**Table 4.24 Eigenvalues, percentages and cumulative percentages for the measured and derived data across four low pH soil environments combined for 2011/12 and 2012/13 seasons**

PC	Eigenvalues	As percentages	Cumulative percentages
1	2.2	18.5	18.5
2	1.8	15.7	34.2
3	1.5	13.1	47.4
4	1.5	12.5	59.8
5	1.3	11.3	71.1
6	1.2	10.0	81.1
7	1.1	9.7	90.8
8	1.1	9.2	100.0

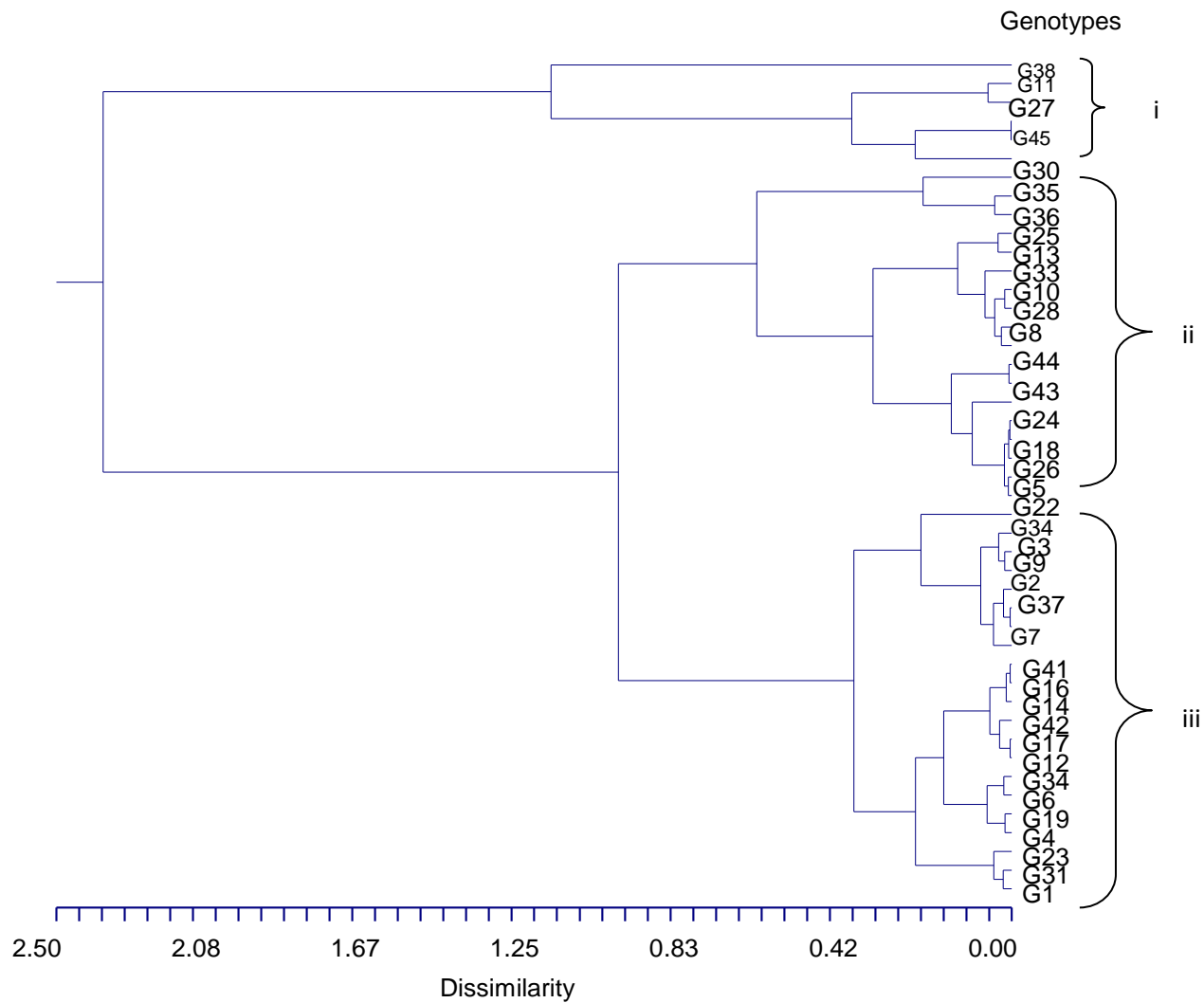
**Table 4.25 Phenotypic and genotypic variances for grain yield and other traits at four optimal environments**

Trait	$\sigma^2_g$	$\sigma^2_p$	H <sup>2</sup> b
AD	2.26	4.34	0.52
DS	2.19	4.34	0.51
EPP	3E-04	0.001	0.27
GY	669.20	2E+05	0.01

$\sigma^2_g$  = genetic advance,  $\sigma^2_p$  = phenotypic variance, H<sup>2</sup>b = broad sense heritability, AD = days to anthesis, DS = days to silking (days), EPP = ears per plant (#), GY = grain yield (kg ha<sup>-1</sup>), # = number.

#### 4.5.23 Clustering of the maize genotypes evaluated at four low pH and four optimal environments combined for the 2011/12 and 2012/13 seasons

The dendrogram was constructed using the UPGMA cluster analysis method based on morpho-agronomic data across optimal and low pH environment Figure 4.3. Cluster two genotypes comprised of well adapted released OPVs G44 (ZM523) and G43 (ZM309) alongside some four of the top ten based on mean grain yield across optimal and low pH environment (Table 4.22) G30, G33, G8 and G5. Though Cluster three was larger than cluster one, the two did not show any clear pattern of clustering. Both groups of genotypes from CIMMYT-Colombia and CIMMYT-Zimbabwe had equal chances to be grouped in the two clusters.



**Figure 4.3 Dendrogram based on Euclidean distance and UPGMA clustering using morphological data for genotypes at four low pH and four optimal environments combined for 2011/12 and 2012/13 seasons**

## 4.6 Discussion

Low pH soil is one of the most constraining edaphic factors contributing to low crop production. Even liming is known to be inefficient as it is restricted to the top soil layer while the sub-soil surface layers with toxic Al remain acid (Custódio *et al.*, 2002). In the present study, soil analysis from the 30 cm soil profile indicated that the sites had different soil pH levels. Lunyangwa low pH site had the most acidic soils as compared to Tsangano and Bembeke low pH sites. Bvumbwe was classified as having moderately acidic soils based on the Soil Test Interpretation Guide (Horneck *et al.*, 2011). In terms of inherent soil fertility with due considerations to resource poor farmers, all the sites had a low N concentration in the top soil. The across site analysis for low pH sites, optimal sites and combined across environments and seasons indicated highly significant effects for most of the traits (Tables 4.3, 4.11 and 4.18). This was an indication that genetic variability existed and suggested the possibility of selection and further improvement. The across sites ANOVA for low pH environments revealed that sites were significantly different and this confirmed soil analysis results. Similarly the ANOVA for optimal sites were also significantly different owing to the fact that Chitedze and Meru were in the mid-altitude ecology while Baka and Chitala were in the low-land ecology. Seasons were also significantly different for a number of traits, suggesting that selection from one season's data may not be reliable but across season data could provide reliable information about the genotypes. Partitioning of variation into four sources of interactions followed the same trend such that the effects of GxE, GxY, ExY, and GxExY were also significant for a number of traits.

Different sources of variation made different contributions to total variation with respect to various traits (Tables 4.4, 4.12 and 4.29). On average, environments made the highest contribution to total variation in all tested environments. Viana *et al.* (2009) indicated that spatial heterogeneity in experimental areas is a common fact and is related to the processes of soil genesis.

In general, the effects of low pH soil contributed to reduction in grain yields and yield components under low pH (Tables 4.5 and 4.7). The combined mean reduction was 69.9% and this is consistent with other findings (Welcker *et al.*, 2005). Plant height reduction, reduced number of ears per plant and shelling percentage reduction under low pH soil conditions could be due to the indirect effect of the impaired nutrient uptake by maize plants as a result of inhibition of root development. This confirms results of Duque-Varga *et al.* (1994) who found that root inhibition leads to low water and nutrient uptake and low maize yields. The mean grain yield in combined analysis for low pH sites was 1.5 t ha<sup>-1</sup> (Table 4.8). This is just slightly above the average maize yields for the country (1.3 t ha<sup>-1</sup>) (MOA, 1994). The mean grain yield trial in the combined ANOVA for optimal conditions was 3.3 t ha<sup>-1</sup> (Table 4.15) and for the combined environments was 2.4 t ha<sup>-1</sup> (Table 4.22). Comparison of the glasshouse hydroponic trial and field trial results indicated that of the top 10 yielding genotypes, SYN DTE-STY-W-B performed well and ranked first in terms of RTi with a NSRL of 2.5 cm and this was followed by VPO717 with RTi (1.0) and NSRL (1.7 cm). These results also confirm that the best performer in the field may not be the most tolerant to low pH stress because low pH is considered a complex stress which is associated with diseases and other stresses.

The partitioning of variance into its components allows plant breeders to estimate the relative importance of the various determinants of the phenotype, in particular the role of heredity versus environment (Duvick, 1986; Volenec *et al.*, 2002). In the present study (Tables 4.6, 4.13 and 4.20), the obtained phenotypic variances ( $\delta^2p$ ) were higher than genotypic variances ( $\delta^2g$ ) at all the environments with low pH sites recording relatively lower values for both variance components.

According to Dabholkar (1992) heritability of a character is classified as low (5-10%), medium (10-30%) and high (30-60%). In the present study, results for low pH environments combined across seasons recorded high heritability values for anthesis date, days to 50% silking, husk cover, grain texture, 100 seed weight and plant vigour. At optimal environments 18 traits had high heritability values. Only root lodging (1.97%) had low value while anthesis-silking interval (18.82) and husk cover (13.98%) had medium heritability values. The results for

optimal environments were consistent with what was reported by Aminu and Izge (2012) and Bello *et al.* (2012).

Genetic advance shows the degree of gain that can be obtained in a character under a particular selection pressure. High genetic advance coupled with high heritability estimates offers the most suitable condition for selection (Bello *et al.*, 2012). Results for the optimal environment (Table 4.14) indicated higher genetic advance potential for grain yield and plant height and this was consistent with what was reported by Vashistha *et al.* (2013).

Phenotypic correlation is the association between two characters that can be directly observed, or can be determined from measurements of two characters in a number of individuals of the population (Falconer and Mackay, 1996). In the present study, correlation analysis was performed to check if grain yield was associated with some yield and yield components under low pH stress, optimal and across environments (Table 4.9; Table 4.17; Table 4.23). Significant and positive correlation between grain yield with both number of ears per plant and shelling percentage was observed in all combined analyses. In the present study low values of significant correlation coefficients were recorded for certain traits as was expected due to the large dataset used, but only values of 0.2 and larger were discussed in order to emphasize the most important correlations. In this study low pH stress contributed to lower values of significant correlation coefficients due to the stress effect at phenotypic level. Positive and significant correlation was observed for 100 seed weight under low pH environment only and not in the combined environment (Table 4.23). The results were consistent with other reports (Alvi *et al.*, 2003, Sofi and Rather, 2007; Sumathi and Muralidharen, 2010).

Anthesis date was positively and significantly correlated with grain yield at low pH and optimal environments except when combined across both environments. This was probably due to differences in ecology for the low-land optimal conditions. Low altitudes are associated with high temperatures and short seasons such that late maturing genotypes tend to perform poorly because they don't have adequate time to accumulate adequate carbohydrates.

Under low pH conditions, higher number of days to anthesis date were recorded as compared to optimal environments (mean AD value of 83 for low pH versus 65 for optimal). The AD results suggest that selection for low pH should be done under low pH conditions because what could be classified as early under low pH may not be tolerant to other stresses like fungal diseases due to prolonged humid conditions and longer seasons in low pH environments. Reports from other researchers (Brun and Dudley, 1989; Byrne *et al.*, 1995; Bänziger *et al.*, 1997) indicated that selection under stress is more effective in maize, than indirect selection under optimal conditions.

Negative correlation was observed for grain yield and ear height across low pH and combined low pH and optimal environments and this was consistent with other reports (Srećkov *et al.*, 2010). Yield was also negatively correlated with plant vigour due to the fact that a higher score in this study for vigour refers to poor vigour hence the reverse should be true that grain yield was positively correlated with plant vigour. The two are measured in different direction such that a score of one is the best for plant vigour in the scale of 1-5 while for grain yield the highest yielding genotype should record the highest number of weight units. It implies that an increase in plant vigour is associated with an increase in yield and a decrease in vigour is associated with decrease in yield. Stem lodging was also positively and significantly correlated with grain yield which was probably due to the indirect effect of low pH on the stalks of maize plants. According to Kochian *et al.* (2005) Al rapidly and effectively inhibits the influx of  $Ca^{2+}$  into cells by modulating the activity of transporters and by changing the membrane potential. Stalks of maize plants under low pH are likely to be weak due to low levels of Ca which causes low rigidity of cell walls and may not be able to support the weight of maize ears; hence higher yields were associated or correlated with stem lodging under low pH soil environments.

Clustering of maize genotypes based on morphological data across low pH environments showed that maize genotypes had similar chances of being grouped in any of the four clusters. The top five genotypes in terms of mean grain yield performance were comprised of two from CIMMYT-Colombia and three from CIMMYT-Zimbabwe. On the other hand when the genotypes were clustered using across optimal and low pH environment morphological data, the well adapted released OPVs G44 (ZM523) and G43 (ZM309) alongside some four of the top ten based on mean grain yield across optimal and low pH environment G30, G33, G8 and

G5. This suggested that the four entries and the rest in this cluster are likely to be adapted to Malawi like the released ZM523 and ZM309. The two were the first drought tolerant OPVs to be released in the country.

#### **4.7 Conclusions and recommendations**

Low pH soil is one of the abiotic factors contributing to low yields in Malawi. In this study it contributed to reduction in grain yields and yield components for the maize genotypes which were evaluated. The mean reduction in yield and yield components of 69.9% due to impaired nutrient uptake confirmed the various reports on the effect of low pH stress on yield of maize. In this study, genotypes LPHpop16, LPHpop3, VPO739, VPO5173 and Low N Pool C3-B had relatively better performance under low pH soil conditions. It is interesting to note that among the top 10 genotypes it was SYN DTE –STY-W-B that ranked first in terms of RTi with a NSRL of 2.5 cm in the glasshouse hydroponic experiment and this was followed by VPO717 with RTi (1.0) and NSRL (1.7 cm). These OPVs will be crossed to disease tolerant lines to generate source populations for inbred lines extraction for use in breeding programme for low pH tolerance.

Phenotypic traits associated with grain yield, such as plant vigour, 100 seed weight, shelling percentage, number of ears per plant, ear height and plant height can be used alongside grain yield when selecting germplasm for tolerance to low pH stress. Traits associated with reproduction such as anthesis date and days to 50% silking tend to be influenced by lower temperatures under low pH environments such that early maturing genotypes mature late and selection may not be effective when done across optimal and low pH environments. In other words these results suggested that selection for low pH tolerance could be effectively carried out *in situ* and not indirectly under optimal environments. There is need to establish the cut off point for significant correlation coefficients under stress as the relationship is affected at phenotypic level as opposed to optimal environment.

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## CHAPTER 5

### **Genotype x environment interactions and stability analysis for tropical and sub-tropical maize genotypes in Malawi**

#### **5.1 Abstract**

Maize, which is the staple food crop in Malawi, is widely cultivated in both marginal and favourable arable land, resulting in low yields in some environments with a high GEI. In this study 45 maize genotypes were evaluated across eight locations for two years using an (0.1) alpha lattice design with three replications. The objective was to study the GEI and stability of the tropical and sub-tropical maize genotypes. In the AMMI biplot, genotypes LPHpop21, VPO52, VPO72, VPO744 and VPO96 were identified as stable. VPO97 was identified as the most unstable genotype. Chitala optimal site was identified as the most discriminating environment for the genotypes, while Chitedze optimal was identified as a suitable environment. Clustering of genotypes at cut off point 1.0 indicated a cophenetic correlation of  $r_{cop} = 0.82$  with four clusters and the results were similar to that of AMMI such that cluster III was comprised of genotypes that were stable, except LPHpop21 which was in its own cluster and was considered to be the most stable and best performer in terms of grain yield. Similarly clustering of environments at cut off point 1.0 indicated a cophenetic correlation of  $r_{cop} = 0.84$  with three clusters that grouped the lowest pH sites in cluster I and the second most low pH sites in cluster II. All optimal sites were grouped in the third cluster. Clustering identified the low nitrogen site as similar to the lowest pH sites in terms of its environmental mean. The stable genotypes identified in this study will be used as base populations in the breeding programmes to generate new inbred lines.

#### **5.2 Introduction**

Maize is an important staple food and world-wide it is a widely grown cereal crop ranging from 58° north to 40° south with respect to latitude, from sea level to 3808 masl in terms of

altitude and under 25.4 to 1016 cm annual rainfall (Brewbaker, 1985; Hallauer and Miranda, 1988). According to “The Guide to Agricultural Production for Malawi” (MOA, 1994), there are two agro-ecologies for maize cultivation in the country namely: low-land or low-altitude (<600 masl) and mid-altitude areas. The low-altitude area mostly covers the lakeshore districts and the Shire Valley Districts. This region has its own challenges which include prolonged dry spells, early cessation of rains, high temperatures and floods. The mid-altitude areas is the main ecology in terms of size and it is 600 - 1300 masl. This is where most of the maize is cultivated and the major environmental challenges include fungal diseases, low pH, low N and witch weed, which is associated with low-soil fertility.

The challenge is to develop varieties of maize with a high grain yield and stable performance in the low pH areas. Some genotypes tend to exhibit good performance in some environments but not in others (Ramagosa and Fox, 1993). The basic cause of differences in yield stability between genotypes is the wide occurrence of GEI. These interactions can be partly understood as a result of different genotypes responding differently to different environmental stresses such as low pH, diseases and other factors. Plant breeders endeavour to develop improved genotypes that are superior not only in grain yield but also in a number of other agronomic and quality characteristics over a relatively wide range of environmental conditions. This interaction is important to geneticists and plant breeders because of the magnitude of the interaction components which provides information concerning the adaptation of a given crop variety (Myers, 2004).

GEI may alter the performance or development of a crop variety, thus the extent of the environmental effect on a trait determines the importance of replicating in time and space such as testing over years and locations. The multilocation evaluation, however, results in GEIs which are difficult to interpret by plant breeders and agronomists and this often reduces the efficiency in selecting the best genotypes (Annicchiarico and Perenzin, 1994). The presence of GEI may mean that a superior variety in one location is not necessarily the best in another environment. Kang *et al.* (1991) suggested that selection based on yield only may not always be adequate when GEI is significant. The analysis of GEI is regarded as an important strategy used by plant breeders to evaluate crop varieties for adaptation and also for making selections

for parents for base populations (Aina *et al.*, 2007). The development of new crop varieties is expensive and time consuming because of the procedure involved in making sure a stable variety is identified.

According to Myers (2004) stability refers to the character of a crop variety that withstands fluctuations of environments. The stability analysis for the interpretation of GEI was first proposed by Yates and Cochran in 1938. Their proposed methodology was based on linear regression of variety yield on experimental mean yield in order to observe varietal stability across varying environments (Finlay and Wilkinson, 1963; Eberhart and Russell 1966). Stability analysis has been adapted for use in comparing agronomic treatments across different environments consisting of the linear regression of treatment mean yield on the environmental mean (Raun *et al.*, 1993). The concept of stability has been defined in many ways by many researchers and several biometrical methods, including univariate and multivariate (Lin *et al.*, 1986; Becker and Leon, 1988; Crossa, 1990). Stability indices are usually univariate while a genotype's response to different environments is considered to be multivariate (Lin *et al.*, 1986). Through multivariate analysis, genotypes with similar responses are observed to cluster together (Crossa, 1990). There are two types of biplots that have been widely used to visualize GEI and these are the AMMI and, genotype and genotype x environment interaction (GGE) biplots (Gauch, 1988; Gauch and Zobel, 1997; Yan *et al.*, 2000; Ma *et al.*, 2004). The main difference between the two approaches is that GGE biplot analysis is based on location centred PCA while AMMI analysis is observed as double centred PCAs. When a number of environments and genotypes are involved it is not always easy to visualize 'which won where' in the AMMI biplot and at times it could be deceptive, as suggested by Yan *et al.* (2007). Still AMMI is regarded as a better tool for presenting conclusions rather than a tool for determining "which won where".

To ensure that suitable varieties are recommended for cultivation by farmers in the country, the Agricultural Technology Clearing Committee (ATTC) developed guidelines for release of varieties in Malawi. The most important rule in relation to GxE is that the candidate variety must have been evaluated for three years and shown stable performance at both on-farm and on-station trials. If it is an introduction, it must have two years of data for on-farm and on-

station trials in the country, supported by regional data. In addition to this rule, the variety release dossiers should clearly specify the environment it is suited for e.g. low-altitude or mid-altitude. In this case, multivariate stability analysis like AMMI and GGE are important tools in selecting a stable variety and making recommendations as to where it is suitable for cultivation.

Stable performance of a variety is also dependent on certain genetic properties. Zivanovic *et al.* (2004) indicated that breeding of F<sub>1</sub> hybrids of maize is successful because it exploits heterosis and increases homogeneity. The uniformity of hybrids consists of: (i) genetic homogeneity and (ii) genetic stability. Genetic homogeneity is focused on maintenance of the identity of genotypes, while genetic stability tends to maintain homeostasis (phenotypic uniformity) in different environments. The level of yield depends on genetic yield potential (all favourable genes incorporated into a cultivar during a breeding process). Stability of yield or of any other trait depends on the ability of a given cultivar to react to changes in the environment which it is subjected to; this is termed as phenotypic plasticity (Frey, 1983). In the present study, the objective was to study the GxE and stability of the tropical and sub-tropical maize genotypes in low pH, low N and optimal conditions.

### **5.3 Materials and methods**

The experimental design, experimental materials and site description are given in Chapter 4 Section 4.3. The low N trial of 2012 was destroyed by livestock just before harvest, hence cluster analysis alone combining with low N data of 2013 was possible using environmental means. The reason for evaluating low N tolerance was to check if other genotypes had additional attributes for this stress apart from that which were pre-described as originating from a low N tolerant source e.g. LOW N POOL C3-B. A genotype which has additional attributes is better placed for high adoption by farmers e.g. a released variety ZM523 is drought tolerant with low N tolerance as an additional attribute and is recognised through the quantities of annual certified seed sales, as widely cultivated compared to other OPVs available in the country.

## **5.4 Data analysis**

### **5.4.1 Analysis of variance**

Statistical analyses were performed using various software packages: GenStat 16<sup>th</sup> Version (2013), Agrobase (2010) and NCSS (Hintze, 2007). The AMMI model, which combines ANOVA with PCA, was used to study the nature of GEI. GxE was partitioned into sources of variation (i) additive main effects for genotypes and environment and (ii) non-additive main effect due to GEI.

### **5.4.2 Stability analysis**

AMMI analysis for mean yield was performed using Agrobase (2010). GGE biplot analysis was conducted using the GGE biplot in GenStat (2013). The model for GGE biplot (Yan and Hunt, 2002) based on single value decomposition (SVD) of the first two principal components (PC) was used.

## **5.5 Results**

### **5.5.1 Analysis of variance for additive main effects multiplicative interaction**

The combined ANOVA of the 45 maize genotypes evaluated for two years across eight locations according to the AMMI model is presented in Table 5.1. The ANOVA indicated highly significant effects ( $p \leq 0.01$ ) for environments, genotypes and GEI. The IPCA's were ordered according to decreasing importance. The F-test was highly significant ( $p \leq 0.01$ ) for the first six IPCA axes and at  $p \leq 0.05$  for the seventh IPCA. The total variation explained ranged from 2.5% for genotypes, 59.4% for environments and 17.5% for GxE. The variation due to GxE was over five times the variation due to genotypes as main effects. The first six IPCA axes explained 75.3% of the GEI. The first IPCA captured 32.8% of the total interaction sum of squares in 8.8% of the interaction degrees of freedom (GxE).

**Table 5.1 AMMI Analysis of variance for grain yield for two years 2011/12 and 2012/13**

Sources	DF	SS	MS	Total variation explained (%)	Eigenvalues	% GxE Explained	Cumulative %
Total	2159	5033813782					
Environments	15	2989647437	199309829.1**	59.4			
Reps within Env	32	159985978.2	4999561.8				
Genotype	44	124590289.1	2831597.5**	2.5			
Genotype x Env	660	879765257.9	13329877.7**	17.5			
IPCA 1	58	288728372.6	4978075.4**		96242790.9	32.82	32.82
IPCA 2	56	136931066	2445197.6**		456436688.7	15.56	48.38
IPCA 3	54	88841154.8	1645206.6**		29613718.3	10.10	58.48
IPCA 4	52	75415838.9	1450304.6**		25138613.0	8.57	67.05
IPCA 5	50	72193861.2	1443877.2**		24064620.4	8.21	75.26
IPCA 6	48	54027784.3	1125578.8**		18009261.4	6.14	81.40
IPCA 7	46	47403253.8	1030505.5*		15801084.6	5.39	86.79
Residual	1408	879824820.1	624875.58				

Grand mean- 2516.39, R- Squared 0.8252, CV = 31.4%, \*P≤0.05, \*\*P≤0.01; IPCA= Interaction principal component axis CV = coefficient of variation,

The second IPCA explained 15.6% of the interaction sum of squares in 8.5% of the interaction degrees of freedom (GxE) (Table 5.1).

Results for IPCA1 and IPCA2 are presented in Table 5. 2 and Table 5.3 and are sorted in terms of environmental mean. G20 ranked first overall. In the biplot in Figure 5.1, low pH environments: Bembeke Turn Off (BKET), Bembeke Office, (BKEO), Lunyangwa (LUN) and Tsangano (TSA) were distributed with the lower yielding environments in quadrants I (top left) and IV (bottom left) (Figure 5.1), while optimal environments: Chitala (CLA), Chitedze (CZE), Meru (MRU) and Baka (BKA) were positioned with the high yielding environments in quadrant II (top right) and III (bottom right). The genotypes categorised under favourable environments with above average means were G20, G18, G13, G26 and G41. Among them G20 was found to be most stable. G42 was identified as the most unstable genotype (quadrant IV) and the other genotypes under low yielding environments are shown in the lower left quadrant of the biplot. With respect to the environments, closer relationships were observed among Bembeke Turn off (BKET), Bembeke Office (BKEO), Lunyangwa (LUN) and Tsangano (TSA). Chitedze (CZE) was identified as a suitable environment as its IPCA score and vector was near to the origin (zero).

### **5.5.2 Genotype and GEI scatter biplot and polygon view of grain yield across eight environments for 20011/12 and 2012/13**

The polygon was constructed from genotypes G20, G45, G42, G2, G40, G16 and G8 as markers (Figure 5.2). Eight lines were drawn starting from the origin and extended beyond the polygon such that the biplot was divided into eight sectors and environments fell into three of them. Bembeke Turn off (BKET) fell in sector 1 delineated by rays 1 and 2 and the vertex genotype was G16. Similarly one environment, Bembeke Office (BKEO) fell into sector three and the vertex genotypes were G28 and G8. All optimal sites fell into sector four and were delineated by rays four and five and the vertex genotype was G20. The remaining two low pH sites: Tsangano (TSA) and Lunyangwa (LUN) fell just at the origin.

**Table 5.2 IPCA1 and IPCA2 scores for the top 20 genotypes based on mean grain yield at eight locations for two seasons**

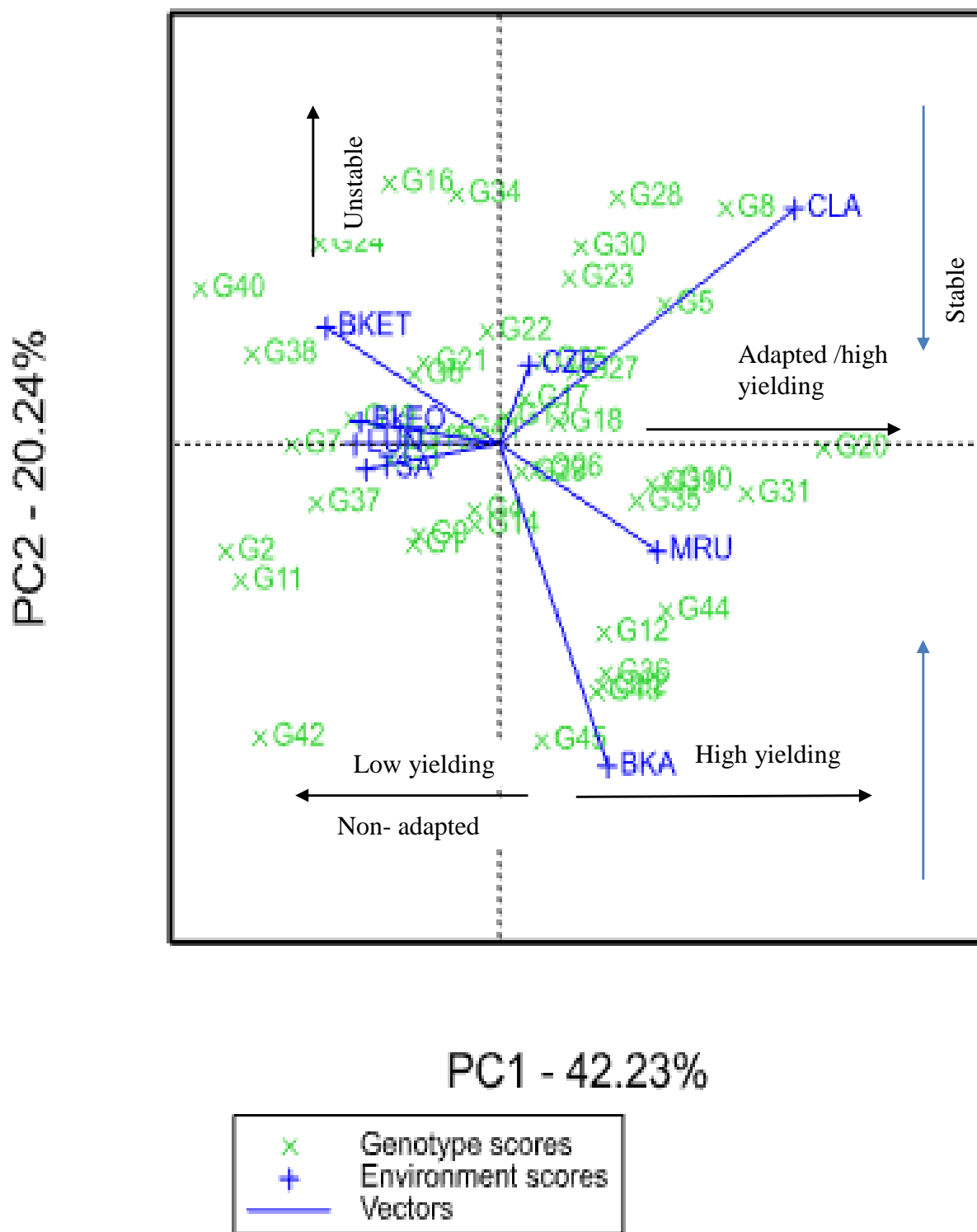
No	Entry code	Mean yield		
		kg ha <sup>-1</sup>	IPCA1	IPCA2
1	G20	2977	23.25322	3.15773
2	G27	2833	7.16857	-6.46885
3	G30	2729	18.05689	-16.53917
4	G8	2728	18.77411	-10.69649
5	G5	2719	15.12165	-8.60019
6	G22	2663	-4.61057	2.08058
7	G33	2646	19.09816	-2.1516
8	G28	2620	0.96947	-4.78308
9	G39	2614	5.95664	2.00067
10	G32	2595	5.91891	13.04379
11	G36	2594	2.29902	17.73102
12	G26	2593	3.39991	1.41781
13	G45	2591	5.21745	9.87007
14	G18	2586	0.03059	3.77821
15	G43	2584	3.63716	14.94491
16	G6	2580	-7.02094	-12.23653
17	G10	2565	10.19885	2.24328
18	G12	2552	5.78089	13.91971
19	G15	2532	-1.59902	-5.52944
20	G44	2522	11.0564	12.81569

**Table 5.3 IPCA1 and IPCA2 scores for the eight environments, ranked based on environmental mean for two seasons**

Environment	Environmental mean	IPCAe[1]	IPCAe[2]
MRU	3964	22.77	14.34
CLA	3618	47.07	-9.58
BKA	3350	10.59	44.64
CZE	2802	-31.12	2.85
BKET	2461	14.44	-38.27
BKEO	1927	-20.15	-8.73
TSA	718	-21.26	-0.35
LUN	516	-22.35	-4.89

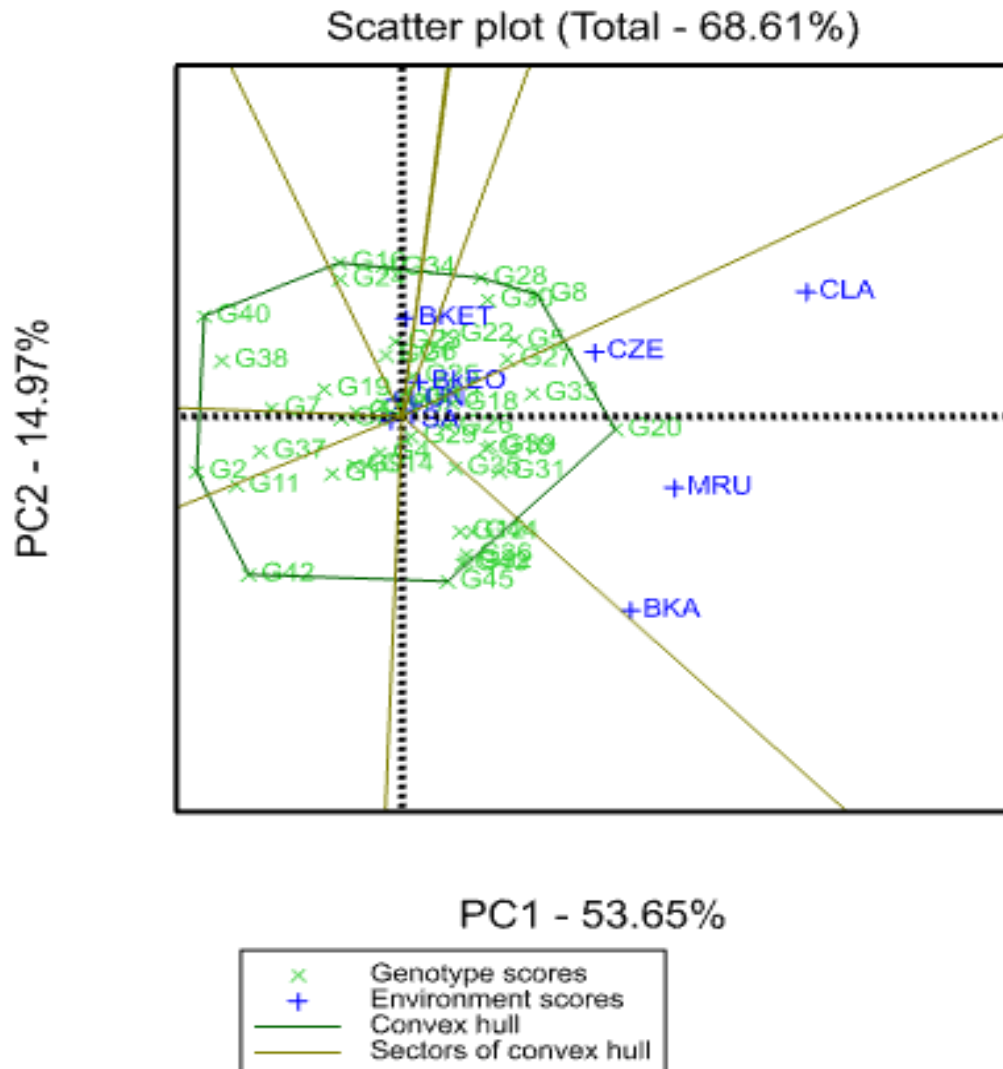
MRU = Meru, CLA = Chitala, BKA = Baka, CZE = Chitedze, BkeT = Bembeke Turn Off, BkeO = Bembeke Office, TSA = Tsangano Low pH site, LUN = Lunyangwa

### Kg\_ha: AMMI biplot (symmetric scaling)



**Figure 5.1 AMMI biplot for yield for genotypes and environments across two seasons 2011/12 and 2012/13**

MRU = Meru, CLA = Chitala, BKA = Baka, CZE = Chitedze, BkeT = Bembeke Turn Off, BkeO = Bembeke Office, TSA = Tsangano Low pH site, LUN = Lunyangwa



**Figure 5.2 Genotype and GEI scatter biplot and polygon view of grain yield across eight environments for 2011/12 and 2012/13 seasons**

MRU = Meru, CLA = Chitala, BKA = Baka, CZE = Chitedze, BkeT = Bembeke Turn Off, BkeO = Bembeke Office, TSA = Tsangano Low pH site, LUN = Lunyangwa

### **5.5.3 GGE comparison biplot across optimal and low pH environments combined for two seasons**

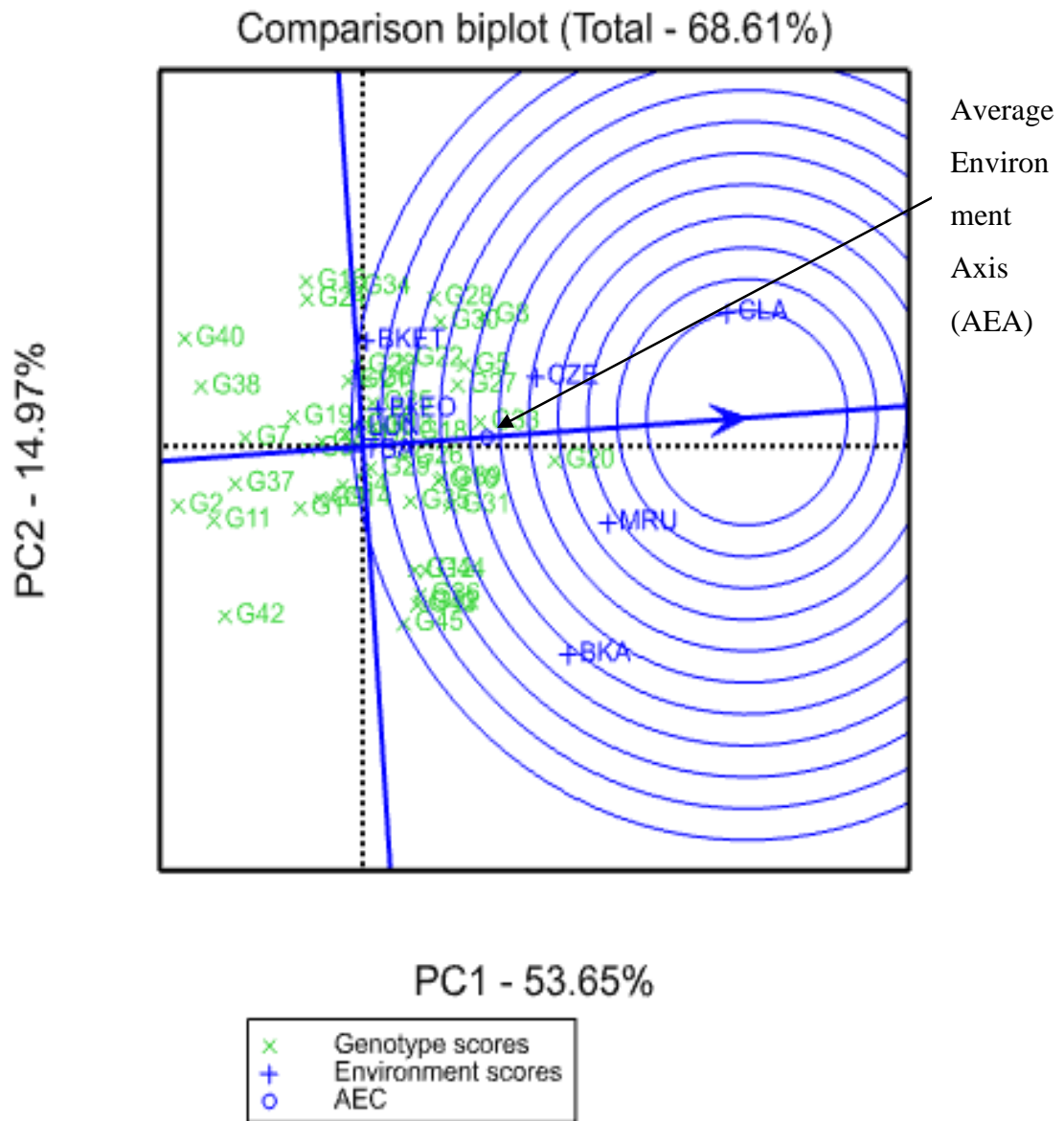
The GGE biplot for ranking of environments based on discriminating ability and the representativeness across environments relative to yield performance is presented in Figure 5.3. The ideal environment was positioned near the centre of the average environment axis (AEA) which is represented by a small circle near the end of the arrow. The arrow at the end of AEA points towards a direction indicating the most informative location. The biplot indicated that environment Chitedze (CZE) was the most representative and discriminative in terms of grain yield performance based on AEA. In terms of genotypes G38, G18 and G26 were close to the small circle (ideal environment).

### **5.5.4 Ranking of genotypes based on both mean yield and stability view of the GGE biplot**

GGE biplot of the genotypes based on both the mean and stability showed the relative mean performance and stability of hybrids across seasons (Figure 5.4). The genotypes G20 and G33 were high yielding based on average environment coordination abscissa (AECa) and average environment coordination ordinate (AECo). The genotypes G18, G13, G26 were near to the AEA which shows that they are very stable but not the highest yielding genotypes.

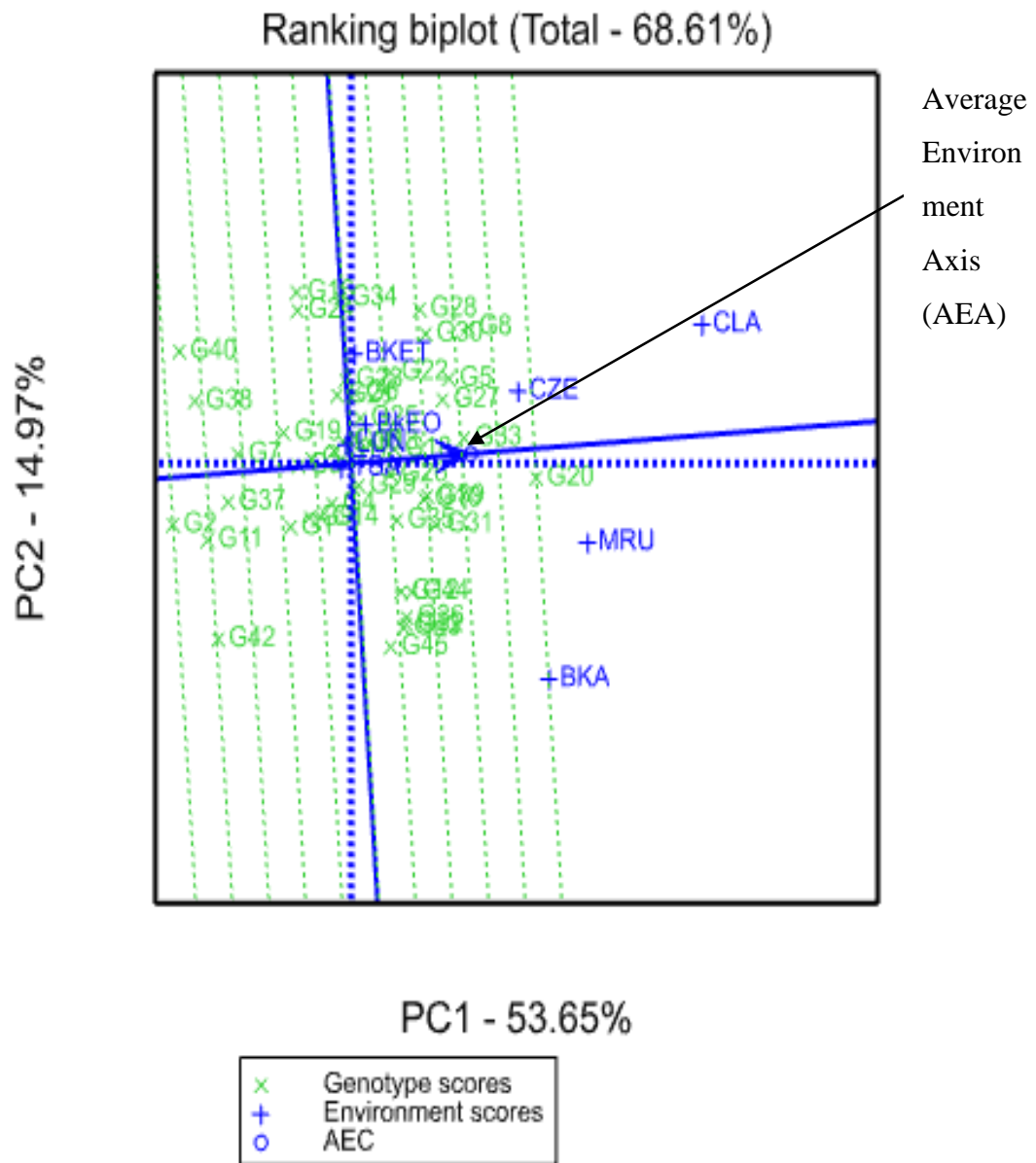
### **5.5.5 Cluster analysis of maize genotypes and environments**

Clustering of genotypes at cut off point of 1.0 produced four clusters (Figure 5.5). Cluster I consisted of 10 genotypes (G1, G14 up to G9) and cluster II consisted of seven genotypes (G11, G2 up to G7), cluster III consisted of 27 genotypes (G10, G12 to G8) and cluster IV consisted of one genotype G20 which was ranked number one by AMMI in terms of mean grain yield performance. Cluster analysis of environments (Figure 5.6) at cut off point 1.0 with a cophenetic correlation ( $r_{\text{cop}} = 0.84$ ) produced three clusters. Cluster I consisted of three stress sites, the low N site, Lunyangwa and Tsangano. Cluster II consisted of two low pH stress sites Bembeke Office and Bembeke Turn Off, Cluster III consisted of four optimal sites Baka, Chitedze, Chitala and Meru.



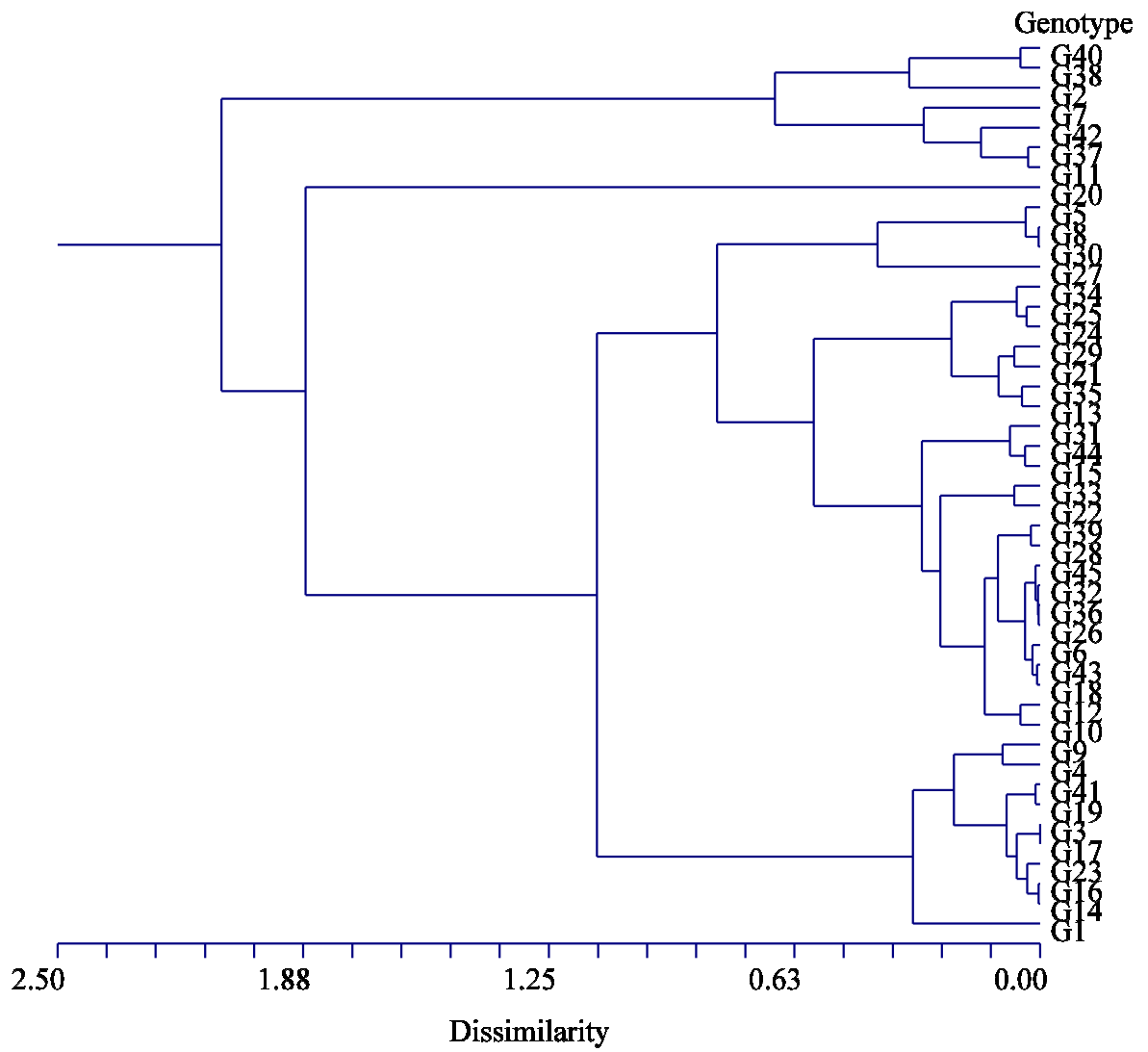
**Figure 5.3 Genotype and GEI comparison biplot of grain yield across eight environments for 2011/12 and 2012/13**

MRU = Meru, CLA = Chitala, BKA = Baka, CZE = Chitedze, BkeT = Bembeke Turn Off, BkeO = Bembeke Office, TSA = Tsangano Low pH site, LUN = Lunyangwa

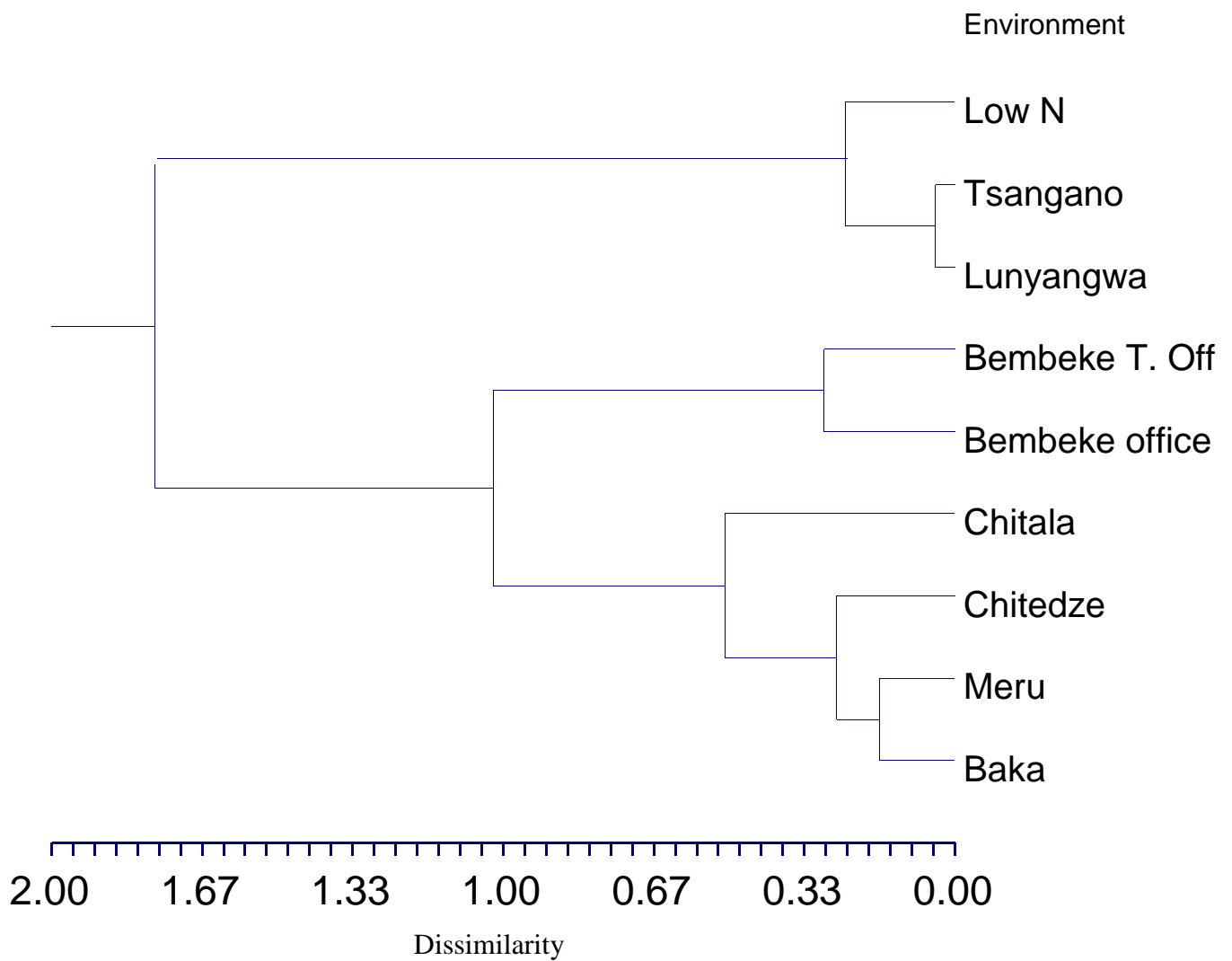


**Figure 5.4 Ranking of genotypes based on both mean yield and stability view of the GGE biplot**

MRU = Meru, CLA = Chitala, BKA = Baka, CZE = Chitedze, BkeT = Bembeke Turn Off, BkeO = Bembeke Office, TSA = Tsangano Low pH site, LUN = Lunyangwa.



**Figure 5.5 Dendrogram of 45 maize genotypes as revealed by UPGMA cluster analysis based on AMMI adjusted mean yields combined for two seasons using Euclidean distance and standard deviation as scaling method**



**Figure 5.6 Dendrogram of nine environments as revealed by UPGMA cluster analysis based on environmental means and Euclidean distance and standard deviation as scaling method**

## 5.6 Discussion

Environments and genotypes were both plotted as vectors and points respectively on the AMMI biplot. Genotypes and environments that were in close proximity are considered to be similar in terms of performance and discrimination of genotypes. The angle between two vectors indicated the degree of association or correlation. Small angles indicated similarity, a 90° angle indicated orthogonality and no association and an angle >90° indicated a negative correlation. The sites Lunyangwa and Tsangano were close to each other and these had the lowest pH. Genotypes G8 (LPHpop9), G28 (LPHpop10) and G30 (LPHpo3) were in close proximity and these were low pH tolerant populations from CIMMYT-Colombia and this demonstrated similarity of the genotypes. The orthogonal projections of genotypes on environment vectors indicate the relative performance of genotypes in a given environment: that is, the greater the projection of the genotype in the positive direction, the better the performance of that genotype in that environment. Drought tolerant (DT) genotypes were close to each other [G44 (ZM523), G12 (TZE YDT STR C4-B), G43 (ZM309) and ZM721]. These genotypes are likely to have some genes in common that were at play. Tsangano, Lunyangwa and Bembeke Office sites were close to each other and these were classified in one group as acidic sites, by use of the Soil Interpretation Guide (Horneck *et al.*, 2011). These sites are also associated with poor performance in terms of maize yields such that 85% yield reduction was reported for Lunyangwa (Munthali and Chilimba, 2004).

When the environment contributes a large percentage of variation, it implies that it was a major factor that influenced yield performance (Issa, 2009). When plotting the genotypes and the environments on the same graph, the association between the hybrids and the environments can be seen clearly. IPCA scores of a genotype in the AMMI analysis are an indication of the stability of a genotype over environments (Gauch and Zobel, 1996; Purchase, 1997). The greater the IPCA scores, either positive or negative, the more specifically adapted a genotype is to certain environments sampled. For instance G27 (Low N Pool C3-B) was close to CZE (Chitedze), indicating that it is a low N tolerant genotype and is able to do much better under ideal conditions probably because of its high N use efficiency (NUE). The present study recorded genotype contribution to total sum of squares as very low at 2.5% and was consistent with findings by Babic *et al.* (2010) who reported

2.2% but was not consistent with findings by Mitrović *et al.* (2011) who reported a somewhat higher 9.17% contribution.

The genotypes categorised under favourable environments with above average means were G20, G18, G13, and G41. Among them G20 (LPHpop21) was found to be more stable and this was from the low pH tolerant populations from CIMMYT-Colombia. Its tolerance to low pH might have contributed to its stable performance across seasons and all locations tested. Genotypes grouped under low yielding environments are shown at the lower left quadrant (IV) of the biplot. G42 (VPO97) from CIMMYT-Zimbabwe was the most unstable genotype identified by the AMMI model (Figure 5.1). Genotypes that are close to each other tend to have similar performance and those that are close to a specific environment indicate their better adaptation to that particular environment. The five IPCA axes can be taken as adequate dimensions of the data, however, only the first two IPCA axes were plotted on the biplots to help investigate the GEI pattern for each genotype. The biplot showed that Chitala was the most discriminating environment for the genotypes as indicated by the longest distance between its marker and the origin and gave information of the performance of the genotypes. This site was associated with genotype G8 (LPHpop 9) which might be specifically adapted to this site. Chitala was used as an optimal site in terms of pH but it is used as random drought screening site classified as low-land tropical zone E by CIMMYT (2008). G8 (LPHpop9) coincidentally was among the low pH tolerant populations from CIMMYT-Colombia and it might be tolerant to random drought. Piñeros *et al.* (2005) and Hartwig *et al.* (2007) reported that low pH negatively influences the use of soil nutrients and induces plants to be more susceptible to drought. It implies that a genotype which is tolerant to low pH is likely to be less susceptible to random drought. However, due to its high IPCA scores, genotype variability at this environment may not exactly reflect the average genotype performance across environments. This is consistent to the findings by Arulselvi and Selvi (2010). For the environments, a closer relationship was observed between Bembeke Turn Off, Bembeke Office, Lunyangwa and Tsangano low pH site. Chitedze was identified as a stable environment as its IPCA score and vector is near to the origin (zero). This site is in the mid-altitude ecology and is in the arable land of Lilongwe Plain with a longer rainfall season than Chitala and these results have confirmed that the country's best yields are obtained in the mid-altitude ecology (MOA, 1994). Generally genotypes with a smaller vector angle have a similar projection, which

designate their proximity in grain yield and performance. Those genotypes that are clustered close to the centre tend to be stable and those far apart unstable.

The GGE biplot construction is carried out by plotting the first PC scores of the genotypes and the environments against their respective scores for PC2 that result from SVD of environment-centered or environment-standardised genotype-by-environment data (GED) (Yan *et al.*, 2000; Setimela *et al.*, 2007). According to Setimela *et al.* (2007), the purpose of the polygon view of the biplot is basically to show which hybrids won in which environments. The genotypes located furthest from the biplot origin demarcate the corners of the polygon. The perpendicular lines that are drawn from the biplot origin divide the biplot into sectors or mega environments. The environments are contained into different sectors such that different sectors contain different winner genotypes (Yan *et al.*, 2007).

The results showed that the polygon was constructed from genotype markers G20, G45, G42, G2, G40, G16 and G8. Among these markers, G40 in the upper left quadrant (I) marked the unstable environment while G42 in the lower bottom left (IV) marked the low yielding environment. Depending on the objective of the research, selection could be carried out in the quadrant with markers G20 (stable environment) and G45 (high yielding environment). Eight lines were drawn starting from the origin and extended beyond the polygon such that the biplot was divided into eight sectors and environments fell into three of them. Bembeke Turn off fell in sector 1 delineated by rays 1 and 2 and the vertex genotypes were G16. Similarly environment Bembeke Office, fell into sector three where the vertex genotypes were G28 and G8. All optimal sites fell into sector four and were delineated by rays four and five and the vertex genotypes was G20. The remaining two low pH sites: Tsangano and Lunyangwa fell just at the origin.

The construction of GGE comparison biplots is done with the aim of ranking environments based on discriminating ability and the representativeness across environments relative to yield performance (Setimela *et al.*, 2007). In this type of biplot, the ideal environment is one positioned near the centre of the AEA which is represented by a small circle near the end of the arrow as indicated in Figure 5.3. The tiny circle in the biplot represents the average environment and is defined by the average PC1 and PC2 scores across the environments. In the present study, the biplot indicated that environment Chitedze was the

most representative and discriminative in terms of grain yield performance based on the AEA and genotypes G38, G18 and G26 were close to the small circle showed that they were stable but not the highest yielding genotypes.

The AEA is the average environment axis and the projection of the genotypes onto this line represents the main effects of the genotypes (Setimela *et al.*, 2007) therefore the AEAa ranks the genotypes according to the mean performance where by a small a is for abscissa. Usually ranking of the genotypes on the AEA is associated with the genotype main effect that is, the AECa approximates the contribution of each genotype to the main effects of genotypes. Similarly, AECo expresses the genotype contributions to GxE and thus it represents the genotype stability across environments where by a small o is for ordination. The AEA from the biplots points towards maize genotypes with high and stable mean grain yield across environments. The genotypes G20 and G33 are high yielding based on AECa and AECo. An “ideal” genotype may not exist in practice but it can be used as a reference for genotype evaluation (Mitrović *et al.*, 2011). A genotype is more desirable if it is located closer to the “ideal” genotype (Kaya *et al.*, 2006). Plant breeders prefer genotypes that are high yielding and also stable across environments. In this ranking biplot, G42 was identified as the most unstable genotype as it was located far from the “ideal genotype”.

The GGE biplot explained 68.6% of the G+GE variation. According to Yan *et al.* (2007), the greater the contribution, the more confidence the researcher would have in the interpretations based on the biplot. However, if a smaller portion of the total variation is explained, it does not necessarily mean that the biplot is useless. GGE biplots provide more information than AMMI biplots while the latter gives important details in the ANOVA, making both useful and both should be used in stability analyses.

According to Ghaderri *et al.* (1980) cluster analysis is the most widely used technique for classifying environments or genotypes into homogeneous groups. It operates on a matrix of dissimilarity or dissimilar indexes for all possible pairs of genotypes or pairs of environments, on which it is being clustered. In the present study, cluster analysis was performed to study the pattern of groupings and environments. The hierarchical clustering report indicated that cluster I consisted of 10 genotypes (G1, G14 up to G9) and cluster II consisted of seven (G11, G2 up to G7), cluster III consisted of 27 genotypes (G10, G12 up

to G8) and cluster IV consisted of one genotype, G20 which was ranked number one by AMMI in terms of mean grain yield performance. Therefore both methods could be used to identify stable genotypes.

Cluster analysis of environments at cut off point 1.0 with a cophenetic correlation of  $r_{cop} = 0.84$  produced three clusters (Figure 5.6). The hierarchical clustering report indicated that cluster I consisted of three environments, low N site, Lunyangwa and Tsangano. Cluster II consisted of two low pH stress sites, Bembeke Office and Bembeke Turn Off. Cluster III consisted of four optimal sites Baka, Chitedze, Chitala and Meru environments. This technique could be useful in general grouping of locations that have stresses and further analysis is required to identify specific stresses at play.

## 5.7 Conclusions

The low pH soil environment had an effect on grain yield performance such that the low pH sites were positioned in the low yielding area in the AMMI biplot. Chitala optimal site was identified as the most discriminating environment for the genotypes, and the most stable environment. Genotypes G20 (LPHpop21), G18 (VPO52), G13 (VPO721) and G41 (VPO96) were positioned in the favourable environments with above average means. Cluster analysis results were similar to AMMI results such that cluster III comprised of genotypes that were stable, except for G20 (LPHpop21) the most stable, which was in its own cluster. G42 (VPO97) was identified as the most unstable genotype.

GGE biplots explained more variation and gave more information than AMMI while the latter gave important details on the ANOVA, making them both useful and both should be used in stability analyses. Similarly clustering of environments classified the lowest pH sites in cluster I and intermediate pH sites in cluster II. All optimal sites were positioned in the third cluster. Clustering identified the low N site as similar to the lowest pH sites in terms of its environmental mean.

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## CHAPTER 6

### **Evaluation of diallel crosses for combining ability between selected tropical and sub-tropical maize lines for low pH tolerance**

#### **6.1 Abstract**

This study was conducted to assess the GCA effects of parental maize inbred lines and SCA effects for the diallel crosses for yield and yield related traits and explore their use in hybrid development for low pH tolerance. Sixty six F<sub>1</sub>s generated by crossing 12 inbred lines were evaluated alongside two hybrid checks at four locations in Malawi. The experimental design was a (0.1) alpha lattice with three replications. Positive and highly significant GCA effects for grain yield were observed for inbred line CZL999601 across low pH and optimal conditions. Negatively and significant GCA effects for grain yield were observed for inbred line CML161 across low pH and optimal conditions. Single cross hybrids CZL999601/CML144, CML144/CML202, CML481/CML288 and CML161/CM172 were identified as good specific combiners for grain yield. The estimates of broad sense heritability were high for days to 50% anthesis, anthesis-silking interval, grain yield and plant vigour. At a cut-off point of 1.0 the UPGMA clustered the inbred lines based on GCA for grain yield into two main clusters through use of Euclidean distance and standard deviation as type of scale. There was a high cophenetic correlation of  $r_{cop} = 0.87$  and the pattern mostly followed the origin of the maize inbred lines such that six out of seven inbred lines from CIMMYT-Colombia (tropical) were grouped in the second cluster. The inbred lines and specific combinations identified in this study will be used in the National Maize Breeding Programme for development of genotypes tolerant to low pH and diseases for yield improvement and subsequent food security in the country.

#### **6.2 Introduction**

Maize is the world's most widely grown cereal and is the primary staple food in many developing countries (Morris *et al.*, 1999). In Malawi, two main types of maize cultivars are cultivated by farmers, hybrids and OPVs. The OPVs include synthetics, composites,

and local types which are locally called Chamakolo. In these types of maize, hybrids have the highest yielding potential, followed by synthetics and composites and then lastly local varieties. The differences are based on the breeding process in case of hybrids, synthetics and composites. The performance of maize genotypes used as parents is evaluated based on the performance of the cross progeny. With respect to hybrids, the parental lines are chosen based on their SCA as measured in their hybrid offspring. Usually two or three selected inbred lines are used in developing single-cross and three-way cross hybrids, respectively.

Synthetics are developed by inter-crossing a larger number of selected parental lines of known superior combining ability, for example inbred lines which are known to give superior hybrid performance when crossed in all combinations. A composite is developed by selecting parental lines of relatively similar maturity periods and mixing the seed from the individuals. In both cases, seed is maintained by allowing full-sib pollination (or plant to plant pollination in the same population). These types are also referred to as open-pollinated because there is no control of pollen movement when planted in isolation for seed increase (bulking), especially for basic and certified seed multiplication. In other words, some form of inbreeding occurs as there is no de-tasselling of the male flowers of the female parent as in hybrid crosses. Hence farmers in Malawi are advised to recycle the OPVs for two seasons and the third season they should look for new seed because of inbreeding depression and genetic drift which occurs with time of recycling the seed and reception of foreign genes from other varieties.

Crop estimates for 2012 showed that hybrids contributed 56.5% to the national total production while composites and synthetics contributed 30.5% and local varieties the least at 13%. In terms of hectareage, hybrids contributed 40%, composites/synthetics 30% and local varieties 30% (MOA, 2012). Combining ability is considered in hybrids more than in synthetics which are based only on GCA. The most well-known synthetic and composite varieties released by the National Maize Breeding Programme in Malawi are Masika and Chitedze Composite A, respectively. In terms of hybrids, it is Malawi maize hybrids number 17 and 18 (MH17, MH18) and these are the first flint three-way cross hybrids which are preferred because of their good pound ability. The two hybrids had a common male parent which is very flint and was identified as a good combiner for flintness.

The concept of GCA and SCA was introduced by Sprague and Tatum (1942) and its mathematical modelling was done by Griffing (1956). The value of any population depends on its potential *per se* and its combining ability in crosses (Vacaro *et al.*, 2002). The variances of GCA and SCA are related to the type of gene action involved. Variance for GCA includes the additive portion while that of SCA includes the non-additive portion of total variance arising largely from dominance and epistatic deviations (Rojas and Sprague, 1952). GCA and SCA are powerful tools used by plant breeders in selecting the best parents for further crosses. Studies on combining ability help breeders in identifying parental lines with good GCA and in detecting hybrids with good SCA (Ndhela, 2012). Devi and Singh (2011) indicated that the best performing genotypes ought to show stable performance across environments in multi-environment trials. However, it is important to bear in mind that heritability is not only influenced by the trait under consideration but is also influenced by the population and environmental conditions which individuals are exposed to, as well as the method of data collection used (Falconer and Mackay, 1996).

In terms of types of genotypes, reports indicate that researchers in the late 1950s and early 1960s recommended the use of maize genotypes with a broader genetic base, because they are more tolerant to stresses than the narrow genetic base genotypes. Lerner (1954), Lewontin (1957) and Allard and Bradshaw (1964) reported that yield stability is influenced in part by the genetic structure of the variety such that more heterogeneous varieties are less affected by environmental differences. Sprague and Federer (1951) found that maize double-cross (DC) hybrids have smaller GEIs and are more stable than single-cross hybrids. Valdivia-Bernal and Hallauer (1991) also reported that more homogeneous generations, inbred lines and  $F_1$ 's had larger GEIs than more heterogeneous generations,  $F_2$  and backcross populations. DC hybrids are rarely used nowadays because of the cost of producing the seed, but with the current maize production constraints, DC hybrids and OPV populations are better placed and the procedure should be to select suitable single-crosses from a diallel trial for the formation of double crosses and also good combiners (high GCA) for formation of synthetics.

Diallel crosses have been widely used in genetic research to investigate the inheritance of important traits among sets of genotypes. These were devised specifically to investigate

the combining ability of the parental lines for the purpose of identifying superior parents for use in hybrid development programmes. It is advisable to carry out indirect selection for related traits which show close correlation with yield and exhibit high heritability because yield is considered a polygenic trait. Analysis of diallel data is usually conducted according to the methods of Griffing (1956) which partition the total variation of diallel data into GCA of the parents and SCA of the crosses (Yan and Hunt, 2002).

A diallel is simple to manipulate in maize and supplies important information to plant breeders about the studied populations for various genetic parameters (Vacaro *et al.*, 2002). In Malawi there is no known maize inbred line or variety that is tolerant to low pH, hence the objective of this study was to evaluate the performance of diallel crosses made with 12 maize inbred lines. Seven were from CIMMYT-Colombia, four were from CIMMYT-Zimbabwe and a well-adapted line from Malawi.

## **6.3 Materials and Methods**

### **6.3.1 Experimental materials description**

The experimental materials comprised of seven low pH tolerant maize inbred lines from CIMMYT-Colombia and five well adapted lines in Malawi from CIMMYT-Zimbabwe (Table 6.1). Seed increase of the parental lines was done at Chitala Research Station in the winter of 2010. Hand pollination by selfing and bulking eight to 10 plants from each parental line was done. Diallel crosses without reciprocals were made during the 2010/11 season at Chitedze Research Station in Lilongwe in summer and at Chitala Research Station and Bwanje Irrigation Scheme during winter by stagger planting. The single crosses pedigrees have a slash separating female and male parent. For example a single cross S410 has a pedigree CML144/CML288. This means that the female was parent number four which was CML144 and the male was parent number 10 which was CML288. The male is always to the right hand side. Even in a three-way cross the male is to the right hand side and the only difference is the double slashes before the male. For instance CML144/CM288// CML481 implies that CML481 which is to the right hand side after two slashes is the male. This was adopted from CIMMYT (CIMMYT, 2012). The trial was

planted at four sites which included Lunyangwa low pH site, Tsangano low pH site, Bembeke low pH site and Chitedze optimal site.

**Table 6.1 Description of 12 maize parental lines used in the diallel crosses and their origin**

<b>NO.</b>	<b>Parental line</b>	<b>Origin</b>	<b>Traits</b>
1	CZL999601	CIMMYT-ZIMBABWE	GLST, LBT, MSVT
2	CML481	CIMMYT-COLOMBIA	Low pH
3	CML359	CIMMYT-COLOMBIA	Low pH
4	CML144	CIMMYT-COLOMBIA	Low pH
5	CML161	CIMMYT-COLOMBIA	Low pH
6	CML172	CIMMYT-COLOMBIA	Low pH
7	CML448	CIMMYT-COLOMBIA	Low pH
8	CML312	CIMMYT-ZIMBABWE	GLST, LBT, MSVT
9	ZL130-23	MALAWI	GLST, LBT, MSVT
10	CML288	CIMMYT-COLOMBIA	Low pH
11	CML202	CIMMYT-ZIMBABWE	GLST, LBT, MSVT
12	CML539	CIMMYT-ZIMBABWE	DT, GLST, LBT, MSVT

GLST = gray leaf spot tolerant, LBT = leaf blight tolerant, MSVT = maize streak virus tolerant, DT = drought tolerant

### 6.3.2 Experimental procedures and design

Sixty six single-cross hybrids plus two checks were laid out in a (0.1) alpha lattice design (0,1) with three replications. Plot size was two rows of 5.1 m per plot with 17 stations per row, one seed was planted per station and two at both ends of the row.

### 6.3.3 Description of sites

The description of sites as well as management of the trials was given in the materials and methods section 4.3 of Chapter 4. The list of phenotypic and agronomic traits investigated and measuring procedures are the same as in Table 4.1 (Chapter 4).

## 6.4. Data analysis

Across site analysis was performed using GenStat (2013). Combining ability analyses were carried out using Statistical Analysis Software (SAS, 2013). Genotypes were considered fixed. Dendrogram construction was carried out in NCSS (Hintze, 2007) using UPGMA. Clustering was based on GCA effects for the inbred lines for grain yield, Euclidean distance and standard deviation as scale type.

## 6.5 Results

### 6.5.1 Performance of diallel crosses

Mean squares for performance of diallel crosses for grain yield and other agronomic traits are presented in Table 6.2. Across site analysis for optimal and three low pH sites indicated that all the sources of variation against the measured traits were significant with the exception of the interaction of genotype for shelling percentage and MSV for site. The mean performance across all four sites showed that hybrid CZL999601/CML144 ( $2.4 \text{ t ha}^{-1}$ ) was the best performer, followed by CZL999601/CML202 ( $2.1 \text{ t ha}^{-1}$ ), CZL999601/ZL130-23 and CML481/CML288 ( $2.0 \text{ t ha}^{-1}$ ) (Table 6.3). Across the three low pH sites, CML288/CML202 ( $1.04 \text{ t ha}^{-1}$ ), CML359/CML448 ( $1.0 \text{ t ha}^{-1}$ ) and CML481/CML288 ( $0.9 \text{ t ha}^{-1}$ ) performed relatively better than the other hybrids (Table 6.4). At the optimal site CZL999601/CML144 ( $7.0 \text{ t ha}^{-1}$ ), CZL999601/CML202 ( $5.4 \text{ t ha}^{-1}$ ), CML172/ZL130-23 ( $5.0 \text{ t ha}^{-1}$ ) and CML481/CML288 ( $4.5 \text{ t ha}^{-1}$ ) were among the top ten yielding hybrids (Table 6.5). Hybrids CML481/CML288 showed some consistency in performance across environments while CZL999601/CML144 was suited for optimal conditions. Grain yield was reduced by 78.2%, ear height by 55.3%, number of ears per plant by 54.5%, grain texture by 15.3%, plant height by 41.7%, 100 seed weight by 36.3%, shelling percentage by 18.8% and plant vigour by 62.5% (Table 6.6). Grain yield and plant vigour were the most reduced traits under low pH. Grain texture exhibited the lowest percentage reduction among the measured traits.

## 6.5.2 Genetic variances, phenotypic variances and heritability estimates for the diallel crosses across optimal and three low pH environments in 2011/12

Results for the diallel crosses are presented in Table 6.7. High broad sense heritability were recorded for anthesis date (0.93), anthesis-silking interval (0.87), grain yield (0.86) and leaf blight (0.86). The lowest heritability value was recorded for number of ears per plant (0.14)

**Table 6.2 Mean squares for diallel crosses across optimal and three low pH environments for grain yield and agronomic traits in 2011/12**

Source	Site	Genotype	G x E	MSE	LSD	CV%
GY	4.78E+08**	1.47E+06**	1.07E+06**	3.50E+05	474.8	12.9
AD	34.87**	34.87**	8800**	2.866	2.716	2.15
ASI	599.97**	1.81**	1.888**	0.575	0.61	30.7
PH	496823**	460.8**	495.9*	259.4	25.8	12.9
EH	160708**	280.5**	250.8**	129.1	18.22	21.1
EPP	32.2**	0.072**	0.093**	0.204	0.31	29
RL	1322.7**	10.76**	10.4**	5.87	2.25	118
SL	21.9**	4.5**	4.1**	2.56	1.82	114
GLS	81.8*	0.99**	0.748**	0.18	0.39	25
LB	82.2**	1.49*	1.26**	0.21	0.43	26.4
MSV	0.0001	0.31**	0.42**	0.16	0.08	36.8
RUST	219.3**	0.48*	0.43**	0.15	0.36	21.1
GT	106.1**	0.88**	1.1**	0.27	0.59	25.5
SH	15036**	617.9	620.3**	478	24.8	31.4
VIG	569.76**	1.22**	1.38**	0.31	0.64	16.4
SWT	10523**	94.8**	77.2**	24.8	5.66	19

\*\*P≤0.01; \*P≤0.05; G = genotype, E = environment, MSE = Mean square error, LSD = Least significant difference, CV = coefficient of variation, GY = grain yield (kg ha<sup>-1</sup>), AD = days to anthesis (days), ASI = anthesis-silking interval (days), PH = plant height (cm), EH = ear height (cm), EPP = ears per plant (#), RL = root lodging (#), SL = stem lodging (#), GLS = gray leaf spot disease (1-5), LB = leaf blight disease (1-5), MSV = maize streak virus disease (1-5), Rust = rust disease (1-5), GT = grain texture (1-5), SH = shelling percentage, VIG = vigour (1-5), SWT = 100 seed weight (g), # = number.

**Table 6.3 Mean performance of diallel crosses across optimal and low pH environments in 2011/12 season**

	Code	GY	AD	ASI	DS	EH	EPP	LB	GLS	GT	MSV	PH	RL	RUST	SWT	SH	SL	VIG
Top 10 Genotypes	G3	2379	80.5	1.1	82.6	56.9	0.6	1.3	1.7	2.4	1.0	126.8	2.2	1.8	24.4	70.9	2.4	3.0
	G10	2075	78.2	1.3	80.3	48.2	0.6	2.3	1.2	2.0	1.1	119.1	2.2	2.1	24.9	74.8	1.9	3.7
	G8	1984	80.2	1.4	82.5	55.0	0.7	2.3	1.6	2.0	1.0	125.8	5.2	1.9	27.1	73.4	1.8	2.7
	G19	1919	76.3	1.7	78.7	58.8	0.6	1.3	1.9	1.8	1.0	130.3	0.8	1.4	27.2	71.5	1.2	2.8
	G48	1913	78.8	2.0	81.5	60.0	0.8	2.0	2.4	2.4	1.0	131.3	3.2	2.3	28.1	72.8	0.8	3.5
	G39	1837	79.0	1.4	81.1	57.8	0.6	3.0	2.1	2.4	1.0	120.1	4.0	1.7	27.1	74.3	1.4	3.0
	G2	1811	77.8	1.6	80.2	56.1	0.6	2.3	2.6	1.7	1.0	132.3	1.7	2.3	29.8	70.5	1.3	3.0
	G16	1808	80.3	1.3	82.0	46.7	0.6	1.7	1.6	2.2	1.2	105.5	8.0	1.8	27.1	69.8	1.3	2.7
	G7	1657	78.3	1.9	80.7	45.7	0.6	2.7	1.8	1.9	1.1	121.7	4.2	1.5	24.5	70.8	2.1	3.7
	G24	1631	79.1	1.9	81.8	51.4	0.9	1.3	1.9	1.9	1.7	120.0	1.3	1.8	23.5	66.0	0.9	2.8
Bottom 10 Genotypes	G57	965.8	79.8	1.7	82.3	59.5	0.7	1.3	2.0	1.9	1.0	130.0	3.5	2.3	26.8	67.1	0.4	3.4
	G54	963	81.8	1.2	83.9	61.5	0.6	2.0	1.0	2.1	1.3	131.7	1.3	1.7	35.7	74.8	0.9	3.3
	G47	943	80.2	2.1	82.5	57.6	0.7	1.0	1.8	2.3	1.0	124.9	2.5	2.2	25.1	75.3	0.8	3.8
	G55	940.8	80.3	1.7	82.8	56.7	0.6	3.0	2.0	1.3	1.2	129.4	2.7	2.0	26.4	70.1	0.4	3.4
	G60	842.8	78.5	1.8	81.1	55.0	0.7	1.0	1.6	1.8	1.0	130.1	4.5	1.8	20.1	71.8	2.6	4.6
	G62	780.3	80.6	2.2	83.5	53.3	0.7	1.0	1.7	1.6	1.0	122.5	1.0	1.7	27.2	66.4	1.8	3.7
	G41	778	80.3	1.8	82.6	46.4	0.6	4.0	1.7	2.3	1.0	113.6	2.3	1.7	24.8	68.5	0.4	4.0
	G44	733	79.5	0.9	81.4	65.3	0.7	4.0	1.7	2.2	1.0	133.7	4.8	2.2	23.2	61.3	0.4	3.4
	G43	711	82.0	2.2	84.9	48.1	0.7	2.0	1.7	1.7	1.1	123.9	0.5	1.7	28.2	65.0	1.7	3.8
	G40	646	77.5	1.9	80.2	47.8	0.7	2.0	1.0	2.3	1.0	124.8	1.2	1.3	27.4	60.9	0.7	4.3
	<b>Mean</b>	<b>1366</b>	<b>79</b>	<b>2</b>	<b>82</b>	<b>54</b>	<b>1</b>	<b>1.0</b>	<b>2</b>	<b>2</b>	<b>1</b>	<b>125</b>	<b>3</b>	<b>2</b>	<b>26</b>	<b>70</b>	<b>1</b>	<b>3</b>
	<b>LSD</b>	<b>478.8</b>	<b>1.4</b>	<b>0.6</b>	<b>1.4</b>	<b>9.1</b>	<b>0.2</b>	<b>1.7</b>	<b>0.4</b>	<b>0.6</b>	<b>0.6</b>	<b>12.9</b>	<b>2.3</b>	<b>0.6</b>	<b>8.4</b>	<b>17.7</b>	<b>1.5</b>	<b>0.6</b>
	<b>MSE</b>	<b>3.50E+05</b>	<b>2.9</b>	<b>0.6</b>	<b>3.2</b>	<b>129.1</b>	<b>0.0</b>	<b>0.4</b>	<b>0.2</b>	<b>0.3</b>	<b>0.2</b>	<b>259.</b>	<b>5.9</b>	<b>0.2</b>	<b>24.8</b>	<b>620.3</b>	<b>2.6</b>	<b>0.3</b>

LSD = Least significant difference, MSE = Mean square error, GY = grain yield (kg ha<sup>-1</sup>), AD = days to anthesis (days), ASI = anthesis-silking interval (days), DS = days to silking (days), EH = ear height (cm), EPP = ears per plant (#), LB = leaf blight disease, (1-5), GLS = gray leaf spot disease (1-5), GT = grain texture (1-5), MSV = maize streak virus disease (1-5), PH = plant height (cm), RL = root lodging (#), Rust = rust disease (1-5), SWT = 100 seed weight (g), SH = shelling percentage, SL = stem lodging (#), VIG = vigour (1-5), # = number.

**Table 6.4 Mean performance of diallel crosses across three low pH environment in 2011/2012 season**

	<b>Entry</b>	<b>GY</b>	<b>AD</b>	<b>ASI</b>	<b>DS</b>	<b>EH</b>	<b>EPP</b>	<b>LB</b>	<b>GLS</b>	<b>GT</b>	<b>MSV</b>	<b>PH</b>	<b>RL</b>	<b>RUST</b>	<b>SWT</b>	<b>SH</b>	<b>SL</b>	<b>VIG</b>
Top 10 Genotypes	G64	1040.7	77.7	2.7	80.3	34.1	0.4	2.0	2.3	1.5	1.0	101.0	0.5	1.8	16.5	65.6	2.5	5.0
	G25	1007.7	81.9	3.4	85.3	32.6	0.8	1.7	1.3	2.2	1.0	92.7	0.5	2.0	21.8	70.3	1.3	4.7
	G19	999.3	77.8	3.0	80.8	47.8	0.5	1.3	2.3	1.7	1.0	113.1	0.5	1.6	21.6	66.8	1.5	4.3
	G10	970.7	79.6	2.6	82.1	40.4	0.5	2.3	1.3	1.9	1.0	104.1	0.5	2.6	20.4	69.5	2.3	5.0
	G18	958.3	83.0	3.2	86.2	44.4	0.5	2.0	2.0	1.9	1.1	109.7	0.5	2.0	17.2	65.0	1.5	4.3
	G6	953.0	77.4	3.0	80.4	43.8	0.5	2.7	2.1	2.6	1.0	113.4	0.5	2.3	18.9	73.6	1.0	5.0
	G8	945.3	82.9	3.1	86.0	41.5	0.5	2.3	1.8	2.1	1.0	103.6	0.5	2.3	24.0	70.2	1.5	4.3
	G51	896.0	80.3	3.0	83.3	45.0	0.6	3.0	2.0	2.1	1.0	100.4	0.5	2.5	20.3	65.4	1.0	4.0
	G1	894.3	83.4	2.9	86.3	46.8	0.5	2.3	1.3	2.2	1.0	109.8	0.5	2.4	22.7	62.0	2.2	4.3
	G20	872.0	79.3	2.9	82.2	43.6	0.6	2.7	2.1	2.1	1.0	110.5	0.5	2.3	23.0	73.4	1.2	4.3
Bottom10 Genotypes	G50	478.0	81.0	2.7	83.7	35.6	0.5	4.0	1.5	2.0	1.0	95.3	0.5	2.3	20.0	50.0	1.5	5.0
	G57	471.7	81.0	3.0	84.0	50.3	0.6	2.0	2.5	1.6	1.0	113.8	1.0	3.0	25.0	63.0	0.5	4.0
	G47	468.3	83.7	2.7	86.3	55.0	0.6	3.0	1.8	2.2	1.0	112.2	0.5	2.8	23.8	72.8	1.0	4.0
	G46	463.7	79.3	2.7	82.0	44.1	0.4	2.0	2.0	2.6	1.0	113.9	1.0	3.0	25.6	35.9	3.0	5.0
	G44	458.3	80.7	2.3	83.0	46.4	0.6	2.0	2.0	2.3	1.0	105.8	0.5	2.8	20.5	57.5	0.5	4.0
	G41	435.0	83.0	3.0	86.0	33.3	0.4	4.0	2.0	2.3	1.5	94.4	0.5	2.0	22.2	65.2	0.5	5.0
	G56	426.3	79.3	3.7	83.0	36.0	0.5	1.0	1.8	1.9	1.0	86.7	0.5	2.0	12.7	62.5	1.0	4.0
	G54	373.3	83.0	2.7	85.7	47.3	0.4	1.0	1.5	1.9	1.0	111.7	0.5	2.0	32.4	69.8	1.0	5.0
	G58	360.3	85.0	2.3	87.3	38.3	0.7	3.0	1.5	2.2	1.0	102.5	1.0	2.0	17.1	61.4	1.0	5.0
	G62	266.0	82.7	3.3	86.0	35.7	0.6	4.0	2.0	1.6	1.0	98.3	0.5	2.0	25.0	61.6	2.5	5.0
	<b>Mean</b>	<b>699.9</b>	<b>81.0</b>	<b>2.9</b>	<b>83.9</b>	<b>41.1</b>	<b>0.5</b>	<b>2.1</b>	<b>1.9</b>	<b>2.0</b>	<b>1.1</b>	<b>105.5</b>	<b>0.6</b>	<b>2.2</b>	<b>22.2</b>	<b>65.7</b>	<b>1.6</b>	<b>4.6</b>
	<b>LSD</b>	<b>293.3</b>	<b>3.1</b>	<b>0.7</b>	<b>0.4</b>	<b>12.9</b>	<b>0.3</b>	<b>0.5</b>	<b>0.8</b>	<b>0.8</b>	<b>0.7</b>	<b>19.3</b>	<b>0.3</b>	<b>0.7</b>	<b>6.9</b>	<b>57.8</b>	<b>1.6</b>	<b>0.6</b>
	<b>MSE</b>	<b>100135</b>	<b>3.7</b>	<b>0.5</b>	<b>4.0</b>	<b>61.4</b>	<b>0.0</b>	<b>0.2</b>	<b>0.2</b>	<b>0.3</b>	<b>0.2</b>	<b>145.1</b>	<b>0.0</b>	<b>0.2</b>	<b>18.3</b>	<b>1295</b>	<b>1.0</b>	<b>0.1</b>
	<b>CV (%)</b>	<b>45.2</b>	<b>2.4</b>	<b>24.6</b>	<b>2.4</b>	<b>19.0</b>	<b>39.3</b>	<b>26.9</b>	<b>25.0</b>	<b>26.0</b>	<b>42.5</b>	<b>11.4</b>	<b>36.4</b>	<b>19.4</b>	<b>19.0</b>	<b>52.1</b>	<b>63.8</b>	<b>7.7</b>
	<b>SE</b>	<b>316.4</b>	<b>1.9</b>	<b>0.7</b>	<b>2.0</b>	<b>7.8</b>	<b>0.2</b>	<b>0.4</b>	<b>0.5</b>	<b>0.5</b>	<b>0.5</b>	<b>12.1</b>	<b>0.2</b>	<b>0.4</b>	<b>4.3</b>	<b>36.0</b>	<b>1.0</b>	<b>0.4</b>
	<b>Min</b>	<b>266.0</b>	<b>77.3</b>	<b>2.1</b>	<b>80.3</b>	<b>28.7</b>	<b>0.4</b>	<b>1.0</b>	<b>1.0</b>	<b>1.0</b>	<b>1.0</b>	<b>86.7</b>	<b>0.5</b>	<b>1.5</b>	<b>12.7</b>	<b>35.9</b>	<b>0.5</b>	<b>4.0</b>
	<b>Max</b>	<b>1040.7</b>	<b>85.7</b>	<b>3.9</b>	<b>88.4</b>	<b>57.7</b>	<b>0.8</b>	<b>4.0</b>	<b>3.0</b>	<b>2.9</b>	<b>3.5</b>	<b>123.2</b>	<b>1.0</b>	<b>3.0</b>	<b>32.4</b>	<b>83.2</b>	<b>3.5</b>	<b>5.0</b>

LSD = Least significant difference, MSE = Mean square error, CV = coefficient of variation, SE = error, Min = minimum, Max = maximum, GY = grain yield (kg ha<sup>-1</sup>), AD = days to anthesis (days), ASI = anthesis-silking interval (days), DS = days to silking (days), EH = ear height (cm), EPP = ears per plant (#), LB = leaf blight disease (1-5), GLS = gray leaf spot disease (1-5), GT = grain texture (1-5), MSV = maize streak virus disease (1-5), PH = plant height (cm), RL = root lodging (#), Rust = rust disease (1-5), SWT = 100 seed weight (g), SH = shelling percentage, SL = stem lodging (#), VIG = vigour (1-5), # = number.

**Table 6.5 Mean performance of diallel crosses at the optimal environments in 2011/12**

	<b>Entry</b>	<b>GY</b>	<b>AD</b>	<b>ASI</b>	<b>DS</b>	<b>EH</b>	<b>EPP</b>	<b>GLS</b>	<b>GT</b>	<b>MSV</b>	<b>PH</b>	<b>RL</b>	<b>RUST</b>	<b>SWT</b>	<b>SH</b>	<b>SL</b>	<b>VIG</b>
Top 10 genotypes	G3	6955	74.0	0.7	74.7	89.4	1.2	1.8	2.8	1.0	173.3	3.0	1.2	42.7	81.9	4.7	1.0
	G10	5386	74.0	1.0	75.0	71.3	1.0	1.0	2.3	1.2	163.8	3.7	1.0	38.6	85.3	1.0	2.3
	G48	5218	74.0	2.0	76.0	85.9	1.1	1.3	2.3	1.0	180.4	5.0	1.5	37.0	80.7	1.3	2.0
	G2	5210	73.0	0.3	73.3	82.7	1.0	2.7	2.3	1.0	178.1	5.7	1.8	38.0	82.7	0.3	2.0
	G8	5099	72.0	0.0	72.0	95.6	1.2	1.2	1.8	1.0	191.7	8.3	1.0	36.3	83.9	2.3	1.0
	G39	5026	75.0	1.3	76.3	98.1	1.0	1.3	2.7	1.0	194.8	4.0	1.0	40.7	83.8	0.3	1.0
	G16	4976	76.0	0.3	76.3	70.8	1.0	1.0	2.3	1.0	124.2	1.3	1.2	38.2	79.9	1.0	1.3
	G45	4713	74.0	2.0	76.0	88.2	1.1	1.0	1.8	1.0	174.1	9.7	1.2	34.0	82.3	0.0	2.3
	G19	4676	72.0	0.3	72.3	92.0	1.0	1.3	2.2	1.0	181.8	5.7	1.0	43.8	86.8	0.7	1.2
	G24	4640	73.3	2.7	76.0	81.1	1.3	1.3	1.8	1.0	168.9	5.0	1.0	37.5	78.3	1.7	1.0
Bottom10 genotypes	G62	2323	74.3	1.7	76.0	106.2	1.1	1.0	1.5	1.0	194.7	4.7	1.0	33.8	80.6	0.3	2.3
	G55	2239	77.0	0.0	77.0	92.9	1.1	1.0	2.2	1.0	186.3	2.7	1.0	31.7	75.6	0.3	2.8
	G31	2142	71.0	0.0	71.0	101.0	1.0	1.7	2.7	1.0	187.7	4.3	1.0	32.5	83.1	0.7	2.3
	G35	1835	76.0	0.7	76.7	95.8	1.2	1.0	1.7	1.2	174.3	10.0	1.2	33.7	74.5	0.0	2.2
	G41	1805	72.0	0.3	72.3	85.5	1.0	1.0	2.5	1.2	170.7	2.3	1.2	29.8	75.1	0.3	3.0
	G60	1622	69.0	1.3	70.3	84.0	1.0	1.2	2.2	1.2	172.7	10.3	1.0	27.7	75.2	0.7	4.2
	G44	1556	76.0	0.7	76.7	122.2	1.1	1.0	2.0	1.0	216.9	1.0	1.0	31.5	72.5	0.3	2.8
	G61	1456	74.0	0.0	74.0	104.8	1.0	1.3	1.7	1.0	190.3	8.0	1.3	24.5	77.0	0.0	4.2
	G43	1381	74.0	0.7	74.7	84.3	1.0	1.0	2.2	1.0	180.2	3.0	1.0	29.5	79.4	0.0	3.5
	G40	757	72.0	3.7	75.7	88.1	0.9	1.0	2.2	1.0	177.9	8.0	1.0	27.9	70.0	0.0	4.5
	<b>Mean</b>	<b>3450.8</b>	<b>73.6</b>	<b>1.0</b>	<b>74.6</b>	<b>91.0</b>	<b>1.1</b>	<b>1.3</b>	<b>2.2</b>	<b>1.0</b>	<b>179.1</b>	<b>5.3</b>	<b>1.1</b>	<b>34.5</b>	<b>79.4</b>	<b>0.8</b>	<b>2.3</b>
	<b>LSD</b>	<b>1649.9</b>	<b>0.8</b>	<b>1.4</b>	<b>1.3</b>	<b>328.2</b>	<b>0.2</b>	<b>0.5</b>	<b>0.8</b>	<b>0.4</b>	<b>38.7</b>	<b>6.7</b>	<b>1.1</b>	<b>10.2</b>	<b>10.5</b>	<b>3.8</b>	<b>1.1</b>
	<b>P</b>	<b>0.001</b>	<b>0.001</b>	<b>0</b>	<b>0</b>	<b>0.63</b>	<b>0.165</b>	<b>0.001</b>	<b>0.36</b>	<b>0.16</b>	<b>0.75</b>	<b>0.002</b>	<b>0.078</b>	<b>0.001</b>	<b>0.05</b>	<b>0.35</b>	<b>0.001</b>
	<b>CV (%)</b>	<b>31.9</b>	<b>0.7</b>	<b>74.7</b>	<b>0.8</b>	<b>19.7</b>	<b>12.4</b>	<b>22.8</b>	<b>23.7</b>	<b>21.3</b>	<b>13.2</b>	<b>82.9</b>	<b>0.2</b>	<b>18.0</b>	<b>8.0</b>	<b>230.1</b>	<b>30.8</b>
	<b>Min</b>	<b>757.0</b>	<b>69.0</b>	<b>0.0</b>	<b>70.3</b>	<b>70.8</b>	<b>0.9</b>	<b>1.0</b>	<b>1.5</b>	<b>1.0</b>	<b>124.2</b>	<b>1.0</b>	<b>1.0</b>	<b>24.5</b>	<b>70.0</b>	<b>0.0</b>	<b>1.0</b>
	<b>Max</b>	<b>6955.0</b>	<b>77.0</b>	<b>3.7</b>	<b>77.0</b>	<b>122.2</b>	<b>1.3</b>	<b>2.7</b>	<b>2.8</b>	<b>1.2</b>	<b>216.9</b>	<b>10.3</b>	<b>1.8</b>	<b>43.8</b>	<b>86.8</b>	<b>4.7</b>	<b>4.5</b>

LSD = Least significant difference, CV = coefficient of variation, Min = minimum, Max = maximum, GY = grain yield (kg ha<sup>-1</sup>), AD = days to anthesis (days), ASI = anthesis-silking interval (days), DS = days to silking (days), EH = ear height (cm), EPP = ears per plant (#), GLS = gray leaf spot disease (1-5), GT = grain texture (1-5), MSV = maize streak virus disease (1-5), PH = plant height (cm), RL = root lodging (#), Rust = rust disease (1-5), SWT = 100 seed weight (g), SH = shelling percentage, SL = stem lodging (#), VIG = vigour (1-5), # = number.

**Table 6.6 Estimated percent reduction for salient phenotypic traits for diallel crosses under low pH versus optimal conditions**

Trait	Low pH	Optimal	Percentage of optimal	% Reduction
GY	699.9	3205.2	21.8	78.2
EH	41.1	92.0	44.7	55.3
EPP	0.5	1.1	45.5	54.5
GT	1.9	2.2	84.7	15.3
PH	105.5	180.9	58.3	41.7
SWT	22.2	34.9	63.7	36.3
SH	65.7	81.0	81.2	18.8
VIG	4.6	2.2	37.5	62.5

GY = grain yield (kg ha<sup>-1</sup>), EH = ear height (cm), EPP = ears per plant (#), GT = grain texture (1-5), PH = plant height (cm), SWT = 100 seed weight (g), SH = shelling percentage, VIG = vigour (1-5), # = number.

**Table 6.7 Genetic variances, phenotypic variances and heritability estimates for the diallel crosses across optimal and three low pH environments in 2011/12**

Trait	Genetic variance ( $\delta^2_g$ )	Phenotypic variance( $\delta^2_p$ )	Heritability( $H^2_b$ )
GY	311866.67	361866.73	0.86
AD	10.67	11.53	0.93
ASI	0.41	0.47	0.87
DS	10.65	11.71	0.91
EH	50.37	65.43	0.77
EPP	0.01	0.07	0.14
LB	0.42	0.49	0.86
GLS	0.27	0.33	0.82
GT	0.2	0.27	0.74
MSV	0.05	0.11	0.45
PH	47.1	67.16	0.70
RL	1.63	1.97	0.83
Rust	0.11	0.17	0.65
SH	31.57	46.63	0.68
SL	1.96	3.03	0.65
SWT	21.33	28.39	0.75
VIG	0.30	0.36	0.83

$\sigma^2_g$  = genotypic variance,  $\sigma^2_p$  = phenotypic variance,  $H^2_b$  = broad sense heritability, GY = grain yield (kg ha<sup>-1</sup>), AD = days to anthesis (days), ASI = anthesis-silking interval (days), DS = days to silking (days), EH = ear height (cm), EPP = ears per plant (#), LB = leaf blight, GLS = gray leaf spot disease (1-5), GT = grain texture (1-5), MSV = maize streak virus disease (1-5), PH = plant height (cm), RL = root lodging (#), Rust = rust disease (1-5), SH = shelling percentage, SL = stem lodging (#), SWT = 100 seed weight (g), VIG = vigour (1-5), # = number

### 6.5.3 Combining ability and inheritance

Combined ANOVA results across all sites are presented in Table 6.8. Environment mean squares were significant for all traits measured except for maize streak virus. Genotype mean squares were significant for all traits excluding root and stem lodging as well as for grain texture and shelling percentage. GCA was significant for grain yield, anthesis date, anthesis-silking interval, days to silking, plant and ear height, and gray leaf spot, leaf blight, maize streak virus, rust, and plant vigour. The interaction of GCA by site was significant for grain yield, anthesis date, root lodging, gray leaf spot, leaf blight, maize streak virus, rust, grain texture, shelling percentage and plant vigour. SCA was significant for all traits measured except shelling percentage. The interaction between SCA effects and site was again significant for all traits apart from number of ears per plant, stem lodging and 100 seed weight. The calculated ratios of GCA/SCA were either higher or closer to 1.0 for the individual traits (Tables 6.8 and 6.9) suggesting the presence of additive and non-additive gene action. Under low pH soil environments (Table 6.9) additive gene action was predominant in the inheritance of grain yield, number of ears per plant, shelling percentage, 100 seed weight and plant vigour because the GCA/SCA ratios were greater than unity while non-additive gene action was predominant in the inheritance of anthesis-silking interval, plant and ear height, grain texture, stem and root lodging, gray leaf spot disease and rust disease. However, the SCA effects were not significant for shelling percentage and ears per plant.

The mean squares due to GCA and SCA were higher under optimal than under low pH conditions and across sites (Table 6.10). However, the mean squares due to GCA and SCA was higher under low pH than under optimal conditions except for root and stem lodging. With respect to the relative contribution of GCA and SCA to total variation, GCA sum of squares for grain yield contributed relatively more to variation under optimal conditions (32.2%) than across environments (28.1%) (Table 6.11). Similarly the sum of squares due to SCA contributed the highest amount of variation under optimal (67.8%) compared to low pH conditions and across sites. A similar trend was observed for other traits. Under low pH the percentage contribution ranged from 0.01 to 9.21 (Table 6.11).

**Table 6.8 Combined analysis of variance for GCA and SCA for diallel crosses for grain yield and other agronomic traits across optimal and three low pH environments in 2012**

Source	Site	Genotypes	GCA	GCA x Site	SCA	SCA x Site	Error	GCA/SCA ratio
DF	3	65	11	33	54	162	195	
GY	833.60**	2.94**	12.40**	1.86**	3.53**	1.44**	0.45	3.51
AD	345.02**	3.92**	4.36**	3.90**	4.87**	6.20**	10.16	0.90
ASI	81605.70**	8.35**	16.43**	-0.70	13.31**	7.53**	3.51	1.23
DS	258820**	11.10**	109.24**	-18.89	36.38**	5.66**	1.24	3.00
PH	1709.46**	1.93**	1.74**	1.77	2.13**	1.76**	279.90	0.82
EH	1132.16**	2.54**	1.76**	1.92	2.15**	1.89**	132.80	0.82
EPP	38.90**	1.58**	1.66	-7.40	1.71**	-18.16	89.27	0.97
RL	118.80**	1.24	1.23	1.83**	1.89**	1.46**	7.65	0.65
SL	44.70**	1.27	1.61	1.69	1.17*	1.10	2.00	1.38
GLS	429.14**	4.91**	5.72**	5.49**	5.38**	3.62**	0.19	1.06
LB	251.47**	3.76**	3.86**	4.88**	3.70**	3.70**	0.32	1.04
MSV	0.02	1.72**	2.17**	3.94**	1.85**	2.58**	0.015	1.17
RUST	1394.02**	3.09**	3.76**	2.12**	3.30**	2.94**	0.15***	1.14
GT	28.55**	1.01	1.05	1.76**	1.46*	1.47**	3.92	0.72
SH	36.10**	1.29	1.61	2.16**	1.17	1.35**	599.50	1.38
SWT	277.60**	1.68**	1.12	0.450	2.30**	0.48	44.76	0.49
VIG	1316.1**	2.38**	1.93*	3.19**	2.91**	3.24**	0.43	0.66

\*\*\*P≤0.001; \*\*P≤0.01; \*P≤0.05; GY = grain yield (kg ha<sup>-1</sup>), AD = days to anthesis (days), ASI = anthesis-silking interval (days), DS = days to silking (days), PH = plant height (cm), EH = ear height (cm), EPP = ears per plant (#), RL = root lodging (#), SL = stem lodging (#), GLS = gray leaf spot disease (1-5), LB = leaf blight disease, MSV = maize streak virus disease (1-5), Rust = rust disease (1-5), GT = grain texture (1-5), SH = shelling percentage, SWT = 100 seed weight (g), VIG = vigour (1-5), # = number.

**Table 6.9 Combined analysis of variance for GCA and SCA for diallel crosses for grain yield and other agronomic traits across three low pH environments in 2012**

Source	Site	Genotypes	GCA	GCA x Site	SCA	SCA x Site	Error	GCA/SCA Ratio
DF	2	65	11	22	54	108		
GY	223.70**	0.48**	1.09**	0.32**	0.57*	0.05*	0.18	1.92
AD	573.40**	43.88**	45.70**	53.50**	44.30**	89.30**	11.34	1.03
ASI	379684.90**	36.59**	16.00**	13.95**	44.20**	38.40**	3.65	0.36
DS	471947.80**	28.80**	52.70**	29.90**	34.98**	34.40**	7.44	1.51
PH	331735.00**	601.70**	154.57	686.00**	686.70**	503.90**	172.90	0.23
EH	49538.45**	367.60**	307.40**	150.70**	379.70**	203.60**	62.37	0.81
EPP	159.00**	35.70	40.00	54.00*	34.90	41.45	32.68	1.15
RL	44.40**	2.94**	2.16**	0.97	3.15**	1.50**	7.70	0.69
SL	172.70**	0.56**	0.18	0.27	0.64**	0.31**	2.00	0.28
GLS	115.10**	0.92**	0.80*	0.76**	0.96**	0.47**	0.21	0.83
LB	114.96**	1.036**	1.23**	1.32**	1.01**	0.68**	0.33	1.22
MSV	0.20**	0.17**	0.28**	0.24**	0.15**	0.19**	0.03	1.87
Rust	317.50**	2.50**	1.60	2.40*	2.70**	1.90*	1.40	0.59
GT	152.00**	0.99**	0.80**	0.60**	1.06**	0.60**	0.49	0.75
SH	2679.30*	877.93	851.16	-315.60	839.61	-357.79	749.20	1.01
SWT	5964.70**	76.39**	120.42**	-50.39	74.15**	-21.60	22.39	1.62
VIG	429.10**	2.16**	61.23**	1.97**	45.39*	1.98**	1.80	1.35

\*\*P<0.01; \*P<0.05; GY = grain yield (kg ha<sup>-1</sup>), AD = days to anthesis (days), ASI = anthesis-silking interval (days), DS = days to silking (days), PH = plant height (cm), EH = ear height (cm), EPP = ears per plant (#), RL = root lodging (#), SL = stem lodging (#), GLS = gray leaf spot disease (1-5), LB = leaf blight disease, MSV = maize streak virus disease (1-5), Rust = rust disease (1-5), GT = grain texture (1-5), SH = shelling percentage, SWT = 100 seed weight (g), VIG = vigour (1-5), # = number.

**Table 6.10 Mean squares for GCA and SCA effects under different environments**

Trait	Across		Optimal		Low pH	
	GCA	SCA	GCA	SCA	GCA	SCA
GY	6.19**	1.76**	2423935.00**	1039389.32**	1.09**	0.57*
AD	43.63**	49.41**	6.23**	6.96**	45.70**	44.30**
ASI	57.76**	46.70**	0.71**	0.82**	16.00**	44.20**
DS	135.50	45.13**	7.77**	6.48**	52.7**	34.98**
EH	234.33	284.98**	107.00	100.53	154.57	34.90
EPP	148.24	152.50**	0.01	0.01	40.00	34.90
LB	1.25**	1.51**	0.49	0.73**	1.23**	1.01**
GLS	1.08**	1.02**	0.30**	0.17**	0.80*	0.96**
GT	4.11	5.74**	0.11	0.10	0.99**	1.06**
MSV	0.33**	0.28**	0.02	0.02	0.28**	0.15**
PH	488.42	596.17**	190.78	159.43	601.70**	686.00**
RL	9.41	14.46**	11.23*	10.26**	2.16**	3.15**
RUST	0.58**	0.51	0.02	0.03	2.50**	2.70**
SWT	50.18	103.27**	18.40	39.90	120.40**	74.2**
SH	964.10	704.60**	32.96**	17.05	851.16	839.61
SL	3.24	2.34	3.05	1.82	0.18	0.64**
VIG	0.84**	1.26**	0.68	0.72**	61.23**	45.39**

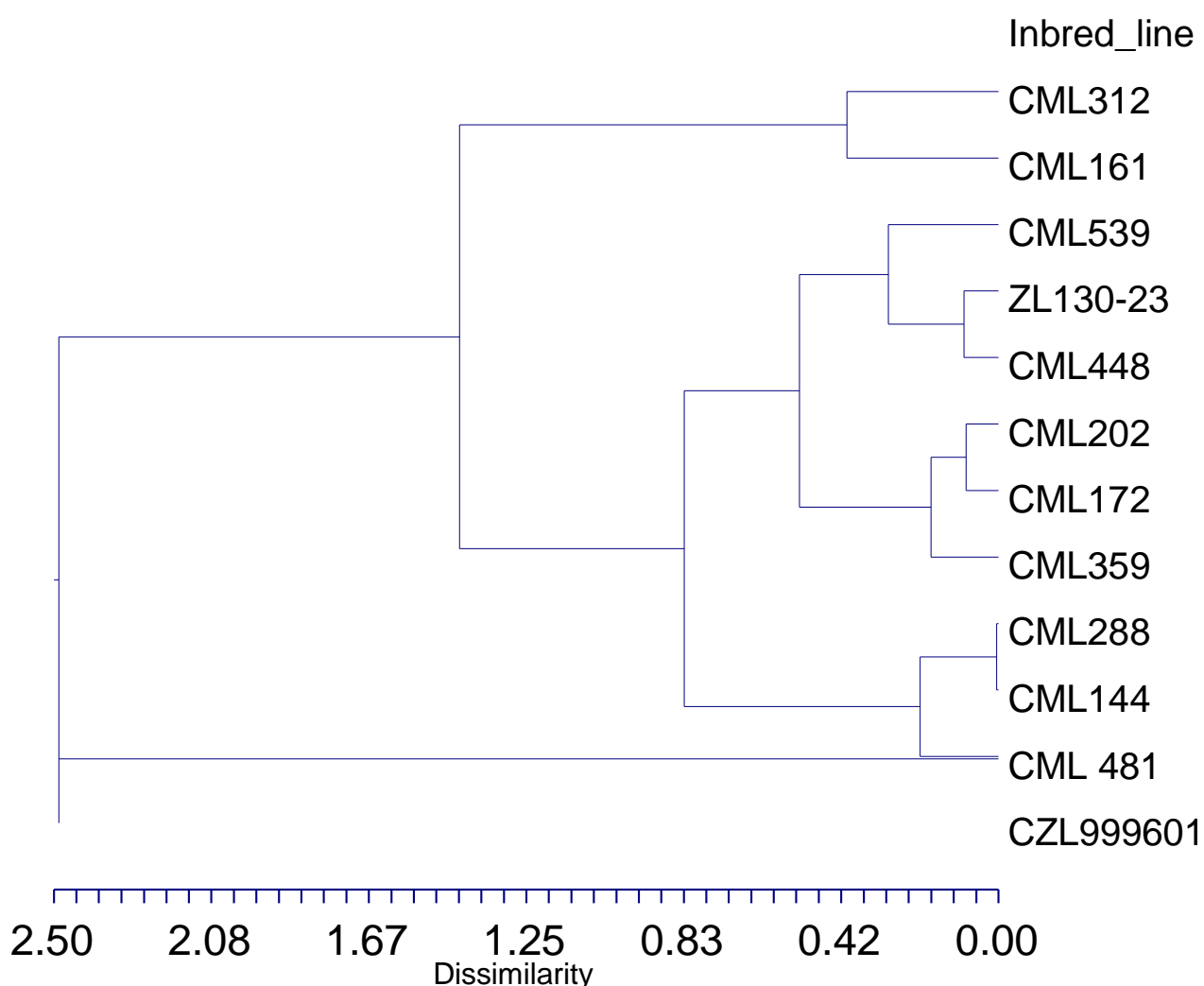
\*\*P≤0.01; \*P≤0.05; GSA = general combining ability, SCA = specific combining ability, GY = grain yield (kg ha<sup>-1</sup>), AD = days to anthesis (days), ASI = anthesis-silking interval (days), DS = days to silking (days), EH = ear height (cm), EPP = ears per plant (#), LB = leaf blight disease, GLS = gray leaf spot disease (1-5), GT = grain texture (1-5), MSV = maize streak virus disease (1-5), PH = plant height (cm), RL = root lodging (#), Rust = rust disease (1-5), SWT = 100 seed weight (g), SH = shelling percentage, SL = stem lodging (#), VIG = vigour (1-5), # = number.

**Table 6.11 Relative percent contribution of sum of squares for GCA and SCA to total sum of squares across environments**

Trait	Environment	Sum of squares		Percentage of		Percentage of	
		GCA	Total SS	total SS	SCA	Total SS	total SS
<b>GY</b>	Across	68.10	242.00	28.10	95.10	242.00	39.30
	Optimal	26663289.00	82760312.20	32.20	56127023.00	82760312.20	67.80
	Low pH	1.09	54.96	2.00	0.57	54.96	1.03
<b>EH</b>	Across	2577.70	67027.00	3.80	15389.00	67027.00	23.00
	Optimal	1177.20	6605.90	17.80	5428.70	6605.90	82.20
	Low pH	307.40	8915.74	3.44	379.70	8915.74	4.30
<b>EPP</b>	Across	1630.60	6680.30	24.40	8236.20	6680.30	123.30
	Optimal	0.10	0.50	17.40	0.40	0.50	82.80
	Low pH	40.00	8003.60	0.45	34.90	8003.60	0.01
<b>GT</b>	Across	45.20	1519.30	3.00	309.80	1519.30	20.40
	Optimal	1.20	6.40	19.30	5.20	6.40	80.70
	Low pH	0.80	8766.00	0.50	1.06	8766.00	0.01
<b>PH</b>	Across	5372.60	133576.10	4.00	32192.90	133576.10	24.10
	Optimal	2098.60	10707.80	19.60	8609.30	10707.80	80.40
	Low pH	154.57	108297.00	0.14	686.70	108297.00	0.63
<b>SWT</b>	Across	552.00	10894.20	5.10	5576.40	10894.20	51.20
	Optimal	202.40	2357.20	8.60	2154.80	2357.20	91.40
	Low pH	120.42	8770.00	1.37	74.15	8770.00	0.85
<b>SH</b>	Across	10605.10	222803.70	4.80	38049.10	222803.70	0.40
	Optimal	362.50	1282.90	28.30	920.40	1282.90	71.70
	Low pH	851.16	9116.80	9.34	839.61	9116.80	9.210
<b>VIG</b>	Across	9.20	168.20	5.50	68.00	168.20	40.40
	Optimal	7.50	46.20	16.20	38.70	46.20	83.80
	Low pH	61.23	5597.50	1.09	45.39	5597.50	0.81

GY = grain yield (kg ha<sup>-1</sup>), EH = ear height (cm), EPP = ears per plant (#), GT = grain texture (1-5), PH = plant height (cm), SWT = 100 seed weight (g), SH = shelling percentage, VIG = vigour (1-5).

Twelve maize inbred lines were clustered based on the GCA effects for grain yield. Two main clusters were observed (Figure 6.1) with a high cophenetic correlation of  $r_{cop} = 0.87$ . The pattern mostly followed the origin of the maize inbred lines such that six out of seven inbred lines from CIMMYT-Colombia (tropical) were grouped in the second cluster. The first cluster comprised of inbred lines with low GCA for grain yield while the second cluster was comprised of inbred lines of medium GCA and the third cluster had CZL999601 which had the highest GCA for grain yield.



**Figure 6.1 Dendrogram of 12 maize inbred lines based on GCA effects for grain yield across four environments for 2011/12 season**

### **6.5.3.1 Estimated general combining ability effects for 12 inbred lines for grain yield and agronomic traits across low pH and optimal environments in 2011/12**

A positive, high and significant GCA effect for yield was recorded for inbred line CZL999601 (0.55) (Table 6.12). Also positive and high but not significant values were observed for grain yield for inbred lines by CML481 (0.18) followed by CML144 (0.13) and CML 288 (0.13) and significant negative GCA was recorded for inbred line CML161 (-0.38). For days to anthesis positive and significant GCA values were observed for lines ZL130-23 (1.2) and CML312 (1.15). Negative and highly significant GCA was recorded for inbred line CML288 (-0.73). Selecting for earliness to maturity should be done by selecting inbred lines with low and negative GCA effects. Positive and high values for GCA effects were observed for anthesis-silking interval for inbred lines CML288 (1.06) and CML481 (0.75). Negative and lowest GCA values were recorded for inbred lines CML161 (-1.1) and CML172 (-0.9). For stress tolerance breeding, lines with negative GCA for this trait would be preferred.

Positive and high GCA effects were observed for number of ears per plant in lines CZL999601 (1.26), CML481 (1.24), ZL130-23 (1.12) and CML288 (1.08). When selecting for prolificacy, lines with high and positive GCA for this trait would be preferred. Positive and high GCA effects were observed for grain texture for inbred lines ZL130-23 (0.36) followed by CML312 (0.17). When selecting for flint and semi-flintiness, lines with negative GCA should be selected since a score of 1 is better than a score of 5 in 1-5 scale. In terms of diseases, positive and high GCA effects were observed for gray leaf spot for lines CML172 (0.15), CML288 (0.13) and CML202 (0.11). Inbred line CML448 (-0.16) had negative and significant GCA. Selecting for tolerance to this disease should be done by selecting lines with negative GCA. Positive and significant GCA effects were observed for MSV in lines CML288 (0.10). Positive GCAs values were also observed for inbred lines CML161 (0.08) and CML35 (0.08). The lowest and negative GCA was observed for lines (CML202 (-0.08), CZL999601 (-0.06) and CML539 (-0.04). Selecting lines for tolerance to this disease could be achieved by selection lines with negative GCA. For leaf blight disease, high and positive GCA's were recorded for inbred lines CML172 (1.7) followed by CML481 (1.2) and the lowest GCA was recorded for line CML539 (-3.3).

**Table 6.12 Estimated general combining ability effects for 12 inbred lines for grain yield and agronomic traits across low pH and optimal environments in 2011/12**

Line	Name	GY	AD	ASI	DS	EH	EPP	GLS	GT	LB	MSV	PH	RL	Rust	SWT	SH	SL	VIG
G1	CZL999601	0.55**	-0.21	-0.17	-0.53	-0.43	1.26	0.05	-0.13	-0.4	-0.06	0.71	0.18	0.04	0.68	5.19	0.22	-0.04
G2	CML481	0.18	-0.26	0.75	-1.26	1.21	1.24	-0.02	-0.24	1.2	-0.04	1.25	0.39	-0.08	-0.25	-3.31	0.38*	-0.2
G3	CML359	0.04	-0.63	0.6	-0.18	0.44	0.43	-0.03	-0.05	0.4	0.08	3.42	-0.21	-0.03	-0.48	-4.12	0.23	-0.06
G4	CML144	0.13	-0.32	0.69	-1.25	1.04	0.29	0.03	-0.28	1.0	-0.02	1.19	-0.36	-0.06	0.96	-0.34	0.02	-0.05
G5	CML161	-0.38**	0.12	-1.1	0.93	0.69	-1.21	0.01	0.22	0.7	0.08	-1.62	-0.23	-0.06	-0.51	-0.18	-0.16	0.16
G6	CML172	-0.02	-0.13	-0.91	1.06	1.69	-1.19	0.15	-0.09	1.7	0.03	-0.58	0.33	0.09	0.56	6.27*	-0.02	-0.06
G7	CML448	-0.15	-0.27	-0.29	-0.08	-1.73	0.48	-0.16*	-0.12	-1.7	-0.04	-2.3	-0.41	-0.04	1.1	-1.81	-0.16	0.05
G8	CML312	-0.28	1.15*	0.45	0.67	-0.38	-2.29	0.00	0.17	-0.4	0.00	-0.16	0.44	0.1	-0.58	1.34	0.07	0.24
G9	ZL130-23	-0.13	1.20*	-0.87	2.01	1.11	1.12	-0.02	0.36	1.1	-0.01	-0.45	-0.06	0.09	-0.45	-0.63	-0.18	0.02
G10	CML288	0.13	-0.73**	1.06	-1.64	-0.9	1.08	0.13	-0.07	-0.9	0.10*	0.97	0.26	-0.15	0.68	-1.6	-0.11	0.07
G11	CML202	0.01	-0.29	0.04	-0.47	0.56	-0.2	0.11	0.05	0.6	-0.08	1.97	-0.43	0.05	-0.62	-1.46	-0.16	-0.01
G12	CML539	-0.07	0.39	-0.24	0.74	-3.31	-1.01	-0.23	0.16	-3.3	-0.04	-45	0.09	0.04	-1.09	0.65	-0.14	-0.11

\*\*P<0.01; \*P<0.05; GY = grain yield (kg ha<sup>-1</sup>), AD = days to anthesis (days), ASI = anthesis-silking interval (days), DS = days to silking (days), EH = ear height (cm), EPP = ears per plant (#), GLS = gray leaf spot disease (1-5), GT = grain texture (1-5), LB = leaf blight disease, MSV = maize streak virus disease (1-5), PH = plant height (cm), RL = root lodging (#), Rust = rust disease (1-5), SWT = 100 seed weight (g), SH = shelling percentage, SL = stem lodging (#) VIG = vigour (1-5), # = number.

Similarly, selecting for tolerance to this disease could be done by selecting lines with negative GCA effects. With respect to rust, positive and high GCA effects were observed for lines CML312 (0.1), CML172 (0.09) and ZL130-23 (0.09). The lowest and negative GCA was observed for line CM288 (-0.15). Selecting lines for tolerance to this disease would be achieved by selecting lines with negative GCA.

High GCA effects were observed for plant height in inbred lines CML359 (3.42), CML202 (1.97), followed by CML481 (1.25). The negative and lowest GCA was observed for line CML539 (-45.0). For ear height a high GCA effects were observed for inbred lines CML172 (1.69) followed by CML481 (1.21). Negative and lowest GCA was observed for line CML539 (-3.31). Where tall varieties are preferred, lines with high and positive GCA could be preferred. Positive and significant value for GCA effects was observed for stem lodging in inbred lines CML481 (0.38), inbred line CML359 (0.23) also had a high value. The lowest and negative GCA values were recorded for lines ZL130-23 (-0.18) followed by CML448, CML202 and CML161 all with a value of -0.16. High GCA effects were observed for root lodging for CML312 (0.44), CML481 (0.39) and CML172 (0.33). The lowest and negative GCA were recorded for lines CML202 (-0.43) and CML448 (-0.41). When selecting for tolerance for both root lodging and stem lodging, lines with negative GCA would be preferred. High GCA effects were observed for plant vigour for inbred lines CML312 (0.24), CM161 (0.16), and negative and lowest GCA was recorded for CML539 (-0.11). In terms of 100 seed weight, high GCA effects were observed for inbred lines CML448 (1.1), CML144 (0.96) and CML288 (0.68). The lowest and negative GCA values were observed for lines CML539 (-0.09), CML202 (-0.62). With respect to shelling percentage, high GCA effects were observed for inbred line CML172 (6.27), followed by CZL999601 (5.19). The lowest and negative GCA was observed for lines CML359 (-4.12) and CML481 (-3.3). For plant vigour, 100 seed weight, and shelling percentage, inbred lines with higher and positive GCAs would be preferred.

### **6.5.3.2 Estimated specific combining ability effects for 12 inbred lines for grain yield and agronomic traits across low pH and optimal environments in 2011/12**

Results for the estimated SCA effects in the diallel crosses are presented in Appendix 12. The promising specific combinations for grain yield were CZL999601/CML144 (0.94), CML144/CML202 (0.73) and CML481/CML288 (0.70). The poorest combinations were CZL130-23/CML202 (-0.65) and CZL999601/CML481 (-0.65). When breeding for high yield, positive SCA values are desirable. For days to 50% pollen shedding, the best specific combinations were CML161/CML288 (4.03), CML144/ZL130-23 (3.8), followed by CML312/CML288 (3.09). The poor combinations were CML359/CM288 (-6.71), CML202/CML539 (-3.7) and CML161/ZL130-23 (-2.56). Selecting for earliness to maturity should be done by selecting inbred lines and hybrids with low and negative GCA and SCA effects, respectively. The highest SCA effects were observed for anthesis-silking interval for crosses and the best combinations were CML161/CML288 (4.14), CML359/CML288 (3.9) and CML144/CML539 (3.39). Low SCA values were recorded for the following hybrids: CML288/CML539 (-3.55), ZL130-23/CML288 (-3.5), CML288/CML202 (-3.12) and CML144/CML161 (-2.9). For stress tolerance, hybrids with negative SCA for this trait would be preferred.

Positive and high SCA effects were observed for number of ears per plant for hybrids CML448/CML288 (16.6) CML144/CML202 (7.4) and ZL130-23/CML288 (6.37) and the poor combinations were CZL999601/CML481 (-5.37), ZL130-23/CML288 (-4.91) and CML481/CML448 (-4.51). When selecting for prolificacy, lines and hybrids with high and positive GCA and SCA for this trait would be preferred. Significant and high SCA effects were observed for grain texture for hybrids ZL130-23/CML539 (2.75), CML161/CML202 (2.51) followed by CML312/ZL130-23 (2.19). The poorest combinations were ZL130-23/CML202 (-0.95), CML481/ZL130-23 (-0.84) and CML161/CML539 (-0.83) but when selecting for flint and semi-flintiness, lines and hybrids with negative GCA and SCA, respectively, could be selected. In terms of diseases, positive and high GCA effects were observed for gray leaf spot for hybrids CZL999601/CML359 (0.86), CM172/ZL130-23 (0.64), and CML144/CML202 (0.62). Hybrids CZL999601/CML202 (-0.61), CML161/CML448 (-0.52) and CZL999601/CML481 (-0.48) had negative SCA. Selecting for tolerance to this disease could be done by selecting lines and hybrids with negative

GCA and SCA, respectively. Positive and high SCA effects were observed for MSV in hybrids CML359/CML288 (0.92), CML161/CML172 (0.47) and CML172/ZL130-23(0.23). The lowest and negative SCA was observed for CML161/CML288 (-0.27), CML359/CML161 (-0.25), and CML359/CML172 (-0.20). Selecting hybrids for tolerance to this disease could be achieved by selection the lines and hybrids with negative GCA and SCA, respectively. For leaf blight disease, high and positive SCA's were recorded for hybrids CML481/CML144 (0.88) and CML172/CML202 (0.83) followed by CML172/CML312 (0.77) and the lowest SCA's were recorded for hybrids CML312/CML202 (-0.6), CML481/CML288 (-0.6) and ZL130-23/CML288 (-0.59). Similarly, selecting for tolerance to this disease could be done by selecting lines and hybrids with negative GCA and SCA effects, respectively. With respect to rust disease, positive and high SCA effects were observed in hybrids CML172/CML448 (0.51), CZL999601/CML359 (0.44) and CML202/CML539 (0.42). The lowest and negative SCA's were observed for hybrid CML448/ZL130-23 (-0.49), CZL999601/CML312 (-0.46) and CML144/CML202 (-0.44). Selecting lines for tolerance to this disease could be achieved by selection hybrids with negative SCA.

Positive and high SCA effects were observed for plant height in hybrids CML144/CML202 (18.64), CML448/CML288 (13.18), followed by CML448/CML202 (12.83). Negative and lowest SCA's were observed for hybrid CML481/CML448 (-16.84), CML202/CML539 (-13.13) and CML144/ZL130-23 (-13.03). For ear height, positive and high SCA effects were observed for hybrids CML448/CML288 (12.49) followed by CML448/CML202 (8.12) and CML172/CML288 (7.96). Negative and lowest SCAs were observed for hybrids CML161/CML312 (-8.88), CML288/CML539 (-8.39) and CML202/CML539 (-8.15). Where tall varieties are preferred, lines and hybrids with high and positive GCA and SCA could be preferred. In terms of lodging, positive and high SCA effects were observed for stem lodging in hybrids CM481/CML359 (2.38) followed by CML202/CML539 (1.24) and CZL999601/CML144 (1.20). The lowest and negative SCA were recorded for hybrids CZL999601/CML481 (-0.72), CZL999601/CML359 (-0.68) and CML481/CML288 (-0.61). Positive and high SCA effects were observed for root lodging for CML172/CML448 (3.29), CML481/CML448 (2.82) and CML144/CML288 (2.35). The lowest and negative SCA were recorded for hybrids CML172/CML539 (-2.60), CML161/CML448 (-0.70) and CML481/CML228 (-1.60). When selecting for tolerance

for both root lodging and stem lodging, inbred lines and hybrids with negative GCA and SCA, respectively could be preferred.

Positive and high SCA effects were observed for plant vigour for hybrids, Z130-23/CML288 (1.08), CML312/CML539 (1.04), CZL999601/CML448 (0.99) and negative and lowest SCA's were recorded for hybrids CZL999601/ZL130-23 (-0.70), CML172/CML481 (-0.60) and CML481/ZL130-23 (-0.60). In terms of 100 seed weight, positive and high SCA effects were observed for hybrid CML288/CML539 (9.44). Also high and positive values were observed for inbred lines CML312/CML202 (5.84), CML359/CML161 (5.57), and CML448/ZL130-23 (5.04). The lowest and negative SCA were observed for hybrids ZL130-23/CML539 (-6.05), CML448/CML539 (-4.86) and CML161/CML202 (-4.81). With respect to shelling percentage, positive and high SCA effects were observed for hybrids, CZL999601/CML172 (41.02) followed by CML481/CML144 (10.49) and CML288/CML539 (9.86). The lowest and negative SCAs were observed for hybrids CML172/CML448 (-15.15), CML448/CML288 (-12.54) and CZL999601/CML539 (-10.72). For plant vigour, 100 seed weight, and shelling percentage inbred lines and hybrids with higher and positive GCA's and SCA's, respectively, would be preferred.

#### **6.5.4 Pearson's correlation coefficients for diallel crosses between grain yield and other agronomic traits at optimal environment**

Pearson correlation was carried out to study the associations between grain yield and some agronomic traits as well as among themselves. The results are presented in Table 6.13. Grain yield was positively and significantly correlated with anthesis date (0.23), days to 50% silking (0.22) and highly significantly correlated with 100 seed weight (0.31). It was highly significantly negatively correlated with leaf blight disease ( $r = -0.29$ ). In terms of diseases, gray leaf spot was positively and highly significantly correlated with rust ( $r = 0.34$ ). Plant vigour showed positive and significant correlation with leaf blight disease (0.40). Days to 50% pollen shed (days to anthesis) was highly significantly and positively correlated with 100 seed weight across optimal ( $r = 0.27$ ).

**Table 6.13 Pearson's correlations coefficients under optimal conditions**

	GY	AD	ASI	DS	PH	EH	RL	SL	EPP	SH	GT	LB	GLS	Rust	MSV	VIG
AD	0.23*															
ASI	-0.01	-0.16														
DS	0.22*	0.90**	0.25*													
PH	-0.19*	-0.07	0.08	-0.04												
EH	-0.11	-0.03	0.01	-0.03	0.80**											
RL	-0.03	-0.02	0.03	0.00	-0.04	-0.1										
SL	0.05	-0.01	-0.08	-0.04	-0.12	-0.1	-0.13									
EPP	-0.14	-0.01	-0.07	-0.04	-0.06	-0.06	0.03	0.05								
SH	0.20	-0.26**	-0.03	-0.27**	-0.13	-0.12	0.12	0.00	-0.06							
GT	-0.10	-0.01	-0.04	-0.03	-0.01	-0.01	-0.12	0.05	-0.01	0.02						
LB	-0.29**	-0.11	-0.03	-0.12	0.07	0.08	0.19	0.17	0.02	0.09	-0.01					
GLS	0.10	-0.26**	0.01	-0.25	0.01	-0.05	0.08	0.00	-0.06	0.16	0.23	-0.06				
Rust	0.06	0.02	0.00	0.02	0.04	0.00	0.03	0.23	0.01	0.11	0.11	-0.05	0.34**			
MSV	-0.18	0.11	-0.06	0.09	-0.05	-0.12	0.16	-0.04	0.04	0.02	-0.01	0.21	-0.05	-0.06		
VIG	-0.49**	-0.14	-0.02	-0.14	0.12	0.04	0.06	0.06	0.15	-0.09	0.15	0.40**	-0.22	0.00	0.24	
SWT	0.31**	0.27**	-0.06	0.24	-0.06	-0.05	-0.21	0.11	0.16	-0.03	0.07	-0.24	-0.02	-0.02	-0.05	-0.29**

\*\*P<0.01; GY = grain yield (kg ha<sup>-1</sup>), AD = days to anthesis (days), ASI = anthesis-silking interval (days), DS = days to silking (days), PH = plant height (cm), EH= ear height (cm), RL = root lodging (#), SL= stem lodging (#), EPP = ears per plant (#), GLS = gray leaf spot disease (1-5), GT = grain texture (1-5), LB = leaf blight disease, MSV = maize streak virus disease (1-5), Rust = rust disease (1-5), SWT = 100 seed weight (g), SH = shelling percentage, VIG = vigour (1-5), # = number.

### 6.5.5 Pearson's correlation coefficients for diallel crosses between grain yield and other agronomic traits at low pH environments

Results are given in Table 6.14. Grain yield was positively and significantly correlated with shelling percentage ( $r = 0.28$ ), 100 seed weight ( $r = 0.57$ ), plant height ( $r = 0.81$ ), ear height ( $r = 0.64$ ), stem lodging ( $r = 0.35$ ) and grain texture ( $r = 0.34$ ). GY was negatively but significantly correlated with anthesis date ( $r = -0.31$ ), anthesis-silking interval ( $r = 0.70$ ), root lodging ( $r = -0.32$ ), and plant vigour ( $r = -0.46$ ). With respect to plant vigour, the lower the score the better the plant vigour while the higher the grain weight the higher the yield. So with correlation analysis this is negative association while in real sense genotypes with high plant vigor (low score) gives higher yield and this is a positive association. Correlation among other traits showed that plant height was correlated with ear height ( $r = 0.85$ ), 100 seed weight ( $r = 0.59$ ) but was negatively and significantly correlated with plant vigour ( $r = -0.46$ ), root lodging ( $r = -0.26$ ). Grain texture was positively and significantly correlated with shelling percentage ( $r = 0.36$ ), 100 seed weight ( $r = 0.3$ ). It was negatively correlated with plant vigour ( $r = -0.52$ ), and rust disease ( $r = -0.37$ ). Shelling percentage was positively and significantly correlated with 100 seed weight ( $r = 0.54$ ), but was negatively and significantly correlated with root lodging ( $r = -0.30$ ), rust disease ( $r = -0.23$ ), and plant vigour ( $r = -0.4$ ).

## 6.6 Discussion

Effective selection of inbred lines for the production of maize hybrids requires prior information of the inbred line *per se* and the behaviour of the line in a particular hybrid combination. Malawi has a humid sub-tropical climate, while Colombia has a tropical climate. In this study, both inter and intra-cross combinations from tropical and sub-tropical maize inbred lines indicated good yield performance under optimal condition and across sites. The top yielding hybrid was a cross between sub-tropical and tropical inbred lines. Under low pH conditions, the top yielding hybrid was a cross involving tropical inbred lines, suggesting that tropical germplasm was more tolerant to low pH than sub-tropical germplasm. The results were similar to what was reported by Magnavaca *et al.* (1987) who reported that Brazilian maize inbred lines (tropical) were generally more

**Table 6.14 Pearson's correlation coefficients and level of significance under low pH for diallel crosses**

	GY	AD	ASI	DS	PH	EH	RL	SL	GT	LB	GLS	Rust	MSV	VIG	SWT	EPP
AD	-0.31**															
ASI	-0.70**	0.25**														
DS	-0.48**	0.96**	0.50**													
PH	0.81**	-0.20**	-0.68**	-0.35**												
EH	0.64**	-0.10**	-0.47**	-0.25**	0.85**											
RL	-0.32**	0.13**	0.56**	0.27**	-0.26**	-0.02										
SL	0.35**	-0.09	-0.05	-0.10*	0.34**	0.33**	0.33**									
GT	0.34**	-0.09	-0.39**	-0.19**	0.25**	0.07	-0.47**	-0.16**								
LB	0.66**	-0.20**	-0.42**	-0.26**	0.63**	0.60**	0.12**	0.47**	0.09							
GLS	0.64**	-0.20**	-0.36**	-0.25**	0.65**	0.69**	0.19**	0.42**	0.03	0.77**						
Rust	-0.05	0.06	0.38**	0.16**	-0.04	0.22**	0.79**	0.38**	-0.37**	0.33**	0.41**					
MSV	0.34**	-0.06	-0.04	-0.06	0.30**	0.42**	0.40**	0.39**	-0.11	0.57**	0.64**	0.60**				
VIG	-0.46**	0.13**	0.69**	0.31**	-0.46**	-0.15**	0.86**	0.23**	-0.52**	-0.03	0.04	0.82**	0.36**			
SWT	0.57**	-0.20**	-0.50**	-0.28**	0.59**	0.45**	-0.23**	0.19**	0.30**	0.43**	0.44**	-0.10	0.20**	-0.40**		
EPP	0.04	0.40**	0.01	0.36**	0.16**	0.14**	-0.01	0.05	0.07	0.00	0.04	0.01	0.02	-0.08	-0.02	
SH	0.28**	-0.03	-0.28**	-0.10*	0.29**	0.19**	-0.30**	0.01	0.36**	0.11	0.08	-0.23**	-0.03	-0.40**	0.54**	0.14**

\*\*P<0.01; GY = grain yield (kg ha<sup>-1</sup>), AD = days to anthesis (days), ASI = anthesis-silking interval (days), DS = days to silking (days), PH = plant height (cm), EH= ear height (cm), RL = root lodging (#), SL= stem lodging (#), EPP = ears per plant (#), GLS = gray leaf spot disease (1-5), GT = grain texture (1-5), LB = leaf blight disease, MSV = maize streak virus disease (1-5), Rust = rust disease (1-5), SWT = 100 seed weight (g), SH = shelling percentage, VIG = vigour (1-5), # = number.

tolerant than USA inbred lines to Al effects. In terms of grain yield for the hybrids *per se*, the results were similar to what was reported by Pérez (2008).

Maize yields were greatly reduced across low pH sites (78.2%), followed by plant vigour (62.5%) and ear height (55.3%). The results for grain yield reduction were consistent with findings from other researchers. Welcker *et al.* (2005) reported that soil acidity reduces maize yields by up to 70% on 8 million hectares in developing countries and that on these soils maize yield is reduced due to Al or Mn toxicity and Ca, Mg, P and Mo deficiencies (Aldrich *et al.*, 1975; Granados *et al.*, 1993).

The results for combining ability and inheritance studies showed that both additive and non-additive gene action were involved and this is consistent with what was reported by Badawy (2013) who indicated that the ratio of GCA and SCA revealed the presence of additive and non-additive types of gene action. The concept of the ratio of GCA/SCA was described by Baker (1978) that when this ratio is more than a unit, there is preponderance of additive gene action, but if it is less than a unit the preponderance is towards non-additive gene action. However, the results were not consistent with what was reported by Iqbal *et al.* (2007) who indicated that none of the cross combinations exhibited desirable significant SCA effects for all the characters measured. In this study higher GCA than SCA effects for grain yield were observed in both sub-tropical and tropical inbred lines CZL999601 and CML481, respectively. In addition these two lines had good GCA effects for other traits, for instance, CZL999601 was also a good general combiner for number of ears per plant and shelling percentage while CML481 was also a good general combiner for traits such as ear height, ears per plant and plant height. While for other traits some inbred lines showed higher GCA effects for plant vigour (CML312), 100 seed weight (CML448) and shelling percentage (CML172), gray leaf spot (CML202) as well as anthesis dates (ZL130-23) which were either sub-tropical in the case of CML312, CML202 and ZL130-23 or tropical inbred lines in the case of CML448 and CML172. This suggested the breeding of DC hybrids as an option to combine a number of important traits for low pH tolerance.

At a cut of point of 1.0, the UPGMA clustered the inbred lines into two main clusters through use of GCA effects for the inbred lines for grain yield with Euclidean distance and standard deviation as scale type. At a high cophenetic correlation of  $r_{\text{cop}} = 0.87$ , the pattern

mostly followed the origin of the maize inbred lines such that six out of seven inbred lines from CIMMYT-Colombia (tropical) were grouped in the second cluster. Srdic *et al.* (2007) reported similar results, that cluster analysis using SCA effects was in good agreement with the origin of ten inbred lines, and was very useful in confirming predicted heterotic patterns.

With respect to SCA, the higher specific combinations for yield, plant vigour and 100 seed weight were observed in inter-crosses; CZL999601/CML144 and CML144/CML202. The former had a negative SCA for gray leaf spot disease, stem lodging implying that it was tolerant while the latter was also a good specific combination for plant height, ear height and number of ears per plant. This also suggested the breeding of DC hybrids as an option to combine a number of important traits for low pH tolerance which could broaden the genetic base.

It was found that under stress the relationship between characteristics is affected by the environment at phenotypic level (Chaubey and Singh, 1994; Ojo *et al.*, 2006). In this study, grain yield at optimal environment was positively and significantly correlated with anthesis date, shelling percentage, stem lodging and 100 seed weight. Late maturing genotypes have adequate time to accumulate carbohydrates, leading to high yields. Shelling percentage and 100 seed weight are yield related traits such that high yielding genotypes tend to show high values for these traits. These results were consistent with what was reported by Chinnadurai and Nagarajan (2011), while under low pH grain yield was negatively correlated with anthesis date. The results under low pH were consistent with findings from other researchers (Magorokosho *et al.*, 2003; Kaonga *et al.*, 2007; Ndhela, 2012) who reported negative correlation under drought stress. Under stress silk emergence is delayed, increasing the anthesis-silking interval (Kaonga and Nhlane, 2004). In the present study, it could be due to cold stress associated with low pH sites so that the plants require a higher number of days to attain the required heat units (day degree concept) which delays flowering but is not related to the amount of carbohydrate accumulation in the grain as is the case with late maturing maize genotypes.

Grain yield was negatively correlated with root lodging and leaf blight disease. In the case of root lodging, Al toxicity causes short, thick and under developed roots and plants, thus

reducing nutrient uptake (Sasaki *et al.*, 1996). Other findings indicate that Al toxicity is the main problem because it inhibits maize root growth, reducing the water and nutrient uptake and interferes in different physiological processes of crop development (Roy *et al.*, 1988). Foy *et al.* (1978) reported that low pH contributes to solubilise Al and make it available in the soil for plant assimilation, causing severe damage to non-adapted genotypes. In this case highly susceptible varieties are likely to have low yield and be susceptible to lodging. Diseases affect photosynthetic area and result in low carbohydrate accumulation in the grain, hence the negative correlation.

Correlation among the traits showed that grain texture was significantly and positively correlated with shelling percentage but was negatively and significantly correlated with plant vigour. Grain texture, refers to hardness (flintiness) or softness (dent) of the kernel. Flint kernels have higher percentage of amylopectin starch formed by branched chain of glucose molecules of high molecular weight. Branching reinforces the caryopsis while soft kernels have relatively higher percentage of amylose starch formed by straight chain of glucose molecules. Regular corn contains 72-76% amylopectin and 24-28% amylose (International Starch Institute, 2001). In terms of diseases, gray leaf spot was positively and significantly correlated with rust. This is a common scenario where different disease pathogens tend to occur together and this calls for breeding for horizontal resistance through the use of inbred lines with negative GCA for such diseases. Plant vigour was positively correlated with leaf blight at optimal conditions but was significantly and negatively correlated with 100 seed weight. In other words it was positively correlated with 100 seed weight as a score of 1.0 for plant vigour is better than a score of 5. The results are consistent with Welcker *et al.* (2005) who reported positive correlation under acid soils. Plant vigour is likely to have been affected in a similar manner to grain yield under low pH soils such that hybrids that had good vigour produced relatively better yields than those with low vigour. Low plant vigour under low pH results in low biomass. Kochian *et al.* (2005) indicated symptoms of Al toxicity as reductions in biomass and the number as well as the length of the roots, often combined with an increase in the mean radius and root volume; and the uptake of water and mineral nutrients, resulting in severe losses of root elongation and ultimately productivity.

## 6.7 Conclusions

Low pH soil is one of the most important abiotic factors contributing to low maize yields in some parts of Malawi. In this study, inbred lines CZL999601 and CML481 were identified to have higher GCA for grain yield. Inbred lines CML312, CML448, CML172, and CML202 were also identified to have the desired GCA for salient traits like plant vigour, 100 seed weight, shelling percentage and gray leaf spot disease respectively. Single cross hybrids CML99601/CML144, CML144/CML202, CML481/ CML288 and CML161/CM172 were identified as good specific combinations for grain yield and could be used in the formation of DCs which are more resilient to stress. The inbred lines and specific combinations identified in this study will be used in the development of maize hybrids and synthetics tolerant to low pH and diseases.

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## CHAPTER 7

### General conclusions and recommendations

Maize is the number one food crop in Malawi with an annual requirement of 2.4 million tons per year. Its production is constrained by a number of abiotic factors and most of them are edaphic factors like low pH soils. This study looked at the performance of maize genotypes from different genetic backgrounds for tolerance to acid soils. The genotypes were obtained from two breeding institutions in the tropical and sub-tropical regions. Two approaches were employed to evaluate their performance under low pH soil environments. These involved the use of potassium aluminium sulphate [ $KAl(SO_4)_2$ ] in a nutrient solution in a glasshouse hydroponic experiment and field trials. Significant differences were observed among the genotypes tested using  $KAl(SO_4)_2$  such that IWDC3SYNF2-B, VPO52 and LPHpop4 were found to be tolerant to low pH. The field trial results indicated that low pH soils made a significant contribution to the yield and yield component reduction of 69.9%. Some genotypes that were among the top ten for field trials were also listed among the top ten in the hydroponic experiment. Phenotypic traits associated with grain yield, like plant vigour, 100 seed weight and numbers of ears per plant were negatively affected by the low pH soil environment and can be used alongside grain yield when selecting maize genotypes which are tolerant to acid soils. Soil analysis identified Lunyangwa Research Site soils as the most acidic of the low pH sites characterised. However, Tsangano Research Site was at a higher altitude than Lunyangwa (1524 masl versus 1342 masl) and was cooler than the latter (28.8°C versus 32.0°C max temperatures). In view of this, dendrogram clustering based on environmental means generated by AMMI identified Tsangano as the most stressed site of the two most acidic sites, i.e., Tsangano and Lunyanwa. As a recommendation, field trials should be accompanied by glasshouse screening for effective selection for tolerance to acid soils bearing in mind the complexity of the field environment.

With respect to GxE and stability analysis, genotypes LPHpop21, VPO52, VPO72, VPO744 and VPO96 were stable across low pH and optimal soil conditions. While genotype VPO97 was identified as the most unstable genotype. Chitala optimal low-land site was identified as the most discriminating environment for the genotypes as it was

located at the longest distance between its marker and the origin on the GGE biplot. Chitedze optimal was identified as a stable environment as its IPCA score and vector was near to the origin (zero).

Inheritance studies identified inbred lines CZL999601 and CML481 which had good GCA for grain yield. It was found that additive and non-additive gene action was the mode of genetic inheritance for tolerance to acid soils for grain yield and some yield related traits, recording a GCA/SCA ratio above unity while characteristics such as grain texture, stem and root lodging, gray leaf spot disease and rust disease recorded a GCA/SCA ratio below unity. SCA analysis identified four specific combinations CML99601/CML144, CML144/CML202, CML481/ CML288 and CML161/CM172 as good combinations for acid tolerance and these single-cross hybrids will be used in the development of three way and double crosses for further low pH research projects.

## SUMMARY

In Malawi maize is grown even in marginal lands, on steep slopes, wet lands, rocky areas and low pH soils due to the high human population which exert pressure on the land. The objectives of this study were to investigate genetically diverse maize genotypes for tolerance to low pH soil conditions. In the hydroponic experiment genotypes IWDC3SYNF2-B, VPO52, and LPHpop 4 had relatively higher nett seminal root length and were considered tolerant, and DT-YSTR SYNTHETIC-B, TZE-WPOPDTTC2STR-B, TZE-YDTSTRC4-B, LPHpop3, LPHpop13, and LPHpop14 were sensitive or susceptible to Al toxicity. Under field conditions, genotypes LPHpop16, LPHpop3, VPO739, VPO5173 and LOW N POOL C3-B were identified to be relatively tolerant to low pH soil conditions. SYNDTE-STY-W-B ranked first in terms of root tolerant index (RTi) with a good NSRL in the glasshouse hydroponic experiment and this was followed by VPO717 which also had a relatively a better root tolerance index and nett seminal root length.

Phenotypic traits associated with grain yield, such as plant vigour, seed size (100 seed weight), shelling percentage, number of ears per plant, ear height and plant height can be used alongside grain yield when selecting germplasm for tolerance to low pH stress. In general, the effects of low pH soil conditions contributed to reduction in grain yields and yield components. The combined mean yield reduction due to low pH soil in this study was 69.9%. From AMMI and GGE analysis, genotypes LPHpop21, VPO52, VPO72, VPO744 and VPO96 were identified as the most stable. VPO097 was identified as an unstable genotype. Chitala low-land optimal site was identified as the most discriminating environment in terms of genotypes while Chitedze mid-altitude optimal environment was identified as a stable environment.

The diallel study revealed that additive and non-additive gene actions were at play in the expression of some of the traits like grain yield, number ears per plant, shelling percentage, 100 seed weight and plant vigour, while non-additive gene action was predominant in the inheritance of characteristics such as anthesis-silking interval, plant and ear height, grain texture, stem and root lodging and gray leaf spot disease. Positive and highly significant GCA effects for grain yield were observed for inbred line CZL999601 across low pH and optimal conditions. While negative and significant GCA effects for grain yield were

observed for inbred line CML161 across low pH and optimal conditions. SCA results indicated that single-cross hybrids CML999601/CML144, CML144/CML202, CML481/CML288 and CML161/CM172 were best for grain yield across low pH and optimal conditions. At a cut-off point of 1.0 with a cophenetic correlation of  $r_{cop} = 0.87$ , the UPGMA clustered the inbred lines based on GCA for grain yield into two main clusters through use of Euclidean distance and standard deviation as type of scale. The pattern mostly followed the origin of the maize inbred lines such that six out of seven inbred lines from CIMMYT-Colombia (tropical) were grouped in the second cluster. The open pollinated inbred line varieties and specific combinations (single-crosses generated) identified in this study will be used in the National Maize Breeding Programme for development of genotypes tolerant to low pH and diseases for yield improvement and subsequent food security in the country.

**Key words:** low pH, hydroponic, cophenetic correlation, diallel crosses, phenotypic traits, GCA, SCA

## OPSOMMING

In Malawi word mielies selfs in marginale grond, op steil hellings, vleilande, klipperige gebiede en in lae pH grond verbou, weens die hoë bevolking wat druk op die land plaas. Die doelwitte van hierdie studie was om geneties diverse mielie genotipes vir verdraagsaamheid vir lae pH grondtoestande te ondersoek. In die hidroponiese eksperiment het genotipes IWDC3SYNF2-B, VPO52 en LPHpop4 relatiewe hoër netto seminale wortel lengtes gehad en word as verdraagsaam beskou en DT-YSTR SYNTHETIC-B, TZE-WPOPDTC2STR-B, TZE-YDTSTRC4-B, LPHpop 3, LPHpop13, en LPHpop14 was sensitief of vatbaar vir Al toksisiteit. Onder veldtoestande, is genotipes LPHpop16, LPHpop3, VPO739, VPO5173 en LOW N POOL C3-B as relatief verdraagsaam vir lae pH grondtoestande geïdentifiseer. In die glashuis hidroponiese eksperiment was SYNDTE-STY-W-B eerste in terme van wortel tolerante indeks met 'n netto seminale wortel lengte van 2.5 cm en is gevolg deur VPO717 met 'n wortel tolerante indeks van 1.0 en netto seminale wortel lengte van 1.7 cm.

Fenotipiese eienskappe wat geassosieer word met graanopbrengs, soos groeikrag, saadgrootte (100 saad gewig), saad persentasie, die aantal koppe per plant, kop hoogte en plant hoogte kan gebruik word saam met graanopbrengs wanneer kiemplasma geselekteer word vir verdraagsaamheid vir lae pH stres. In die algemeen dra die effek van lae pH grondtoestande by tot die verlaging in graanopbrengste en opbrengs komponente. Die gekombineerde gemiddelde opbrengs verlaging as gevolg van lae pH grond in hierdie studie was 69.9%. Deur die AMMI en GGE analise is LPHpop21, VPO52, VPO72 is VPO744 en VPO96 geïdentifiseer as die mees stabiele genotipes. VPO097 is geïdentifiseer as 'n onstabiele genotipe. Chitala lae-ligging optimale omgewing is geïdentifiseer as die mees onstabiele omgewing in terme van genotipes terwyl Chitedze middel-ligging optimale omgewing geïdentifiseer is as 'n stabiele omgewing.

Die dialleel studie het getoon dat beide additiewe en nie-additiewe geenaksie 'n rol gespeel het by die uitdrukking van die eienskappe soos graanopbrengs, aantal koppe per plant, saad persentasie, 100 saad gewig en groeikrag, terwyl nie-additiewe geenaksie oorheersend was in die oorerwing van eienskappe soos antese-baard interval, plant en kop hoogte, graantekstuur, stam en wortel omval en blaarvlek siekte. Positiewe en hoogs betekenisvolle

algemene kombineervermoë (GCA) vir graanopbrengs is in die lae pH en optimale omgewings, vir ingeteelde lyn CZL999601 waargeneem, terwyl negatief en betekenisvolle GCA effekte vir graanopbrengs waargeneem is vir ingeteelde lyn CML161 vir lae pH en optimale toestande. Resultate vir spesifieke kombineervermoë (SCA) het aangedui dat enkelkruisbasters CML99601/CML144, CML144/CML202, CML481/ CML288 en CML161/CM172 die beste graanopbrengs oor lae pH en optimale toestande gelewer het. By 'n afsny punt van 1.0 met 'n ko-fenetiese korrelasie van  $r_{cop} = 0.87$ , het die UPGMA kluster, deur die gebruik van Euklidiese afstand en standaardafwyking as skaal tipe, die ingeteelde lyne gebaseer op GCA vir graanopbrengs in twee hoof groepe verdeel. Die groepering het die oorsprong van die ingeteeldelyne gevolg, sodanig dat ses uit sewe ingeteelde lyne van CIMMYT-Kolombië (tropiese) in die tweede kluster groepeer. Die OPVs, ingeteelde lyne en spesifieke kombinasie enkelkruisings wat geïdentifiseer is in hierdie studie sal gebruik word in die Nasionale Mielie Teelprogram vir die ontwikkeling van genotipes tolerant vir lae pH en siektes en om opbrengs te verbeter sodat voedselsekuriteit in die land verseker kan word.

**Sleutel woorde:** lae pH, hidroponiese, ko-fenetiese korrelasie, dialleel kruise, fenotipiese eienskappe, GCA, SCA

APPENDICES

**Appendix 1 Root length measurements and derived data before and seven days after transplanting a glasshouse hydroponic experiment**

	<b>Pedigree</b>	<b>Initial cm</b>	<b>Final cm</b>	<b>Control cm</b>	<b>Rti</b>	<b>% response</b>	<b>RSRLCtrl (cm)</b>	<b>RSRLTrtd(cm)</b>	<b>NSRL cm</b>
1	99TZE FY-STR QPM CO-B	2.8	5.0	6.7	0.7	-25.9	2.03	1.19	2.2
2	DT-WSTR SYNTHETIC-B	8.7	7.4	9.1	0.8	-18.0	0.08	-0.12	0.9
3	DT-YSTR SYNTHETIC-B	2.9	1.7	4.3	0.4	-59.3	0.50	-0.39	0.6
4	EVDT-Y2000 STR QPM CO-B	6.3	7.5	8.9	0.8	-15.7	0.41	0.19	1.2
5	EVD-W 99 STR QPM CO-B	7.2	8.5	10.8	0.8	-20.9	0.50	0.19	1.2
6	IAR-FLINT-Q-B	2.4	2.2	3.5	0.6	-36.2	0.50	-0.04	1.0
7	IWD C3 SYN F2-B	1.4	3.5	5.1	0.7	-32.1	3.20	2.02	3.0
8	LOW N POOL C3-B	6.1	4.3	6.1	0.7	-29.4	0.06	-0.28	0.7
9	MULTICOB EARLY DT-B	7.6	6.4	7.8	0.8	-17.4	0.04	-0.14	0.9
10	OBA SUPER1[9021-18(IITA)]-B	2.5	2.1	4.1	0.5	-46.5	0.67	-0.07	0.9
11	OBATANPA/IWDC2SYNF2/IWDC2SY	1.4	2.9	4.0	0.7	-27.8	1.83	1.06	2.1
12	OBATANPA/IWDC2SYNF2/IWDC2SYNF2-B	3.9	2.8	4.5	0.6	-36.2	0.19	-0.27	0.7
13	OBATANPA/TZLCOMP4C3F2/TZLC	7.0	5.8	8.5	0.7	-28.2	0.21	-0.16	0.8
14	OBATANPA/TZLCOMP4C3F2/TZLCOMP4C3F2-B	3.8	2.6	4.8	0.6	-43.8	0.36	-0.26	0.7
15	POP66 SR/DMR-LSRY/DMR-LSRY	2.6	2.1	3.9	0.6	-44.7	0.50	-0.17	0.8
16	POP66 SR/TZUTSR-WSGY/T	5.2	7.5	9.0	0.8	-16.1	0.74	0.47	1.5
17	SYN DTE STR-Y-B	1.7	2.4	3.5	0.7	-29.1	1.24	0.55	1.6
18	SYN DTE STY-W-B	3.4	8.5	8.2	1.1	5.4	1.39	1.49	2.5
19	TZE COPM3 DTV C2 F2-B	1.4	1.8	2.9	0.7	-34.7	1.02	0.27	1.3
20	TZE E-WPOP X LD(SET2)-B	1.3	2.7	3.7	0.7	-26.4	1.97	1.21	2.2
21	TZE-W POP DTC2 STR-B	4.5	2.7	5.6	0.5	-51.0	0.26	-0.39	0.6
22	TZE-WDT STR QPM-CO-B	8.5	9.4	11.5	0.8	-18.2	0.37	0.11	1.1
23	TZE-YDT STR C4-B	5.2	3.2	4.7	0.8	-20.4	-0.07	-0.38	0.6
24	TZE-YPOP DTC2 STR-B	1.8	2.5	3.0	0.8	-17.5	0.70	0.39	1.4
25	VPO0721	6.1	7.6	9.2	0.8	-15.8	0.50	0.26	1.3
26	VPO5148	2.8	3.2	5.2	0.6	-37.1	1.16	0.46	1.5

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**Appendix 1 continue**

27	VPO5173	2.0	2.5	3.8	0.7	-33.2	1.37	0.57	1.6
28	VPO5179	4.8	3.5	5.8	0.6	-38.5	0.23	-0.24	0.8
29	VPO5187	3.3	4.6	5.7	0.8	-18.0	0.78	0.43	1.4
30	VPO52	1.4	3.4	4.4	0.8	-21.1	3.30	2.47	3.5
31	VPO627	1.7	3.3	4.5	0.7	-27.6	1.68	0.95	1.9
32	VPO630	1.5	2.5	3.9	0.6	-37.0	1.79	0.73	1.7
33	VPO710	5.9	4.8	7.7	0.6	-38.4	0.34	-0.17	0.8
34	VPO712	2.4	2.7	4.2	0.7	-33.8	0.77	0.16	1.2
35	VPO716	2.3	2.8	3.9	0.7	-27.9	0.79	0.27	1.3
36	VPO717	2.9	4.9	4.8	1.0	0.5	0.66	0.70	1.7
37	VPO738	1.9	3.5	4.2	0.8	-16.6			2.0
38	VPO739	5.6	4.8	7.3	0.7	-31.7	0.31	-0.12	0.9
39	VPO741	6.5	6.6	8.7	0.8	-23.3	0.36	0.03	1.0
40	VPO743	3.2	3.5	4.7	0.7	-26.1	0.65	0.21	1.2
41	VPO744	2.2	2.1	3.5	0.6	-38.7	0.66	0.02	1.0
42	VPO76	10.1	10.0	11.1	0.9	-9.0	0.11	-0.01	1.0
43	VPO86	4.2	5.4	7.0	0.8	-23.3	0.86	0.42	1.4
44	VPO96	3.4	4.8	6.6	0.7	-26.8	0.99	0.46	1.5
45	VPO97	2.4	3.3	4.4	0.8	-23.4	0.86	0.43	1.4
46	LPHpop 4	1.4	4.0	5.7	0.7	-27.8	3.31	1.99	3.0
47	LPHpop 3	5.7	3.4	6.1	0.6	-42.7	0.09	-0.41	0.6
48	LPHpop 6	1.8	3.1	4.8	0.7	-30.9	1.73	0.69	1.7
49	LPHpop 8	1.7	2.0	2.9	0.7	-30.8	0.83	0.23	1.2
50	LPHpop 9	6.0	5.6	7.1	0.8	-20.0	0.20	-0.05	0.9
51	LPHpop 10	5.3	8.1	8.0	1.0	4.0	0.50	0.56	1.6
52	LPHpop 11	8.2	7.9	10.4	0.8	-23.9	0.29	-0.02	1.0
53	LPHpop 13	4.3	2.3	4.4	0.5	-46.8	0.06	-0.44	0.6
54	LPHpop 14	6.2	4.0	6.4	0.6	-35.5	0.03	-0.36	0.6
55	LPHpop 15	2.2	2.6	3.7	0.7	-29.9	0.78	0.24	1.2
56	LPHpop 16	3.3	3.8	5.0	0.8	-21.7	0.50	0.17	1.2
57	LPHpop 17	2.8	2.7	4.2	0.7	-35.0	0.50	-0.03	1.0

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<b>Appendix 1 continue</b>									
58	LPHpop 18	1.4	2.4	4.0	0.6	-38.4	2.74	1.10	2.1
59	LPHpop 19	7.4	7.2	9.4	0.8	-22.7	0.29	-0.02	1.0
60	LPHpop 20	1.9	2.9	3.5	0.8	-17.0	0.98	0.65	1.7
61	LPHpop 21	2.7	3.6	5.0	0.7	-29.0	0.91	0.38	1.4
62	LPHpop 23	1.5	1.7	3.2	0.5	-46.2	1.25	0.24	1.2
63	LPHpop 1	3.2	3.0	4.8	0.6	-37.7	0.50	-0.07	0.9
64	LPHpop 2	1.4	2.1	3.7	0.6	-41.9	1.64	0.50	1.5
65	LPHpop 7	1.8	1.5	2.7	0.5	-45.5	0.50	-0.18	0.8
66	LPHpop 12	4.4	3.9	5.6	0.7	-29.3	0.30	-0.07	0.9
67	ZM309	0.8	1.8	3.4	0.6	-38.5	3.82	1.50	2.5
68	ZM523	3.3	2.5	4.5	0.5	-46.3	0.41	-0.25	0.7
69	ZM623	3.2	3.7	5.7	0.7	-33.8	0.80	0.19	1.2
70	ZM721	3.4	4.0	6.3	0.7	-32.0	0.93	0.26	1.3
	<b>Mean</b>	<b>3.73</b>	4.08	<b>5.6</b>	<b>0.7</b>	<b>-29.1</b>	<b>0.89</b>	<b>0.32</b>	<b>1.3</b>
	<b>LSD</b>	<b>0.95</b>	1.2	<b>1.87</b>	<b>0.22</b>	<b>22.01</b>	<b>1.27</b>	<b>0.88</b>	<b>0.88</b>
	<b>Prob</b>	<b>0.001</b>	<b>0.001</b>	<b>0.001</b>	<b>0.001</b>	<b>0.001</b>	<b>0.001</b>	<b>0.001</b>	<b>0.001</b>
	<b>CV (%)</b>	<b>15.8</b>	18.3	<b>20.5</b>	<b>19.2</b>	<b>46.9</b>	<b>88.3</b>	<b>171.5</b>	<b>41.5</b>
	<b>Min</b>	<b>0.8</b>	1.5	<b>2.7</b>	<b>0.4</b>	<b>-59.3</b>	<b>-0.07</b>	<b>-0.44</b>	<b>0.6</b>
	<b>Max</b>	<b>10.1</b>	10	<b>11.5</b>	<b>1.1</b>	<b>5.4</b>	<b>3.82</b>	<b>2.47</b>	<b>3.5</b>
	<b>R-Squared</b>	<b>0.96</b>	0.92	<b>0.85</b>	<b>0.53</b>	<b>0.54</b>	-	-	<b>0.69</b>
	<b>Heritability</b>	<b>0.98</b>	0.96	<b>0.91</b>	<b>0.56</b>	<b>0.58</b>	-	-	<b>0.74</b>

ISRL = initial seminal root length, FSRL = final seminal Root length, Zero Al = Zero Aluminium or control, RTi = root tolerance index, RSRLzero Al = relative seminal root length with zero Aluminium, RSRLTRTD = relative seminal root length treated with Aluminium and NSRL = net seminal root length, LSD = Least significant difference, CV = coefficient of variation, Min = minimum, Max = maximum.

## Appendix 2 Maize genotypes evaluated in the field trials 2011/12 and 2012/13

Genotype number	Genotype
G1	DT-WSTR SYNTHETIC-B
G2	EVD-W 99 STR QPM CO-B
G3	IAR-FLINT-Q-B
G4	IWD C3 SYN F2-B
G5	MULTICOB EARLY DT -B
G6	SYN DTE STY-W-B
G7	OBA SUPER1(9021-18(IITA))-B
G8	LPHpop 9
G9	POP66 SR/DMR -LSRY/DMR-LSRY
G10	POP66 SR/TZUTSR-WSGY/T
G11	LPHpop 7
G12	TZE-YDT STR C4-B
G13	VPO0721
G14	TZE E -WPOP X LD(SET2)-B
G15	VPO5173
G16	VPO717
G17	VPO5187
G18	VPO52
G19	VPO630
G20	LPHpop 21
G21	VPO741
G22	VPO739
G23	LPHpop 18
G24	LPHpop 16
G25	VPO743
G26	VPO744
G27	LOW N POOL C3-B
G28	LPHpop 10
G29	VPO76
G30	LPHpop 3
G31	LPHpop 20
G32	LPHpop 2
G33	VPO710
G34	VPO712
G35	LPHpop 8
G36	OBATANPA/TZLCOMP4C3F2/TZLCOMP4C3F2-B
G37	VPO738
G38	LPHpop 13
G39	LPHpop 15
G40	VPO86
G41	VPO96
G42	VPO97
G43	ZM309 Check 1
G44	ZM523 Check 2
G45	ZM721 Check 3

### Appendix 3 Soil sampling data

Lab no.	Location	Depth (cm)	pH	%OC	%OM	%N	P( $\mu\text{g g}^{-1}$ )	Ca( $\mu\text{g g}^{-1}$ )	Mg( $\mu\text{g/g}$ )	% Al
97135	Tsangano	0-15	5.27	0.20	0.35	0.02	16.30	1.72	0.29	0.4
97136	" "	15-30	5.23	1.78	3.06	0.15	5.68	1.80	0.29	0.8
97137	" "	0-15	5.35	1.69	2.91	0.15	28.32	2.51	0.39	0.4
97138	" "	15-30	5.41	2.16	3.72	0.19	46.77	2.48	0.47	2.4
97139	" "	0-15	5.44	1.81	3.11	0.16	5.64	1.84	0.44	0.4
97140	" "	15-30	5.49	1.98	3.41	0.17	5.36	2.25	0.34	0.8
97141	Bvumbwe	0-15	5.65	1.49	2.56	0.13	44.32	3.22	0.98	0.6
97142	" "	15-30	5.74	1.05	1.81	0.09	62.34	2.90	0.79	0.6
97143	" "	0-15	5.67	1.46	2.51	0.13	69.11	3.03	0.81	0.6
97144	" "	15-30	5.57	1.40	2.41	0.12	41.40	2.46	0.56	0.2
97145	" "	0-15	5.68	1.17	2.01	0.10	80.30	3.88	1.06	0.6
97146	" "	15-30	5.67	1.02	1.76	0.09	83.79	3.08	0.87	0.2
97147	Lunyangwa	0-15	4.38	1.83	3.16	0.16	21.86	0.65	0.13	1.2
97148	" "	15-30	4.62	1.57	2.71	0.14	11.92	0.60	0.11	1.0
97149	" "	0-15	4.59	0.87	1.51	0.08	30.52	0.71	0.13	0.8
97150	" "	15-30	4.42	0.70	1.21	0.06	12.56	0.44	0.101	0.2
97151	" "	0-15	5.11	1.81	3.11	0.16	20.53	2.08	0.36	0.6
97152	" "	15-30	4.49	0.87	1.51	0.08	6.90	0.69	0.13	0.6
97153	Bembeke	0-15	5.10	1.98	3.41	0.17	20.77	2.00	0.41	0.8
97154	" "	15-30	4.95	0.84	1.46	0.07	7.17	0.65	0.11	0.6
97155	" "	0-15	5.15	1.66	2.86	0.14	11.83	2.09	0.43	0.4
97156	" "	15-30	5.06	1.81	3.11	0.16	20.10	1.98	0.38	1.0
97157	" "	0-15	5.14	1.63	2.81	0.14	15.53	2.05	0.46	1.0
97158	" "	15-30	5.10	1.60	2.76	0.14	18.57	2.28	0.49	1.0

#### Appendix 4 Eigenvectors for the measured and derived data at low pH environments across two seasons

	AD	ASI	DS	EH	EPP	LB	GLS	GT	HC	GY	MSV	PH	RE	RL	RUST	SH	SL	SWT
AD	1.000	-0.045	0.941	-0.032	0.058	-0.059	-0.081	0.000	0.137	0.047	-0.123	-0.069	-0.042	0.087	0.187	0.058	0.044	-0.010
ASI	-0.045	1.000	0.295	0.008	0.035	0.050	0.091	0.000	0.176	-0.091	0.095	-0.023	-0.130	-0.072	0.021	0.037	0.044	-0.030
DS	0.941	0.295	1.000	-0.028	0.067	-0.039	-0.046	0.000	0.191	0.014	-0.085	-0.074	-0.084	0.059	0.186	0.068	0.057	-0.010
EH	-0.032	0.008	-0.028	1.000	0.018	0.022	-0.076	0.000	0.012	-0.026	0.058	0.680	0.042	-0.082	-0.040	0.016	0.004	0.182
EPP	0.058	0.035	0.067	0.018	1.000	0.016	-0.125	0.000	-0.149	0.653	-0.082	0.061	-0.068	-0.132	-0.073	0.993	-0.030	-0.130
LB	-0.059	0.050	-0.039	0.022	0.016	1.000	0.380	0.000	-0.063	0.059	0.229	0.008	-0.095	0.025	0.031	0.013	-0.080	-0.030
GLS	-0.081	0.091	-0.046	-0.076	-0.125	0.380	1.000	0.000	-0.031	-0.048	0.249	-0.119	-0.036	0.100	0.040	-0.124	0.038	-0.220
GT	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
HC	0.137	0.176	0.191	0.012	-0.149	-0.063	-0.031	0.000	1.000	-0.225	-0.113	0.039	0.066	0.135	0.176	-0.144	0.052	0.094
GY	0.047	-0.091	0.014	-0.026	0.653	0.059	-0.048	0.000	-0.225	1.000	-0.091	0.007	-0.113	-0.103	-0.101	0.643	0.007	-0.210
MSV	-0.123	0.095	-0.085	0.058	-0.082	0.229	0.249	0.000	-0.113	-0.091	1.000	-0.071	-0.084	0.044	-0.132	-0.087	-0.140	0.048
PH	-0.069	-0.023	-0.074	0.680	0.061	0.008	-0.119	0.000	0.039	0.007	-0.071	1.000	0.040	-0.010	-0.127	0.062	-0.020	0.163
RE	-0.042	-0.130	-0.084	0.042	-0.068	-0.095	-0.036	0.000	0.066	-0.113	-0.084	0.040	1.000	-0.001	0.033	-0.067	-0.000	0.096
RL	0.087	-0.072	0.059	-0.082	-0.132	0.025	0.100	0.000	0.135	-0.103	0.044	-0.010	-0.001	1.000	0.181	-0.143	-0.020	0.061
RUST	0.187	0.021	0.186	-0.040	-0.073	0.031	0.040	0.000	0.176	-0.101	-0.132	-0.127	0.033	0.181	1.000	-0.070	0.142	0.175
SH	0.058	0.037	0.068	0.016	0.993	0.013	-0.124	0.000	-0.144	0.643	-0.087	0.062	-0.067	-0.143	-0.070	1.000	-0.030	-0.120
SL	0.044	0.044	0.057	0.004	-0.035	-0.085	0.038	0.000	0.052	0.007	-0.135	-0.016	-0.003	-0.023	0.142	-0.027	1.000	0.123
SWT	-0.006	-0.026	-0.014	0.182	-0.132	-0.034	-0.217	0.000	0.094	-0.211	0.048	0.163	0.096	0.061	0.175	-0.119	0.123	1.000

AD = days to anthesis (days), ASI = anthesis-silking interval (days), DS = days to silking (days), EH = ear height (cm), EPP = ears per plant (#), LB = leaf blight disease (1-5), GLS = gray leaf spot disease (1-5), GT = grain texture (1-5), HC = husk cover, GY = grain yield (kg ha<sup>-1</sup>), MSV = maize streak virus disease (1-5), PH = plant height (cm), RE = rotten ears (#), RL = root lodging (#), Rust = rust disease (1-5), SH = shelling percentage, SL = stem lodging (#), SWT = 100 seed weight (g), # = number.

**Appendix 5 Eigenvectors from the principal component analysis for grain yield and agronomic at optimal environments across two seasons**

	<b>GY</b>	<b>AD</b>	<b>DS</b>	<b>EH</b>	<b>EPP</b>	<b>LB</b>	<b>GLS</b>	<b>GT</b>	<b>HC</b>	<b>MSV</b>	<b>PH</b>	<b>RL</b>	<b>RE</b>	<b>RUST</b>	<b>SH</b>	<b>SL</b>	<b>SWT</b>	<b>VIG</b>
AD	-0.06	1.00	0.98	0.13	0.09	-0.03	0.00	-0.04	0.06	-0.07	0.07	-0.04	-0.05	-0.06	0.01	-0.03	-0.09	0.11
DS	-0.04	0.98	1.00	0.14	0.08	-0.04	0.02	-0.06	0.08	-0.07	0.06	-0.04	-0.03	-0.05	0.01	-0.01	-0.09	0.28
EH	-0.05	0.13	0.14	1.00	0.04	-0.07	-0.03	0.05	-0.15	0.06	0.63	-0.02	0.10	-0.07	-0.04	-0.04	0.12	0.21
EPP	-0.08	0.09	0.08	0.04	1.00	0.14	0.06	-0.31	-0.01	0.09	0.08	0.04	-0.17	-0.03	-0.04	0.09	0.02	0.00
LB	-0.09	-0.03	-0.04	-0.07	0.14	1.00	0.21	0.03	-0.02	-0.15	-0.01	-0.04	0.05	0.17	0.02	-0.05	-0.07	-0.51
GLS	0.01	0.00	0.02	-0.03	0.06	0.21	1.00	-0.02	-0.04	-0.11	0.15	0.06	-0.02	-0.09	0.01	-0.01	-0.06	0.00
GT	0.04	-0.04	-0.06	0.05	-0.31	0.03	-0.02	1.00	-0.04	-0.12	0.05	0.00	0.01	-0.07	0.01	-0.12	-0.10	0.00
HC	0.10	0.06	0.08	-0.15	-0.01	-0.02	-0.04	-0.04	1.00	-0.16	-0.19	0.17	0.11	0.06	0.01	0.00	-0.06	0.00
GY	1.00	-0.06	-0.04	-0.05	-0.08	-0.09	0.01	0.04	0.10	0.12	-0.09	-0.01	0.03	0.09	0.10	-0.05	0.25	0.11
MSV	0.12	-0.07	-0.07	0.06	0.09	-0.15	-0.11	-0.12	-0.16	1.00	0.05	-0.04	0.00	-0.16	-0.05	0.01	0.14	0.00
PH	-0.09	0.07	0.06	0.63	0.08	-0.01	0.15	0.05	-0.19	0.05	1.00	-0.02	-0.07	-0.20	-0.05	-0.03	0.13	0.00
RL	-0.01	-0.04	-0.04	-0.02	0.04	-0.04	0.06	0.00	0.17	-0.04	-0.02	1.00	-0.04	-0.03	-0.03	-0.07	-0.10	0.13
RE	0.03	-0.05	-0.03	0.10	-0.17	0.05	-0.02	0.01	0.11	0.00	-0.07	-0.04	1.00	0.07	0.03	-0.01	-0.05	0.00
RUST	0.09	-0.06	-0.05	-0.07	-0.03	0.17	-0.09	-0.07	0.06	-0.16	-0.20	-0.03	0.07	1.00	0.01	-0.12	0.21	0.69
SH	0.10	0.01	0.01	-0.04	-0.04	0.02	0.01	0.01	0.01	-0.05	-0.05	-0.03	0.03	0.01	1.00	0.11	-0.04	-0.09
SL	-0.05	-0.03	-0.01	-0.04	0.09	-0.05	-0.01	-0.12	0.00	0.01	-0.03	-0.07	-0.01	-0.12	0.11	1.00	0.17	-0.32
SWT	0.25	-0.09	-0.09	0.12	0.02	-0.07	-0.06	-0.10	-0.06	0.14	0.13	-0.10	-0.05	0.21	-0.04	0.17	1.00	0.00
VIG	0.11	0.11	0.28	0.21	0.00	-0.51	0.00	0.00	0.00	0.00	0.00	0.13	0.00	0.69	-0.09	-0.32	0.00	1.00

**GY = grain yield (kg ha<sup>-1</sup>), AD = days to anthesis (days), DS = days to silking (days), EH = ear height (cm), EPP = ears per plant (#), LB = leaf blight disease (1-5), GLS = gray leaf spot disease (1-5), GT = grain texture (1-5), HC = husk cover, MSV = maize streak virus (1-5), PH = plant height (cm), RL = root lodging (#), RE = rotten ears (#), Rust = rust disease (1-5), SH = shelling percentage, SL = stem lodging (#), SWT = 100 seed weight (g), VIG = vigor, # = number.**

## **Appendix 6 Soil analytical data interpretation guide**

Threshold values

### **i) Phosphorus ug g<sup>-1</sup> (Mehlich3) rating**

<8	very low
9 - 18	low
19 - 25	medium (adequate range)
25 - 33	high (adequate range)
>34	very high

### **ii) Potassium cmol kg<sup>-1</sup>(Mehlich3) rating**

<0.05	very low
0.06 - 0.10	low
0.11 - 0.04	medium (adequate range)
0.50 - 0.80	high
>1.00	very high

### **iii) Zinc ug g<sup>-1</sup> (Mehlich3) rating**

<1.00	very low
1.00 - 1.50	low
1.60 - 2.50	medium (adequate range)
2.5 - 3.00	high (adequate range)
>2.5	very high

### **iv) Boron ug g<sup>-1</sup> (Mehlich3) rating**

<0.70	low
0.8 - 1.40	medium
1.40 - 2.50	high
>2.5	very high

### **v) Copper ug g<sup>-1</sup> (Mehlich3) rating**

<0.30	low
0.4 - 0.8	medium
0.9 - 2.50	high
>2.5	very high

### **vi) Manganese cmol kg<sup>-1</sup> (Mehlich3) rating**

<0.20	very low
0.2 - 0.5	low
0.6 - 3.9	high
>4.0	very high

## **Appendix 6 continued**

NB: Critical levels for Mn is 3.0 ug g<sup>-1</sup> and Ca is 2.0 cmol kg<sup>-1</sup> using Mehlich3 (Melich 1984)

### **vii) Soil pH**

<u>In water</u>	<u>In CaCl<sub>2</sub></u>	<u>Rating</u>
<4.5	<4.0	very strongly acid
4.5 - 5.0	4.0 - 4.45	strongly acid
5.1 - 5.5	4.5 - 4.95	acid
5.6 - 6.05	0 - 5.45	moderately acid
6.1 - 6.5	5.5 - 5.95	slightly acid
6.6 - 7.0	6.0 - 6.45	almost neutral
7.1 - 7.5	6.5 - 6.95	very slightly alkaline
7.6 - 8.0	7.0 - 7.45	slightly alkaline
>8.0	-7.45 - 7.95	alkaline / moderately alkaline
>8.5	>8.00	strongly alkaline

### **vii) Total Nitrogen% Rating**

<0.08	very low
0.08 - 0.12	low
0.12 - 0.2	medium
0.20 - 0.30	high
>0.3	very high

<u>vii) % Carbon</u>	<u>organic matter %</u>	<u>Rating</u>
<0.88	1.5	low
0.88 - 2.35	1.5 - 4.0	medium
>2.35	>4.0	high

Notes: Soil reaction (pH) is determined in water. Non acidifying fertilisers such as CAN are recommended below pH 5.5 in water (4.5 in CaCl<sub>2</sub>). Dolomitic lime application (1-2 t ha<sup>-1</sup>).

Farm yard manure is recommended at 5 tons per ha if the soil test measures less than 1.0% OM (0.58% OC) and 2.5 tons per ha when the soil test is between 1.0 and 1.5% OM (0.58-0.87% OC).

**Appendix 7 Mean performance for grain yield across four optimal environments combined for 2011/12 and 2012/13 seasons**

<b>Genotypes</b>	<b>GY</b>	<b>AD</b>	<b>ASI</b>	<b>DS</b>	<b>EH</b>	<b>EPP</b>	<b>LB</b>	<b>HC</b>	<b>GLS</b>	<b>GT</b>	<b>MSV</b>	<b>PH</b>	<b>RE</b>	<b>RL</b>	<b>Rust</b>	<b>SL</b>	<b>SWT</b>	<b>SH%</b>	<b>VIG</b>
G1	3100	66.1	2.9	67.8	75	1.0	1.8	1.2	1.7	1.6	1.1	171	0.6	0.1	1.7	0.2	30.8	77.3	2.3
G2	2326	62.1	1.2	64.5	69	1.0	2.0	1.3	1.9	1.8	1.3	156	1.0	0.5	1.5	1.5	29.3	67.4	2.1
G3	3014	64.1	0.8	65.7	75	1.2	1.4	1.3	1.7	1.6	1.3	163	0.7	0.5	1.5	1.0	30.1	76.4	2.0
G4	3311	65.0	1.3	66.9	73	1.1	1.9	1.2	1.3	1.8	1.1	165	0.6	0.3	1.7	0.2	28.3	80.6	2.0
G5	3921	68.3	1.0	70.0	87	1.1	1.9	1.9	1.5	1.9	1.1	189	0.7	0.8	1.4	1.0	32.1	79.6	1.9
G6	3505	67.2	0.8	69.0	85	1.2	2.0	1.4	1.9	2.0	1.1	185	0.8	0.9	1.6	0.6	31.2	79.6	1.9
G7	2663	65.6	1.4	67.6	78	1.1	1.9	0.9	1.4	2.0	1.3	171	0.8	0.7	1.5	0.6	30.9	80.0	2.0
G8	3965	65.9	1.1	67.6	85	1.2	1.9	1.6	1.9	1.7	1.2	182	0.7	1.4	2.0	1.2	30.1	80.9	1.9
G9	3254	66.0	0.9	67.4	80	1.1	1.9	1.2	1.5	2.0	1.3	175	0.9	0.6	1.6	0.7	29.3	75.7	1.9
G10	3817	67.7	2.0	70.2	86	1.1	2.0	0.9	1.9	2.0	1.2	183	0.8	0.6	1.7	0.2	31.1	81.8	2.0
G11	2517	67.8	1.0	69.4	76	1.1	2.0	1.3	1.8	1.8	1.4	169	0.7	0.6	1.6	0.5	26.7	77.5	2.1
G12	3687	66.3	1.1	68.0	78	1.1	1.9	1.1	1.8	1.8	1.1	173	0.7	0.4	1.9	0.0	26.1	78.3	2.0
G13	3444	66.5	0.8	68.1	82	1.2	1.6	1.4	2.1	1.8	1.2	173	1.4	0.8	1.3	0.2	28.6	80.8	2.2
G14	3189	65.5	1.7	67.9	82	1.0	2.0	1.1	1.8	1.8	1.0	177	1.1	0.9	1.6	0.2	30.2	79.4	2.0
G15	3319	63.6	1.3	65.6	73	1.1	2.0	1.7	1.7	1.8	1.1	165	0.9	1.2	1.8	0.9	29.5	79.0	2.3
G16	2901	63.2	0.8	64.9	80	1.0	1.8	2.1	1.8	1.8	1.1	173	0.8	0.5	1.8	0.6	32.3	77.7	2.0
G17	3143	65.3	1.3	67.3	75	1.2	1.8	1.5	1.3	1.6	1.1	164	0.5	0.3	1.4	0.8	26.2	83.4	2.4
G18	3633	63.3	1.2	65.3	79	1.2	1.4	1.1	1.5	1.7	1.1	173	1.1	0.9	1.5	1.0	32.1	80.8	2.1
G19	3179	64.9	1.0	66.8	79	1.1	1.9	2.0	1.8	2.0	1.1	167	0.8	1.5	2.1	0.6	29.4	86.5	2.1
G20	4489	65.0	1.0	66.7	81	1.1	1.9	1.3	1.5	1.8	1.2	173	1.1	1.4	1.3	0.4	32.0	83.0	1.5
G21	3307	65.1	1.0	66.7	83	1.2	2.4	1.5	1.6	1.8	1.0	176	0.7	1.1	1.7	1.8	30.9	80.3	2.0
G22	3573	62.7	1.4	64.7	75	1.1	1.9	1.2	1.5	1.8	1.3	170	0.8	0.2	1.7	0.7	30.3	80.1	2.5
G23	3160	61.6	1.2	63.4	70	1.1	2.1	1.1	1.9	2.0	1.0	163	0.7	0.6	2.0	0.6	28.6	79.0	2.0
G24	2983	59.1	1.3	61.1	72	1.1	2.2	1.8	1.3	2.0	1.1	167	0.7	0.7	1.7	0.8	29.1	75.9	2.2
G25	3396	63.3	0.9	65.2	82	1.1	1.7	1.7	1.7	2.1	1.4	177	1.5	0.7	1.8	0.7	30.7	81.0	1.7
G26	3611	64.4	1.2	66.2	75	1.1	2.1	1.3	1.6	2.0	1.1	174	1.3	0.8	1.5	0.2	30.5	77.4	1.9
G27	3965	66.4	1.4	68.4	82	1.2	1.9	1.6	1.7	2.1	1.2	179	0.8	0.3	1.6	0.1	30.7	79.8	1.9
G28	3727	62.0	1.0	63.8	75	1.1	2.0	1.3	1.8	2.1	1.2	171	0.5	1.0	1.7	0.5	27.4	82.6	1.9
G29	3384	66.8	1.3	68.3	78	1.1	2.0	1.6	1.5	2.0	1.4	176	0.9	0.6	1.6	0.5	29.7	79.3	2.1

Appendix 7 continued

G30	3693	65.7	0.9	67.3	82	1.1	1.9	1.5	1.8	1.8	1.2	179	0.8	0.4	1.6	0.4	30.9	79.2	2.0
G31	3820	64.8	1.0	66.6	82	1.1	2.2	1.3	1.8	1.8	1.2	176	0.8	0.3	1.7	1.0	27.6	80.4	2.0
G32	3744	62.2	1.0	64.0	80	1.1	2.0	1.8	1.5	1.9	1.4	178	0.5	0.6	1.6	0.6	28.6	85.4	2.0
G33	4003	61.3	1.2	63.4	75	1.2	1.8	1.5	1.4	1.9	1.6	168	0.7	1.0	1.7	0.9	29.5	82.1	1.9
G34	3223	60.7	0.9	62.5	78	1.2	2.2	1.3	1.6	2.7	1.0	174	0.7	1.8	1.9	0.3	25.9	80.2	2.1
G35	3563	64.2	1.0	65.9	81	1.1	1.8	2.6	1.6	2.0	1.1	178	0.7	0.7	1.6	1.2	29.9	77.8	2.0
G36	3757	62.9	1.1	64.9	83	1.1	1.9	1.3	1.6	2.0	1.3	179	0.8	0.8	1.9	0.7	28.6	79.5	2.0
G37	2594	65.3	1.3	67.2	66	1.1	1.7	1.0	1.5	2.1	1.2	162	0.6	0.2	1.9	0.9	30.4	74.1	2.0
G38	2413	69.0	1.1	70.3	78	1.0	1.9	1.0	1.4	1.9	1.4	175	0.5	0.8	1.4	0.8	30.5	72.8	2.0
G39	3902	64.1	0.6	65.6	70	1.1	2.0	1.2	1.5	1.9	1.1	161	0.9	0.6	1.8	0.8	31.4	79.3	2.1
G40	2295	66.5	1.1	68.0	73	1.1	1.9	1.2	1.4	1.9	1.2	169	0.8	0.6	1.4	0.5	33.2	77.8	2.0
G41	3130	60.4	0.6	62.0	70	1.0	1.5	1.3	1.4	1.5	1.0	164	0.9	0.2	1.9	0.3	27.5	82.2	2.3
G42	2996	63.2	1.0	64.9	71	1.0	1.8	0.9	1.6	1.7	1.2	168	0.5	1.6	1.7	0.5	31.3	78.7	2.2
G43	3750	61.9	0.9	63.8	69	1.1	2.0	1.3	1.6	1.9	1.2	162	0.9	0.2	1.7	1.7	31.0	77.4	1.9
G44	3685	64.3	1.2	66.5	75	1.0	2.0	1.4	1.8	1.7	1.2	172	0.8	0.5	1.5	1.0	32.1	79.4	2.0
G45	3735	66.2	0.8	68.0	89	1.1	1.8	1.4	1.3	2.5	1.7	186	0.7	0.2	1.8	0.9	33.7	76.7	1.8
<b>Mean</b>	<b>3373</b>	<b>64.5</b>	<b>1.1</b>	<b>66.3</b>	<b>77.6</b>	<b>1.1</b>	<b>1.9</b>	<b>1.4</b>	<b>1.6</b>	<b>1.9</b>	<b>1.2</b>	<b>172.3</b>	<b>0.8</b>	<b>0.7</b>	<b>1.7</b>	<b>0.7</b>	<b>29.9</b>	<b>79.2</b>	<b>2.0</b>
<b>LSD</b>	<b>608</b>	<b>1.7</b>	<b>1.3</b>	<b>1.7</b>	<b>8</b>	<b>0.1</b>	<b>0.3</b>	<b>0.8</b>	<b>0.3</b>	<b>0.5</b>	<b>0.3</b>	<b>12</b>	<b>0.4</b>	<b>1.2</b>	<b>0.3</b>	<b>0.7</b>	<b>2.9</b>	<b>5.9</b>	<b>0.3</b>
<b>CV</b>	<b>31.8</b>	<b>4.6</b>	<b>194</b>	<b>4.6</b>	<b>18.2</b>	<b>15</b>	<b>27.1</b>	<b>103</b>	<b>36</b>	<b>40</b>	<b>35.9</b>	<b>12.1</b>	<b>80</b>	<b>302</b>	<b>31.3</b>	<b>169</b>	<b>16.8</b>	<b>13.1</b>	<b>27.3</b>
<b>SE</b>	<b>1072</b>	<b>3.0</b>	<b>2.2</b>	<b>3.1</b>	<b>14.1</b>	<b>0.2</b>	<b>0.5</b>	<b>1.4</b>	<b>0.6</b>	<b>0.9</b>	<b>0.4</b>	<b>20.8</b>	<b>0.7</b>	<b>2.1</b>	<b>0.5</b>	<b>1.2</b>	<b>5.1</b>	<b>10.4</b>	<b>0.6</b>
<b>Min</b>	<b>2295</b>	<b>59</b>	<b>1</b>	<b>61</b>	<b>66</b>	<b>1</b>	<b>1</b>	<b>1</b>	<b>1</b>	<b>1</b>	<b>1</b>	<b>156</b>	<b>1</b>	<b>0</b>	<b>1</b>	<b>0</b>	<b>26</b>	<b>67</b>	<b>2</b>
<b>Max</b>	<b>4489</b>	<b>69</b>	<b>3</b>	<b>70</b>	<b>89</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>2</b>	<b>3</b>	<b>2</b>	<b>189</b>	<b>2</b>	<b>2</b>	<b>2</b>	<b>2</b>	<b>34</b>	<b>86</b>	<b>2</b>

LSD = Least significant difference, CV = coefficient of variation, SE = error, Min = minimum, Max = maximum, GY = grain yield (kg ha<sup>-1</sup>), AD = days to anthesis (days), ASI = anthesis-silking interval (days), DS = days to silking (days), EH = ear height (cm), EPP = ears per plant (#), LB = leaf blight disease (1-5), HC = husk cover, GLS = gray leaf spot disease (1-5), GT = grain texture (1-5), MSV = maize streak virus disease (1-5), PH = plant height (cm), RE = rotten ears (#), RL = root lodging (#), Rust = rust disease (1-5), SL = stem lodging (#), SWT = 100 seed weight (g), SH = shelling percentage, VIG = vigour, # = number.

**Appendix 8 Mean performance for grain yield and other agronomic traits across four low pH environments combined for 2011/12 and 2012/13 seasons**

<b>Genotypes</b>	<b>GY</b>	<b>AD</b>	<b>ASI</b>	<b>DS</b>	<b>EH</b>	<b>EPP</b>	<b>LB</b>	<b>HC</b>	<b>GLS</b>	<b>GT</b>	<b>MSV</b>	<b>PH</b>	<b>RE</b>	<b>RL</b>	<b>Rust</b>	<b>SL</b>	<b>SWT</b>	<b>SH%</b>	<b>VIG</b>
G1	1417	84.1	2.8	87.0	52.0	0.9	2.1	1.3	2.4	2.4	1.2	136	1.4	1.7	1.2	1.2	23	67	2.8
G2	1282	82.4	2.3	84.8	48.2	0.8	2.4	1.6	1.8	2.4	1.2	127	0.9	2.5	1.4	1.7	22	65	3.0
G3	1562	81.4	2.5	84.0	49.4	0.8	2.1	1.7	2.0	2.5	1.2	132	0.8	1.9	1.1	1.7	26	75	2.6
G4	1372	83.5	3.4	87.3	48.6	0.8	2.3	2.2	2.2	2.7	1.1	127	1.7	1.8	1.4	0.9	23	68	2.4
G5	1517	85.9	2.0	87.9	44.3	0.8	2.3	2.1	1.9	2.4	1.2	129	1.2	1.8	1.3	1.5	24	71	2.2
G6	1655	82.0	2.0	83.1	44.4	0.9	2.4	1.3	2.7	2.8	1.2	129	1.6	1.4	1.2	0.7	22	68	2.7
G7	1527	80.4	3.3	83.0	46.2	0.8	2.3	1.2	2.1	2.7	1.2	301	1.4	1.7	1.1	1.1	24	73	2.9
G8	1488	83.3	2.5	85.8	45.4	0.8	2.7	1.1	2.0	2.6	1.2	128	0.9	1.4	1.0	1.8	23	65	2.6
G9	1537	84.6	2.3	86.9	46.9	0.9	2.2	0.8	1.9	2.5	1.2	132	1.0	1.4	1.4	1.0	22	66	2.9
G10	1314	84.6	2.8	87.5	46.2	1.0	2.3	1.4	2.1	2.9	1.3	123	0.9	2.3	1.4	1.3	19	68	2.5
G11	1502	82.7	2.1	84.8	38.0	0.8	2.1	1.5	2.3	2.5	1.2	120	0.9	1.2	1.3	1.5	20	72	2.8
G12	1417	82.5	2.7	85.0	43.8	0.9	2.4	1.3	2.7	2.9	1.3	129	1.3	1.7	1.5	1.0	20	68	3.1
G13	1489	82.0	3.5	85.8	50.1	0.9	2.4	1.9	2.1	2.8	1.3	127	1.4	1.4	1.2	2.2	22	69	3.0
G14	1362	83.6	2.7	86.3	46.5	0.8	2.2	2.5	2.3	2.3	1.2	148	0.9	2.0	1.0	1.3	22	68	2.7
G15	1746	82.5	3.5	86.0	48.6	0.9	2.3	1.7	2.4	2.7	1.1	132	1.2	2.2	1.5	1.4	22	70	2.8
G16	1650	80.8	3.1	82.9	38.9	0.8	1.9	1.9	2.1	2.6	1.3	116	1.0	1.3	1.4	1.6	24	71	2.7
G17	1434	82.9	3.4	86.3	46.0	0.9	2.4	1.9	2.3	2.7	1.2	124	1.5	2.0	1.2	1.5	23	71	2.9
G18	1539	83.3	2.0	85.3	50.4	0.8	2.2	1.6	1.9	2.5	1.2	128	0.9	1.6	1.2	2.2	22	70	2.6
G19	1501	82.9	1.7	84.3	48.8	0.8	2.2	1.6	2.2	2.8	1.2	127	1.2	2.0	1.4	1.4	21	74	3.2
G20	1466	84.2	2.6	87.1	45.7	0.8	1.8	1.9	2.3	2.6	1.2	122	0.9	1.7	1.2	1.3	24	72	2.4
G21	1602	83.8	2.8	86.6	42.6	0.9	2.3	1.7	2.0	2.9	1.1	126	1.0	1.8	1.4	1.0	22	71	2.6
G22	1753	81.6	2.5	84.8	48.2	0.9	2.2	2.1	2.1	2.4	1.2	126	1.2	1.7	1.2	1.1	23	70	2.7
G23	1496	82.6	2.1	85.4	43.8	0.9	2.2	1.8	2.2	2.3	1.3	122	1.6	2.2	1.2	1.2	24	71	3.0
G24	1792	79.7	3.0	83.0	51.2	0.8	2.4	1.6	2.3	2.4	1.1	134	1.4	2.3	1.5	1.2	24	73	2.7
G25	1401	84.5	2.7	86.4	50.8	0.9	2.2	1.5	1.9	2.5	1.2	141	1.1	1.5	1.3	1.1	23	69	2.5

**Appendix 8 continued**

G26	1575	84.5	3.2	87.7	47.5	0.8	2.6	2.2	2.1	1.9	1.3	125	1.2	1.3	1.2	0.9	23	68	2.7
G27	1701	84.7	2.0	86.7	49.8	0.9	2.1	1.0	1.9	2.8	1.2	129	1.2	1.3	1.1	1.7	27	69	2.4
G28	1513	84.5	2.3	86.8	47.9	0.8	2.1	1.7	2.1	3.0	1.2	127	0.9	2.0	1.4	1.1	24	67	2.7
G29	1491	85.2	2.4	87.4	52.4	0.9	2.2	2.4	1.8	2.4	1.5	139	1.0	1.3	1.1	1.4	22	70	3.2
G30	1765	82.5	2.9	85.4	44.0	0.9	1.9	2.4	2.1	2.3	1.3	119	1.1	2.0	1.3	0.6	24	74	2.1
G31	1195	80.3	2.6	82.9	45.4	0.9	2.0	2.3	2.1	2.6	1.3	123	1.3	2.2	1.3	0.9	22	64	3.1
G32	1448	80.7	2.7	83.8	51.6	0.8	2.1	1.0	2.1	2.7	1.4	132	1.3	1.8	1.2	1.1	23	68	2.7
G33	1385	83.0	3.0	86.5	46.6	0.8	2.0	1.2	2.1	2.5	1.2	127	1.0	2.4	1.3	1.3	22	70	2.5
G34	1599	81.6	3.0	84.0	44.7	0.8	2.0	1.1	1.8	1.8	1.3	123	0.9	1.7	1.3	1.1	22	70	2.7
G35	1396	86.3	3.0	89.3	45.2	0.8	2.1	2.3	2.3	3.2	1.1	126	1.4	2.0	1.2	1.1	26	69	2.8
G36	1432	83.6	3.2	86.4	46.0	0.7	2.3	1.9	2.3	2.3	1.3	123	1.5	2.4	1.5	0.9	21	70	2.6
G37	1409	82.5	2.2	85.1	47.1	0.8	1.9	2.6	2.1	2.8	1.2	129	1.1	1.3	1.3	1.4	20	71	2.7
G38	1436	85.8	1.6	87.4	46.8	0.8	2.2	1.9	2.1	2.2	1.3	121	1.5	1.5	1.1	1.1	22	71	2.6
G39	1429	82.5	3.4	86.1	49.6	0.8	2.0	1.3	2.2	2.4	1.3	130	1.2	2.7	1.2	1.7	22	68	2.8
G40	1475	84.8	2.3	87.3	47.4	0.8	2.2	2.1	1.8	2.3	1.2	130	0.9	2.3	1.3	0.7	21	69	2.5
G41	1376	80.3	2.8	83.5	40.6	0.8	2.0	1.9	3.7	2.6	1.3	118	1.0	1.8	1.2	1.0	21	67	2.8
G42	1406	82.7	2.8	85.9	53.9	0.8	2.3	1.8	1.6	2.5	1.2	139	1.5	1.8	1.2	1.2	22	68	3.2
G43	1418	84.6	3.1	89.0	39.5	0.9	2.1	2.6	2.0	2.0	1.4	121	1.3	1.6	1.2	1.0	22	66	3.2
G44	1359	83.9	2.4	86.7	48.1	0.9	2.1	1.2	2.1	2.4	1.3	135	0.9	1.8	1.4	1.0	23	67	3.3
G45	1447	83.8	2.9	86.7	45.4	0.8	1.9	1.5	1.9	2.4	1.2	133	1.0	1.3	1.1	0.7	25	66	2.5
<b>Mean</b>	<b>1491</b>	<b>83.1</b>	<b>2.7</b>	<b>85.8</b>	<b>46.8</b>	<b>0.9</b>	<b>2.2</b>	<b>1.7</b>	<b>2.1</b>	<b>2.5</b>	<b>1.2</b>	<b>131.9</b>	<b>1.2</b>	<b>1.8</b>	<b>1.3</b>	<b>1.2</b>	<b>22.6</b>	<b>69.3</b>	<b>2.7</b>
<b>LSD</b>	<b>358</b>	<b>2.4</b>	<b>1</b>	<b>2.4</b>	<b>7.7</b>	<b>0.3</b>	<b>0.4</b>	<b>0.8</b>	<b>0.7</b>	<b>0.4</b>	<b>0.3</b>	<b>58</b>	<b>0.7</b>	<b>0.9</b>	<b>0.3</b>	<b>0.9</b>	<b>3</b>	<b>8</b>	<b>0.4</b>
<b>CV</b>	<b>42.4</b>	<b>5.1</b>	<b>67</b>	<b>5</b>	<b>28</b>	<b>54</b>	<b>35</b>	<b>83</b>	<b>59</b>	<b>28</b>	<b>37</b>	<b>77</b>	<b>103</b>	<b>92.1</b>	<b>44.6</b>	<b>127.6</b>	<b>20.3</b>	<b>19</b>	<b>27.4</b>
<b>SE</b>	<b>631</b>	<b>4.2</b>	<b>1.8</b>	<b>4.3</b>	<b>13.6</b>	<b>0.5</b>	<b>0.8</b>	<b>1.4</b>	<b>1.3</b>	<b>0.7</b>	<b>0.5</b>	<b>101.5</b>	<b>1.2</b>	<b>1.7</b>	<b>0.6</b>	<b>1.6</b>	<b>4.6</b>	<b>13</b>	<b>0.8</b>
<b>Min</b>	<b>1195.0</b>	<b>79.7</b>	<b>1.6</b>	<b>82.9</b>	<b>38.0</b>	<b>0.7</b>	<b>1.8</b>	<b>0.8</b>	<b>1.6</b>	<b>1.8</b>	<b>1.1</b>	<b>116.0</b>	<b>0.8</b>	<b>1.2</b>	<b>1.0</b>	<b>0.6</b>	<b>19.0</b>	<b>64.0</b>	<b>2.1</b>
<b>Max</b>	<b>1792.0</b>	<b>86.3</b>	<b>3.5</b>	<b>89.3</b>	<b>53.9</b>	<b>1.0</b>	<b>2.7</b>	<b>2.6</b>	<b>3.7</b>	<b>3.2</b>	<b>1.5</b>	<b>301.0</b>	<b>1.7</b>	<b>2.7</b>	<b>1.5</b>	<b>2.2</b>	<b>27.0</b>	<b>75.0</b>	<b>3.3</b>

LSD = Least significant difference, CV = coefficient of variation, SE = error, Min = minimum, Max = maximum, GY = grain yield (kg ha<sup>-1</sup>), AD = days to anthesis (days), ASI = anthesis-silking interval (days), DS = days to silking (days), EH = ear height (cm), EPP = ears per plant (#), LB = leaf blight disease (1-5), HC = husk cover, GLS = gray leaf spot disease (1-5), GT = grain texture (1-5), MSV = maize streak virus disease (1-5), PH = plant height (cm), RE = rotten ears (#), RL = root lodging (#), Rust = rust disease (1-5), SL = stem lodging (#), SWT = 100 seed weight (g), SH = shelling percentage, VIG = vigour, # = number.

**Appendix 9 Mean performance for grain yield and agronomic traits for low N environment 2012/13 season**

Genotypes	GY	Rank	AD	ASI	DS	EH	EPP	LB	GLS	GT	HC	PH	RL	RUST	SL	SWT	SH%
G1	826	12	68.7	2.3	71.0	33.6	0.8	3.5	1.0	2.3	0.3	77.3	6.3	1.0	3.3	32.0	64.5
G2	614	27	65.3	2.0	67.3	19.3	0.7	4.0	1.7	2.2	0.3	60.7	8.3	1.0	0.3	25.7	59.0
G3	467	37	66.3	3.0	67.0	38.3	1.1	3.0	2.0	2.3	1.7	73.0	5.0	1.2	1.0	27.0	60.0
G4	465	38	67.0	1.0	69.7	32.0	0.8	3.7	1.5	2.2	0.7	77.7	6.0	1.0	0.3	29.7	50.0
G5	396	42	72.3	1.7	75.0	38.6	0.6	2.2	1.2	2.0	0.0	72.7	3.3	1.0	1.7	29.0	51.0
G6	493	33	68.3	1.7	74.0	31.0	0.6	3.0	1.5	2.2	0.7	76.3	1.7	1.0	1.0	29.7	43.0
G7	721	22	68.0	3.0	70.0	37.6	0.7	3.3	1.8	2.0	0.0	74.3	6.0	1.0	1.7	30.7	56.7
G8	504	31	68.0	2.3	71.0	37.6	0.8	2.8	1.0	1.5	0.0	80.7	1.0	1.0	0.3	35.4	52.2
G9	1146	1	68.0	2.0	70.3	56.6	0.9	3.2	1.3	1.7	1.3	108.3	2.3	1.0	2.0	30.0	74.5
G10	1145	2	72.3	1.3	73.0	37.3	0.8	2.8	1.5	1.7	0.0	72.3	4.3	1.0	1.0	34.4	70.8
G11	744	21	69.7	2.7	71.0	43.0	0.8	3.3	1.7	2.0	0.0	88.3	5.0	1.0	2.0	25.7	68.9
G12	471	34	67.7	2.3	70.3	44.0	0.8	3.3	1.8	2.3	0.0	77.3	7.3	1.0	1.7	24.7	57.2
G13	1113	3	69.0	1.3	71.3	35.6	0.7	3.3	1.7	2.2	0.7	82.3	4.7	1.0	0.7	28.7	70.3
G14	640	25	66.3	1.7	67.7	34.3	0.7	2.7	1.3	2.5	0.0	78.0	3.0	1.0	1.0	28.0	63.9
G15	377	43	66.0	2.3	67.7	32.6	0.5	3.5	1.3	2.0	0.0	69.3	2.0	1.0	1.7	27.7	62.4
G16	788	18	65.7	3.3	68.0	31.0	0.7	3.0	1.7	1.5	2.3	62.7	6.0	1.0	2.3	30.0	59.2
G17	820	14	66.3	0.7	69.7	43.0	0.8	3.0	1.8	2.2	0.0	86.0	1.7	1.0	0.0	28.4	69.4
G18	432	39	65.7	2.7	66.3	22.3	0.6	3.3	1.2	1.7	0.7	59.7	6.0	1.0	2.0	30.4	60.8
G19	974	6	67.7	2.0	70.3	41.3	1.0	4.0	1.2	2.0	2.3	81.3	2.7	1.0	2.3	31.4	63.9
G20	959	7	65.7	1.3	67.7	45.3	0.7	3.0	1.8	2.7	1.0	92.0	6.3	1.0	1.3	30.0	56.7
G21	938	8	66.0	1.0	67.3	33.3	0.7	3.2	2.2	1.5	0.3	70.0	4.7	1.0	2.3	28.7	68.3
G22	678	24	63.3	1.3	64.3	49.6	0.8	3.7	1.3	1.5	0.7	100.0	3.7	1.0	4.0	28.0	63.3
G23	324	44	62.0	1.3	63.3	35.6	0.7	3.5	1.2	2.0	0.7	69.7	7.3	1.0	4.0	25.7	55.6
G24	1008	4	62.3	1.0	63.7	36.6	0.8	2.5	1.7	2.5	0.0	65.0	1.7	1.0	0.0	28.7	67.3
G25	790	16	64.0	2.7	65.0	32.3	1.0	2.8	1.3	2.2	0.0	67.7	2.7	1.0	1.3	34.4	65.5

**Appendix 9 continued**

G26	502	32	68.3	1.3	71.0	35.3	0.6	2.7	1.3	2.5	0.0	80.7	1.3	1.0	2.3	30.0	57.2
G27	924	10	68.0	1.3	69.3	43.3	0.8	3.3	1.5	2.3	1.3	88.7	4.3	1.0	0.3	28.4	67.9
G28	799	15	66.0	2.3	67.3	43.3	0.9	3.3	1.8	3.0	0.3	95.0	6.3	1.0	2.3	27.4	58.3
G29	506	29	71.3	0.7	73.7	18.6	0.6	2.7	1.0	1.7	0.3	45.7	4.0	1.0	1.7	26.7	61.6
G30	820	13	67.7	3.0	70.7	47.0	0.6	3.3	1.3	1.8	0.7	81.3	7.0	1.0	0.0	30.0	61.1
G31	538	28	68.0	2.3	71.0	33.6	1.3	3.5	1.2	2.0	0.0	79.0	5.7	1.0	2.7	26.7	51.8
G32	431	40	63.7	2.3	66.0	33.0	0.9	3.2	1.5	2.0	0.0	67.3	2.3	1.0	1.0	24.7	46.7
G33	755	20	65.7	0.7	68.0	39.3	0.6	3.2	1.2	2.7	0.0	87.3	3.0	1.0	2.0	23.0	61.7
G34	398	41	65.0	1.3	65.7	38.0	0.9	3.7	1.5	2.5	0.0	87.0	7.3	1.2	1.7	30.7	53.3
G35	900	11	65.0	2.0	66.3	45.6	0.9	3.5	1.3	2.2	0.0	94.0	4.3	1.0	0.3	28.0	70.0
G36	789	17	65.3	1.0	67.3	36.6	0.6	2.7	1.5	2.3	0.0	65.3	3.7	1.0	2.0	27.7	62.0
G37	469	36	64.3	2.0	65.3	39.3	0.9	2.5	1.5	2.2	0.3	66.3	1.3	1.0	1.7	29.4	65.3
G38	505	30	69.0	1.3	71.0	26.6	0.7	2.3	1.2	2.0	0.0	56.0	0.3	1.0	0.0	31.7	65.9
G39	615	26	65.0	2.7	66.3	31.6	0.8	3.0	1.5	2.5	0.3	64.3	5.0	1.0	1.7	26.7	59.3
G40	469	35	66.7	1.3	67.7	34.3	0.9	2.3	1.3	1.8	0.3	68.7	2.7	1.0	0.7	30.7	51.9
G41	1000	5	64.0	0.0	65.3	48.6	0.9	3.5	1.3	1.7	0.0	93.3	2.7	1.0	1.3	24.0	68.3
G43	690	23	55.0	2.7	64.3	34.0	1.0	2.7	1.7	1.8	0.3	65.7	2.7	1.0	1.0	29.4	63.3
G44	762	19	65.9	1.6	57.7	47.3	0.8	3.0	1.7	2.5	0.0	91.3	2.7	1.0	1.3	27.0	68.9
G45	938	9	72.3	2.7	67.4	38.1	0.8	3.4	1.0	1.6	0.2	69.2	4.8	1.0	1.4	28.4	61.4
<b>MEAN</b>	<b>696</b>		<b>66.5</b>	<b>1.8</b>	<b>68.3</b>	<b>37.2</b>	<b>0.8</b>	<b>3.1</b>	<b>1.5</b>	<b>2.1</b>	<b>0.4</b>	<b>76.6</b>	<b>4.1</b>	<b>1.0</b>	<b>1.5</b>	<b>28.7</b>	<b>61.1</b>
<b>P</b>	<b>0.73</b>		<b>0.0</b>	<b>0.1</b>	<b>0.0</b>	<b>0.3</b>	<b>0.3</b>	<b>0.0</b>	<b>0.1</b>	<b>0.3</b>	<b>0.3</b>	<b>0.3</b>	<b>0.0</b>	<b>0.5</b>	<b>0.2</b>	<b>0.3</b>	<b>0.8</b>
<b>LSD</b>	<b>695.8</b>		<b>3.1</b>	<b>1.9</b>	<b>3.7</b>	<b>19.6</b>	<b>0.4</b>	<b>0.9</b>	<b>27.5</b>	<b>0.6</b>	<b>1.6</b>	<b>32.8</b>	<b>4.4</b>	<b>0.1</b>	<b>2.4</b>	<b>7.1</b>	<b>0.4</b>
<b>CV</b>	<b>61.8</b>		<b>2.9</b>	<b>63.8</b>	<b>3.3</b>	<b>32.3</b>	<b>31.3</b>	<b>18.7</b>	<b>0.7</b>	<b>27.5</b>	<b>246.7</b>	<b>26.4</b>	<b>65.7</b>	<b>6.0</b>	<b>102.0</b>	<b>15.3</b>	<b>31.3</b>
<b>Min</b>	<b>324.3</b>		<b>55.0</b>	<b>0.0</b>	<b>57.7</b>	<b>18.6</b>	<b>0.5</b>	<b>2.2</b>	<b>1.0</b>	<b>1.5</b>	<b>0.0</b>	<b>45.7</b>	<b>0.3</b>	<b>1.0</b>	<b>0.0</b>	<b>23.0</b>	<b>43.0</b>
<b>Max</b>	<b>1146</b>		<b>72.3</b>	<b>3.3</b>	<b>75.0</b>	<b>56.6</b>	<b>1.3</b>	<b>4.0</b>	<b>2.2</b>	<b>3.0</b>	<b>2.3</b>	<b>108.3</b>	<b>8.3</b>	<b>1.2</b>	<b>4.0</b>	<b>35.4</b>	<b>74.5</b>

LSD = Least significant difference, CV = coefficient of variation, Min = minimum, Max = maximum, GY = grain yield (kg ha<sup>-1</sup>), AD = days to anthesis (days), ASI = anthesis-silking interval (days), DS = days to silking (days), EH = ear height (cm), EPP = ears per plant (#), LB = leaf blight disease (1-5), GLS = gray leaf spot disease (1-5), GT = grain texture (1-5), HC = husk cover, PH = plant height (cm), RL = root lodging (#), Rust = rust disease (1-5), SL = stem lodging (#), SWT = 100 seed weight (g), SH = shelling percentage, # = number.

**Appendix 10 Mean performance combined across two years and across optimal and low pH environment for 2011/12 and 2012/13 seasons**

Genotypes	GY	AD	ASI	DS	PH	EH	EPP	RL	SL	GLS	LB	MSV	RUST	GT	SH%	VIG	RE	HC	SWT
G1	2060.1	75.9	2.2	77.9	143.0	58.3	1.0	2.3	1.5	1.0	2.3	1.1	1.6	2.0	70.3	2.7	0.9	1.2	27.2
G2	1368.4	73.4	1.5	75.2	136.5	57.6	0.9	3.0	1.2	1.0	2.4	1.1	1.7	2.8	58.7	2.8	1.1	1.0	23.5
G3	2240.9	72.4	1.4	73.9	136.4	59.7	1.1	2.2	1.3	1.0	2.0	1.1	1.4	2.2	77.3	2.4	0.9	1.4	25.9
G4	2159.8	73.6	2.4	75.9	136.8	57.8	1.0	2.0	0.8	1.0	2.1	1.1	1.7	2.8	72.1	2.3	1.3	1.5	24.6
G5	2610.7	77.9	1.1	79.1	151.0	61.0	1.0	1.1	1.2	1.0	2.0	0.9	1.6	2.3	69.1	1.9	0.9	1.5	26.9
G6	2222.2	73.2	1.3	74.6	155.8	66.7	1.1	2.5	1.0	1.0	2.3	1.1	1.6	2.7	69.2	2.4	1.2	1.3	25.5
G7	1454.7	73.0	2.3	75.2	273.4	58.6	1.0	1.3	1.0	1.0	2.1	1.1	1.5	2.5	71.6	2.3	1.4	1.1	29.2
G8	2739.4	74.7	1.8	76.5	152.8	66.0	1.1	1.5	1.6	1.0	2.5	1.0	1.4	2.0	70.1	2.4	0.9	1.4	26.3
G9	2293.9	75.3	1.7	76.8	148.6	63.4	1.0	2.6	1.0	1.0	2.1	1.0	1.7	2.5	70.3	2.2	0.9	0.9	26.3
G10	2529.0	77.0	2.1	79.1	147.9	64.8	1.6	1.8	1.0	1.0	1.9	1.1	1.7	3.0	73.5	2.1	1.2	0.8	26.6
G11	1628.9	76.3	1.4	77.6	133.0	52.6	1.0	2.7	1.2	1.0	2.0	1.1	1.7	3.2	74.3	2.8	0.3	1.2	22.3
G12	2121.4	73.9	2.5	76.2	145.7	59.6	1.1	3.0	1.2	1.0	2.2	1.1	1.8	2.0	71.2	2.6	1.1	1.1	22.5
G13	2511.7	74.6	2.5	77.0	142.1	62.1	1.1	1.7	1.0	1.0	2.1	1.2	1.5	3.3	74.8	2.6	1.5	1.4	25.8
G14	2108.0	74.2	1.9	76.1	159.2	60.0	1.0	2.0	1.3	1.0	2.1	1.1	1.4	2.5	68.3	2.1	0.7	1.3	27.5
G15	2244.0	71.5	2.8	74.2	138.2	56.1	1.0	1.4	1.0	1.0	2.4	1.0	1.8	2.5	71.2	2.6	1.2	1.8	24.3
G16	2131.7	69.6	2.2	71.7	136.8	58.3	1.0	1.7	1.2	1.0	2.0	1.1	1.7	3.3	71.1	2.2	1.6	2.6	27.3
G17	2158.6	73.8	2.5	76.3	143.6	61.7	1.1	1.8	0.9	1.0	2.1	1.0	1.5	2.3	73.8	2.6	0.9	1.6	24.1
G18	2600.8	73.9	1.1	75.1	148.4	64.2	1.1	2.0	1.2	1.0	2.2	1.0	1.6	2.3	73.3	2.2	0.7	1.4	26.5
G19	2223.4	74.3	1.2	75.5	142.0	60.9	1.0	1.7	1.2	1.0	2.3	1.1	1.6	1.8	75.0	2.6	0.9	2.1	25.3
G20	2848.8	75.6	1.9	77.4	146.5	65.9	1.0	1.7	0.8	1.0	1.9	1.1	1.6	2.7	72.9	2.0	0.9	1.8	28.1
G21	2350.0	74.1	1.8	75.9	150.8	64.6	1.1	1.7	1.3	1.0	2.3	1.0	1.7	2.8	74.4	2.3	0.9	1.4	26.2
G22	2389.2	71.7	2.8	74.4	146.1	61.3	1.1	2.1	1.3	1.0	2.2	1.1	1.6	2.3	72.4	2.4	1.1	1.4	25.4
G23	2028.7	70.5	2.4	72.8	131.5	52.3	1.0	3.2	1.6	1.0	2.3	1.1	1.8	2.5	70.1	2.8	1.6	1.1	23.7
G24	2662.0	69.9	2.3	72.2	146.0	60.7	1.1	1.3	1.2	1.0	2.5	0.9	1.6	2.0	74.6	2.4	0.7	1.5	25.7
G25	2473.3	72.5	0.8	73.4	146.1	63.1	1.1	1.7	1.1	1.0	2.1	1.1	1.7	2.3	73.9	2.3	1.2	1.4	26.2
G26	2615.8	75.8	2.5	78.1	148.1	62.9	1.0	1.0	1.1	1.0	2.1	1.1	1.5	1.7	69.6	2.3	0.9	1.3	26.2
G27	2934.4	76.7	1.9	78.6	158.2	70.4	1.2	1.8	1.0	1.0	2.2	1.1	1.5	2.3	74.1	1.9	0.9	1.3	26.4
G28	2698.0	72.7	2.1	74.8	149.0	65.7	1.1	2.3	1.4	1.0	2.1	1.1	1.7	2.3	72.7	2.1	0.7	0.9	25.1

**Appendix 10 continued**

G29	2270.6	76.8	1.6	78.3	145.5	60.1	1.0	1.4	1.2	1.0	2.1	1.1	1.5	2.3	71.7	2.6	0.8	1.7	25.4
G30	2554.8	74.3	2.0	76.3	147.8	66.8	1.0	2.0	0.5	1.0	2.1	1.0	1.6	2.0	73.4	2.1	0.9	1.4	25.8
G31	2037.4	73.0	2.1	75.1	140.1	63.4	1.1	2.4	1.2	1.0	2.1	1.1	1.7	2.3	68.6	2.5	1.1	1.3	23.0
G32	2271.4	71.7	2.1	73.7	145.8	62.5	1.1	2.0	1.3	1.0	2.2	1.0	1.8	2.8	71.4	2.3	0.9	1.4	24.5
G33	2424.7	71.9	2.1	74.1	146.9	63.6	1.1	2.0	1.3	1.0	2.0	1.1	1.6	1.5	72.0	2.4	1.0	1.1	23.3
G34	2177.4	70.3	1.8	72.0	144.2	61.0	1.2	3.6	0.9	1.0	2.4	1.1	1.7	2.0	74.2	2.7	0.8	1.2	22.9
G35	2303.4	74.3	2.1	76.3	146.1	61.1	1.0	2.6	1.1	1.0	2.1	0.9	1.6	2.5	75.1	2.5	0.9	2.3	27.2
G36	2509.3	72.1	2.3	74.4	147.7	63.7	1.0	1.7	1.2	1.0	2.1	1.1	1.9	1.5	73.0	2.2	1.2	1.3	25.6
G37	1450.0	71.5	1.8	73.3	134.9	51.8	1.1	1.5	1.0	1.0	1.9	1.1	1.6	2.2	70.1	2.8	0.7	0.8	24.9
G38	1000.1	76.1	1.4	77.3	142.3	59.5	0.9	1.0	0.9	1.0	2.1	0.9	1.4	1.8	66.0	2.2	1.4	0.9	27.0
G39	2518.1	71.4	1.8	73.3	137.9	55.3	1.1	2.1	1.2	1.0	2.1	1.1	1.7	3.0	74.3	2.4	0.9	1.5	26.5
G40	1573.2	76.7	1.7	78.3	140.0	56.7	1.0	1.1	0.5	1.0	1.9	1.1	1.4	3.0	69.6	2.5	0.7	1.3	26.9
G41	2153.2	67.6	2.0	69.7	132.4	51.7	1.0	2.0	1.1	0.7	2.0	1.0	1.8	2.3	76.1	2.7	0.8	1.2	25.0
G42	2109.8	73.8	2.0	75.8	138.9	57.6	1.0	1.3	1.2	0.0	2.1	1.0	1.6	2.3	71.1	2.4	1.4	0.8	25.2
G43	2632.7	70.4	3.1	73.4	146.3	60.1	1.0	1.7	0.9	1.0	2.0	1.0	1.8	1.7	69.5	2.5	0.9	1.4	26.5
G44	2617.6	72.3	2.2	74.6	146.2	61.5	1.0	1.9	1.0	1.0	2.1	1.0	1.6	2.8	70.1	2.6	0.7	1.3	26.7
G45	2764.9	75.7	1.9	77.7	148.6	64.8	1.0	1.6	0.9	1.0	1.9	1.1	1.6	2.7	71.5	2.1	0.8	1.0	28.2
<b>Mean</b>	<b>2261.7</b>	<b>73.5</b>	<b>2.0</b>	<b>70.5</b>	<b>147.2</b>	<b>60.8</b>	<b>1.1</b>	<b>1.9</b>	<b>1.1</b>	<b>71.7</b>	<b>2.1</b>	<b>1.0</b>	<b>1.6</b>	<b>2.4</b>	<b>71.7</b>	<b>2.4</b>	<b>1.0</b>	<b>1.3</b>	<b>25.7</b>
<b>Min</b>	<b>1000.1</b>	<b>67.6</b>	<b>0.8</b>	<b>131.5</b>	<b>51.7</b>	<b>0.9</b>	<b>1.0</b>	<b>0.5</b>	<b>0.0</b>	<b>1.9</b>	<b>0.9</b>	<b>1.4</b>	<b>1.5</b>	<b>58.7</b>	<b>1.9</b>	<b>0.3</b>	<b>0.8</b>	<b>22.3</b>	<b>22.3</b>
<b>Max</b>	<b>2934.4</b>	<b>77.9</b>	<b>3.1</b>	<b>273.4</b>	<b>70.4</b>	<b>1.6</b>	<b>3.6</b>	<b>1.6</b>	<b>1.0</b>	<b>2.5</b>	<b>1.2</b>	<b>1.9</b>	<b>3.3</b>	<b>77.3</b>	<b>2.8</b>	<b>1.6</b>	<b>2.6</b>	<b>29.2</b>	<b>29.2</b>

Min = minimum, Max = maximum, GY = grain yield (kg ha<sup>-1</sup>), AD = days to anthesis (days), ASI = anthesis-silking interval (days), DS = days to silking (days), PH = plant height (cm), EH = ear height (cm), EPP = ears per plant (#), RL = root lodging (#), SL = stem lodging (#), GLS = gray leaf spot disease (1-5), LB = leaf blight disease (1-5), MSV = maize streak virus disease (1-5), Rust = rust disease (1-5), GT = grain texture (1-5), SH = shelling percentage, VIG = vigour, RE = rotten ears (#), HC = husk cover, SWT = 100 seed weight (g), # = number..

## Appendix 11 Genotypes used in genotype x environment interactions and stability analysis

Genotype code	Pedigree
G1	DT-WSTR SYNTHETIC-B
G2	EVD-W 99 STR QPM CO-B
G3	IAR-FLINT-Q-B
G4	IWD C3 SYN F2-B
G5	MULTICOB EARLY DT -B
G6	SYN DTE STY-W-B
G7	OBA SUPER1(9021-18(IITA))-B
G8	LPHpop 9
G9	POP66 SR/DMR -LSRY/DMR-LSRY
G10	POP66 SR/TZUTSR-WSGY/T
G11	LPHpop 7
G12	TZE-YDT STR C4-B
G13	VPO0721
G14	TZE E -WPOP X LD(SET2)-B
G15	VPO5173
G16	VPO717
G17	VPO5187
G18	VPO52
G19	VPO630
G20	LPHpop 21
G21	VPO741
G22	VPO739
G23	LPHpop 18
G24	LPHpop 16
G25	VPO743
G26	VPO744
G27	LOW N POOL C3-B
G28	LPHpop 10
G29	VPO76
G30	LPHpop 3
G31	LPHpop 20
G32	LPHpop 2
G33	VPO710
G34	VPO712
G35	LPHpop 8
G36	OBATANPA/TZLCOMP4C3F2/TZLCOMP4C3F2-B
G37	VPO738
G38	LPHpop 13
G39	LPHpop 15
G40	VPO86
G41	VPO96
G42	VPO97
G43	ZM309 Check 1
G44	ZM523 Check 2
G45	ZM721 Check 3

**Appendix 12 Estimated specific combining ability effects of 12 inbred lines for grain yield and agronomic traits across low pH and optimal environments**

Cross	Pedigree	GY	AD	ASI	DS	EH	EPP	LB	GLS	GT	MSV	PH	RL	Rust	SWT	SH%	SL	VIG
S12	CZL999601/CML481	-0.646	1.74	-1.71	3.57	2.14	-5.37	-0.40	-0.48	0.33	0.07	-5.04	0.47	0.22	-0.25	-0.76	-0.72	0.00
S13	CZL999601/CML359	-0.148	-0.18	-1.98	1.38	3.51	3.08	0.11	0.86	-0.25	-0.11	5.06	-0.80	0.44	1.76	-4.32	-0.68	-0.30
S14	CZL999601/CML144	0.943	2.14	-0.62	2.54	2.66	-4.24	-0.50	-0.04	0.40	-0.01	0.81	-0.20	0.03	3.53	-5.75	1.20	-0.30
S15	CZL999601/CML161	-0.13	-0.57	-1.20	1.13	-0.74	2.61	-0.20	-0.07	-0.16	-0.11	-3.89	-0.10	0.09	0.05	-6.84	-0.28	-0.10
S16	CZL999601/CML172	-0.449	-1.87	1.26	-1.86	0.78	2.84	-0.10	0.18	0.10	-0.05	3.17	-0.80	-0.18	-2.09	41***	0.24	0.10
S17	CZL999601/CML448	-0.097	-2.54	-0.45	-1.17	4.05	-4.36	0.82	0.16	0.23	0.02	6.48	-0.50	0.07	-0.54	-3.28	0.16	0.99
S18	CZL999601/CML312	0.401	-1.61	0.99	-2.47	-6.67	-1.62	-0.10	0.06	-0.29	0.03	-1.25	0.87	-0.46	-0.23	2.73	0.48	0.05
S19	CZL999601/CZL130-23	0.212	0.61	1.15	-0.28	-0.63	0.04	-0.20	-0.10	-0.35	-0.02	-0.94	0.89	-0.06	-0.52	-1.11	0.41	-0.70
S110	CZL999601/CML288	-0.361	0.37	0.95	-2.40	-0.92	2.66	0.69	0.15	0.37	-0.01	-3.07	0.36	0.01	-2.47	-9.89	-0.22	0.47
S111	CZL999601/CML202	0.668	-0.16	-0.97	0.66	-4.62	1.74	-0.10	-0.61	-0.09	0.11	-5.65	0.03	0.14	2.48	-1.08	-0.18	0.30
S23	CML481/CML359	-0.387	0.32	0.10	-0.07	-0.71	2.29	0.65	0.21	0.23	-0.07	0.22	-1.30	0.17	-3.67	2.34	2.38	0.60
S24	CML481/CML144	-0.316	0.65	-1.17	1.90	0.21	-4.37	0.88	-0.35	0.04	-0.03	0.86	1.72	0.21	-1.48	10.49	0.15	0.18
S25	CML481/CML161	0.487	-2.44	-0.75	-1.37	-2.68	4.74	0.06	-0.11	-0.09	0.04	1.21	1.71	-0.07	-1.61	-3.99	0.11	0.30
S26	CML481/CML172	-0.219	0.18	1.79	-0.59	-2.86	4.14	-0.10	-0.13	-0.03	-0.07	-1.19	-1.20	-0.12	-3.49	-6.57	-0.59	0.85
S27	CML481/CML448	0.403	2.14	-0.46	1.28	-6.09	-4.51	-0.50	0.06	0.38	0.00	-16.8	2.82	0.13	1.37	7.76	-0.34	-0.60
S28	CML481/CML312	-0.079	2.44	1.16	1.53	-1.31	-1.56	0.24	0.24	-0.04	0.12	-3.39	-1.50	-0.01	-0.15	-7.11	0.54	-0.10
S29	CML481/CZL130-23	0.595	-0.41	2.57	-2.02	3.59	3.75	-0.30	0.08	-0.84	0.02	11.30	-0.30	-0.17	3.69	-2.41	-0.43	-0.60
S210	CML481/CML288	0.703	-2.18	-1.97	-1.07	4.27	0.47	-0.60	0.16	-0.10	-0.14	7.43	-1.60	-0.28	3.19	1.55	-0.61	-0.50
S211	CML481/CML202	-0.3	-0.84	-1.89	1.58	0.80	3.20	0.02	0.24	0.32	0.04	-0.09	-0.60	0.15	-0.93	6.21	-0.23	-0.20
S34	CML359/CML144	-0.593	1.96	-2.34	5.21	-2.52	-3.54	-0.20	-0.40	0.00	-0.15	-1.07	-0.60	-0.07	-0.79	6.07	-0.59	0.62
S35	CML359/CML161	0.189	0.43	0.03	0.36	6.07	-2.01	-0.30	0.13	-0.19	-0.25	5.18	-0.70	-0.01	5.57	-2.19	0.03	-0.20
S36	CML359/CML172	0.404	0.82	0.85	-0.85	-3.31	-1.38	-0.50	0.04	-0.42	-0.20	-5.69	-0.20	-0.11	2.14	-7.08	-0.22	-0.50
S37	CML359/CML448	0.236	2.42	0.32	1.48	-2.98	3.30	0.38	-0.26	0.06	0.04	-7.54	1.31	-0.09	-2.64	7.71	-0.19	0.10

**Appendix 12 continued**

S38	CML359/CML312	0.012	-1.50	0.20	-2.05	6.58	-0.35	0.44	0.30	-0.18	-0.06	6.24	-1.40	0.05	-1.27	-1.26	-0.42	-0.10
S39	CML359/CZL130-23	-0.032	0.78	-1.53	0.37	3.20	1.97	-0.20	-0.46	-0.24	0.01	3.66	-0.40	-0.22	-2.15	2.12	-0.50	0.04
S310	CML359/CML288	0.144	-6.71	3.90	-5.26	-6.93	1.77	-0.30	-0.41	0.91	0.92	0.69	0.19	-0.02	-2.59	-4.89	0.27	-0.10
S311	CML359/CML202	-0.249	-0.47	-0.10	-0.77	-0.86	-3.04	0.05	0.19	0.20	-0.08	-2.13	0.03	0.04	-0.62	-0.16	-0.19	-0.40
S45	CML144/CML161	-0.234	-1.63	-2.90	0.34	4.24	-1.81	0.09	0.51	0.08	0.07	-2.76	-0.80	0.08	-0.77	-0.35	0.02	0.15
S46	CML144/CML172	-0.218	-1.94	-0.42	-1.42	-5.95	3.23	-0.20	-0.47	0.26	-0.10	-2.22	-0.20	-0.30	1.19	-5.11	-0.01	0.28
S47	CML144/CML448	-0.156	-2.53	-2.04	-0.36	-5.21	3.55	0.56	0.01	0.03	0.14	-7.19	-1.10	0.11	-1.29	6.61	-0.20	0.26
S48	CML144/CML312	0.431	-1.71	1.01	-2.99	2.33	-0.65	-0.30	-0.37	-0.44	-0.07	0.66	2.19	0.14	3.51	-0.84	-0.10	-0.30
S49	CML144/ZL130-23	-0.475	3.80	2.79	0.85	-6.46	1.10	-0.10	-0.03	-0.67	0.00	-13.0	1.09	-0.13	-1.89	-8.34	-0.18	-0.10
S410	CML144/CML288	0.208	-1.12	-0.31	-0.88	-0.11	-3.61	-0.10	0.55	0.25	0.17	-6.27	2.35	0.22	-1.86	-3.25	-0.14	-0.30
S411	CML144/CML202	0.731	-1.30	2.60	-4.03	7.70	7.40	-0.20	0.62	-0.16	0.01	18.64	-0.30	-0.44	3.00	0.15	-0.26	-0.30
S56	CML161/CML172	0.671	0.43	0.29	-0.63	0.46	-0.44	-0.30	0.28	0.12	0.47	-4.05	1.41	-0.19	0.26	2.91	0.06	-0.50
S57	CML161/CML448	-0.359	-0.93	0.00	-0.15	-6.13	-1.99	0.29	-0.52	0.03	-0.13	2.43	-1.70	-0.39	1.24	-2.43	-0.24	0.64
S58	CML161/CML312	-0.12	0.40	1.43	-0.99	-8.88	0.63	0.18	-0.01	-0.17	0.22	-11.0	0.28	-0.14	0.30	2.04	-0.36	0.19
S59	CML161/CZL130-23	0.094	-2.56	-1.92	-1.41	2.94	-2.72	0.31	0.17	-0.78	-0.16	4.70	-0.60	-0.02	-3.97	4.02	0.67	-0.20
S510	CML161/CML288	-0.557	4.03	4.14	0.90	-6.64	-2.65	-0.40	-0.14	-0.52	-0.27	-2.01	-1.20	0.06	2.53	8.84	0.38	0.11
S511	CML161/CML202	-0.558	1.41	0.86	0.20	4.13	3.99	0.00	-0.12	2.5***	-0.09	-0.89	0.11	0.35	-4.81	-8.44	-0.13	-0.10
S67	CML172/CML448	-0.185	-1.34	-0.68	-1.45	-0.41	-2.13	0.16	0.01	0.50	-0.02	6.91	3.29	0.51	-0.25	-15.15	0.40	-0.10
S68	CML172/CML312	-0.301	0.57	-0.26	0.30	1.31	0.76	0.77	0.02	0.17	-0.12	-0.73	0.32	0.15	-0.41	2.33	-0.51	0.24
S69	CML172/CZL130-23	0.521	-0.89	-1.03	0.46	2.25	-2.58	-0.20	0.64	-0.10	0.23	5.94	-0.80	0.33	2.40	1.80	0.08	0.13
S610	CML172/CML288	-0.107	1.54	-0.04	2.03	7.96	-2.56	-0.30	-0.22	-0.29	-0.05	8.02	-0.40	-0.05	1.04	-0.67	0.46	-0.50
S611	CML172/CML202	-0.013	1.59	-0.10	1.27	-4.40	-1.29	0.83	-0.43	-0.08	-0.03	-7.51	0.31	-0.08	-2.44	-9.69	0.06	0.58
S78	CML448/CML312	0.225	-1.04	-1.79	0.78	-0.07	-0.95	-0.50	0.16	0.16	-0.04	0.09	-0.80	0.12	1.21	-0.48	-0.03	-0.40
S79	CML448/CZL130-23	0.13	0.25	0.60	-0.14	-4.27	-4.33	0.00	-0.11	-0.49	0.08	-1.35	0.12	-0.49	5.04	7.08	0.12	-0.40
S710	CML448/CML288	-0.06	3.53	1.27	1.06	12.49	16.6***	-0.40	-0.09	0.13	-0.14	13.18	-1.00	0.03	-0.50	-12.54	0.27	-0.20
S711	CML448/CML202	0.039	1.99	3.10	-1.62	8.12	-2.87	-0.50	0.38	-0.75	0.04	12.83	-0.50	0.16	1.22	0.83	-0.13	0.00
S89	CML312/CZL130-23	-0.161	-1.09	-2.88	1.94	3.81	5.02	-0.20	0.34	2.2***	0.04	4.26	-0.80	0.32	-0.81	-4.94	-0.01	-0.30
S810	CML312/CML288	-0.233	3.09	2.21	0.73	-2.63	-1.53	0.41	-0.41	0.06	-0.13	-6.77	-0.60	-0.05	-4.23	0.89	-0.19	-0.30

**Appendix 12 continued**

S811	CML312/CML202	0.145	2.06	0.62	2.00	1.79	-0.30	-0.60	-0.44	-0.76	0.00	3.61	-1.20	0.02	5.8**	2.21	0.52	-0.10
S910	CZL130-23/CML288	-0.254	-0.79	-3.50	2.16	5.32	-4.91	0.59	-0.34	-0.53	-0.17	-0.65	0.90	0.35	1.55	7.76	0.07	1.08
S911	CZL130-23/CML202	-0.65	1.10	1.94	0.24	-3.31	-3.71	0.34	-0.10	-0.95	0.01	-5.54	1.15	-0.30	2.71	3.13	-0.11	0.24
S112	CZL999601/CML539	-0.393	2.07	2.58	-1.09	0.43	2.62	-0.10	-0.11	-0.29	0.07	4.32	-0.30	-0.29	-1.72	-10.72	-0.41	-0.40
S212	CML481/CML539	-0.241	-1.60	2.33	-4.75	2.65	-2.79	0.03	0.08	-0.18	0.00	5.53	-0.50	-0.23	3.32	-7.50	-0.25	0.07
S312	CML359/CML539	0.422	2.12	0.54	0.20	-2.05	-2.09	-0.20	-0.19	-0.12	-0.07	-4.61	1.75	-0.17	4.25	1.66	0.12	0.18
S412	CML144/CML539	-0.322	1.66	3.39	-1.16	3.10	2.94	0.20	-0.04	0.21	-0.03	11.57	-0.90	0.14	-3.13	0.34	0.10	-0.20
S512	CML161/CML539	0.518	1.41	0.03	1.61	7.23	-0.36	0.30	-0.12	-0.83	0.21	11.10	1.11	0.26	1.20	6.42	-0.26	-0.30
S612	CML172/CML539	-0.105	0.92	-1.66	2.73	4.17	-0.59	-0.10	0.08	-0.23	-0.07	-2.64	-2.60	0.04	1.65	-3.79	0.04	-0.60
S712	CML448/CML539	-0.176	-1.94	0.14	0.29	0.50	-2.32	-0.30	0.22	-0.29	0.00	-9.00	-0.20	-0.16	-4.86	3.89	0.19	-0.30
S812	CML312/CML539	-0.32	-1.62	-2.68	1.21	3.74	0.54	-0.20	0.12	-0.70	0.01	8.29	2.32	-0.13	-3.76	4.45	0.06	1.04
S912	ZL130-23/CML539	0.019	-0.79	1.82	-2.17	-6.44	6.37	0.06	-0.09	2.8***	-0.03	-8.34	-1.00	0.38	-6.05	-9.11	-0.13	0.76
S1011	CML288/CML202	0.053	-2.46	-3.12	0.05	-4.41	-3.54	0.19	0.48	-0.09	-0.05	-3.79	0.17	-0.23	-6.1**	2.35	-0.07	0.36
S1012	CML288/CML539	0.465	0.69	-3.55	2.68	-8.39	-2.73	0.24	0.27	-0.17	-0.14	-6.75	0.17	-0.05	9.***	9.86	-0.20	0.00
S1112	CML202/CML539	0.165	-3.70	-1.41	0.06	-8.15	-3.56	0.20	0.25	0.08	0.03	-13.1	1.50	0.42	1.81	2.36	1.24	-0.30

**GY = grain yield (kg ha<sup>-1</sup>), AD = days to anthesis (days), ASI = anthesis-silking interval (days), DS = days to silking (days), EH = ear height (cm), EPP = ears per plant (#), LB = leaf blight disease (1-5), GLS = gray leaf spot disease (1-5), GT = grain texture (1-5), MSV = maize streak virus disease (1-5), PH = plant height (cm), RL = root lodging (#), Rust = rust disease (1-5), SWT = 100 seed weight (g), SH = shelling percentage, SL = stem lodging (#), VIG = vigour, # = number.**

**Appendix 13 Mean performance of diallel cross progeny across optimal and low pH environments in 2011/12**

	<b>Pedigree</b>	<b>GY</b>	<b>AD</b>	<b>ASI</b>	<b>DS</b>	<b>EH</b>	<b>LB</b>	<b>EPP</b>	<b>GLS</b>	<b>GT</b>	<b>MSV</b>	<b>PH</b>	<b>RL</b>	<b>Rust</b>	<b>SWT</b>	<b>SH%</b>	<b>SL</b>	<b>VIG</b>
1	CZL999601/CML481	3809	69.0	0.0	69.0	91.1	1.5	1.0	1.2	1.8	1.2	163.5	6.0	1.2	33.2	80.0	0.7	2.0
2	CZL999601/CML359	5210	73.0	0.3	73.3	82.7	2.0	1.0	2.7	2.3	1.0	178.1	5.7	1.8	38.0	82.7	0.3	2.0
3	CZL999601/CML144	6955	74.0	0.7	74.7	89.4	1.0	1.2	1.8	2.8	1.0	173.3	3.0	1.2	42.7	81.9	4.7	1.0
4	CZL999601/CML161	3740	74.0	2.0	76.0	102.8	2.3	1.0	1.0	2.0	1.0	195.9	4.3	1.2	38.4	79.1	0.0	2.2
5	CZL999601/CML172	3624	69.0	1.0	70.0	94.3	2.3	1.0	2.3	2.2	1.0	189.5	6.7	1.0	31.2	87.7	2.3	2.2
6	CZL999601/CML448	2663	70.0	2.0	72.0	89.3	4.0	1.0	1.0	2.2	1.0	174.0	3.0	1.0	33.1	80.5	2.0	3.8
7	CZL999601/CML312	4024	69.3	0.7	70.0	92.7	1.5	1.0	1.2	2.0	1.0	195.2	5.0	1.2	35.5	86.5	2.7	2.7
8	CZL999601/ZL130-23	5099	72.0	0.0	72.0	95.6	1.5	1.2	1.2	1.8	1.0	191.7	8.3	1.0	36.3	83.9	2.3	1.0
9	CZL999601/CML288	3779	74.0	0.0	74.0	90.6	3.7	1.1	1.0	2.3	1.0	169.4	10.3	1.2	36.8	83.4	0.7	2.8
10	CZL999601/CML202	5386	74.0	1.0	75.0	71.3	2.0	1.0	1.0	2.3	1.2	163.8	3.7	1.0	38.6	85.3	1.0	2.3
11	CZL999601/CML539	3939	72.0	0.7	72.7	92.3	1.7	1.0	1.0	2.0	1.2	180.3	4.0	1.0	33.3	77.3	0.0	1.0
12	CML481/CML359	3128	71.7	0.7	72.3	99.2	3.2	1.0	1.3	2.3	1.2	188.0	4.7	1.5	31.7	85.0	9.3	2.8
13	CML481/CML144	3628	74.0	1.3	75.3	96.4	3.7	1.1	1.0	1.8	1.0	179.9	1.3	1.0	28.8	79.1	3.3	2.3
14	CML481/CML161	3566	70.0	2.0	72.0	89.2	2.3	1.2	1.0	2.2	1.5	177.8	7.3	1.0	28.0	84.0	2.7	2.7
15	CML481/CML172	2349	67.3	1.0	68.3	100.6	3.3	1.0	1.2	2.0	1.0	184.8	12.0	1.0	25.2	81.9	0.3	3.0
16	CML481/CML448	4976	76.0	0.3	76.3	70.8	1.3	1.0	1.0	2.3	1.0	124.2	1.3	1.2	38.2	79.9	1.0	1.3
17	CML481/CML312	2509	75.3	0.7	76.0	82.2	3.8	1.0	1.0	1.8	1.5	180.0	16.0	1.2	28.2	81.8	3.7	2.3
18	CML481/ZL130-23	3612	73.3	2.3	75.7	90.8	1.7	1.1	1.2	2.2	1.0	188.0	2.0	1.0	45.2	83.6	0.3	1.0
19	CML481/CML288	4676	72.0	0.3	72.3	92.0	1.5	1.0	1.3	2.2	1.0	181.8	5.7	1.0	43.8	86.8	0.7	1.2
20	CML481/CML202	2702	72.0	1.7	73.7	90.5	2.2	1.2	1.8	2.7	1.0	171.5	1.7	1.2	33.8	87.4	0.3	1.7
21	CML481/CML539	2509	70.0	0.7	70.7	79.3	2.0	1.0	1.0	2.3	1.0	175.6	5.7	1.0	34.9	82.3	0.7	1.7
22	CML359/CML144	2484	72.0	2.0	74.0	90.9	1.5	1.0	1.0	2.0	1.0	190.7	2.7	1.0	34.8	80.9	0.3	2.8
23	CML359/CML161	2535	73.0	2.3	75.3	96.5	1.7	1.0	1.2	2.5	1.0	188.7	2.0	1.2	42.2	70.8	0.3	2.0
24	CML359/CML172	4640	73.3	2.7	76.0	81.1	1.3	1.3	1.3	1.8	1.0	168.9	5.0	1.0	37.5	78.3	1.7	1.0
25	CML359/CML448	3073	76.0	0.0	76.0	93.4	3.0	1.0	1.0	2.2	1.5	185.9	2.7	1.0	39.2	82.7	1.3	2.3
26	CML359/CML312	2344	69.0	2.7	71.7	84.3	3.2	1.2	1.5	2.3	1.0	180.0	10.7	1.0	25.3	82.5	1.0	2.3
27	CML359/ZL130-23	2722	74.0	0.0	74.0	109.7	1.3	1.0	1.0	2.5	1.2	197.3	1.7	1.0	32.9	74.7	0.3	2.8
28	CML359/CML288	3621	70.0	2.0	72.0	81.8	1.5	1.0	1.2	1.8	1.2	173.2	3.7	1.0	38.0	81.4	1.7	2.0

**Appendix 13 continued**

29	CML359/CML202	3170	70.3	1.7	72.0	108.6	2.0	1.0	2.2	2.0	1.0	194.3	3.7	1.0	39.7	81.7	0.3	1.2
30	CML359/CML539	3353	76.0	2.0	78.0	97.4	1.5	1.0	1.2	2.2	1.2	188.6	10.0	1.0	34.0	77.6	1.3	1.8
31	CML144/CML161	2142	71.0	0.0	71.0	101.0	2.0	1.0	1.7	2.7	1.0	187.7	4.3	1.0	32.5	83.1	0.7	2.3
32	CML144/CML172	2379	70.0	1.0	71.0	94.3	1.7	1.1	1.0	2.2	1.0	184.5	3.0	1.2	37.4	90.1	1.3	2.8
33	CML144/CML448	2513	71.3	0.3	71.7	74.1	2.3	1.0	1.7	2.0	1.5	151.3	2.0	1.0	31.8	86.3	0.0	2.3
34	CML144/CML312	3280	74.0	0.3	74.3	86.8	1.7	1.0	1.0	2.2	1.0	172.0	2.7	1.3	46.4	83.1	0.7	2.3
35	CML144/ZL130-23	1835	76.0	0.7	76.7	95.8	1.5	1.2	1.0	1.7	1.2	174.3	10.0	1.2	33.7	74.5	0.0	2.2
36	CML144/CML288	3210	70.0	2.0	72.0	88.5	1.7	1.1	2.3	2.5	1.0	178.6	7.3	1.8	31.5	87.2	1.0	1.5
37	CML144/CML202	4172	70.0	1.0	71.0	91.0	1.5	1.1	2.3	2.3	1.0	184.3	5.7	1.0	38.0	83.4	0.3	1.2
38	CML144/CML539	2513	70.0	1.7	71.7	94.7	1.7	1.1	1.0	2.0	1.0	183.4	2.0	1.0	31.9	83.8	1.3	1.2
39	CML161/CML172	5026	75.0	1.3	76.3	98.1	1.2	1.0	1.3	2.7	1.0	194.8	4.0	1.0	40.7	83.8	0.3	1.0
40	CML161/CML448	757	72.0	3.7	75.7	88.1	4.3	0.9	1.0	2.2	1.0	177.9	8.0	1.0	27.9	70.0	0.0	4.5
41	CML161/CML312	1805	72.0	0.3	72.3	85.5	1.3	1.0	1.0	2.5	1.2	170.7	2.3	1.2	29.8	75.1	0.3	3.0
42	CML161/ZL130-23	2652	75.3	0.7	76.0	85.7	2.7	1.0	1.5	1.8	1.0	167.3	3.7	1.0	28.6	80.7	0.7	1.8
43	CML161/CML288	1381	74.0	0.7	74.7	84.3	1.3	1.0	1.0	2.2	1.0	180.2	3.0	1.0	29.5	79.4	0.0	3.5
44	CML161/CML202	1556	76.0	0.7	76.7	122.2	2.7	1.1	1.0	2.0	1.0	216.9	1.0	1.0	31.5	72.5	0.3	2.8
45	CML161/CML539	4713	74.0	2.0	76.0	88.2	1.5	1.1	1.0	1.8	1.0	174.1	9.7	1.2	34.0	82.3	0.0	2.3
46	CML172/CML448	2514	69.3	0.7	70.0	85.9	2.8	1.1	1.0	1.8	1.2	179.9	12.3	1.2	29.7	74.3	0.3	1.5
47	CML172/CML312	2365	69.7	1.3	71.0	65.2	4.0	1.0	2.0	2.7	1.0	162.9	9.0	1.0	28.9	82.7	0.3	3.7
48	CML172/ZL130-23	5218	74.0	2.0	76.0	85.9	2.0	1.1	1.3	2.3	1.0	180.4	5.0	1.5	37.0	80.7	1.3	2.0
49	CML172/CML288	3251	74.0	0.7	74.7	81.8	1.5	1.1	1.2	1.8	1.5	169.4	6.3	1.2	37.9	79.5	0.7	1.8
50	CML172/CML202	3649	76.0	1.3	77.3	104.2	3.2	1.0	1.5	2.2	1.0	194.3	5.3	1.2	29.0	81.4	1.3	2.8
51	CML172/CML539	2775	78.0	2.0	80.0	95.0	1.3	1.0	1.0	2.0	1.0	173.3	0.3	1.0	45.7	77.6	0.3	1.3
52	CML448/CML312	2448	75.7	1.7	77.3	95.0	1.5	0.9	1.0	2.5	1.0	192.3	4.3	1.0	39.7	68.2	1.3	2.7
53	CML448/ZL130-23	2882	74.0	2.0	76.0	109.2	2.0	1.0	1.2	1.7	1.3	199.4	2.7	1.2	42.6	80.2	0.0	1.2
54	CML448/CML288	2732	78.0	0.7	78.7	104.0	1.5	1.1	1.7	2.7	1.0	191.2	3.3	1.0	39.0	84.9	0.7	1.7
55	CML448/CML202	2239	77.0	0.0	77.0	92.9	1.5	1.1	1.0	2.2	1.0	186.3	2.7	1.0	31.7	75.6	0.3	2.8
56	CML448/CML539	3243	69.0	3.7	72.7	93.6	2.0	1.1	1.0	1.7	1.0	182.0	5.3	1.0	44.8	84.9	0.3	2.2
57	CML312/ZL130-23	2448	76.3	1.0	77.3	86.9	1.7	1.1	1.0	3.0	1.3	178.4	5.3	1.0	32.0	79.4	0.3	2.8

### Appendix 13

58	CML312/CML288	3737	69.0	3.0	72.0	111.3	2.3	1.0	1.2	2.2	1.2	187.1	6.0	1.2	33.7	87.6	0.0	1.8
59	CML312/CML202	3820	72.0	0.7	72.7	97.6	1.5	1.0	1.0	2.5	1.0	182.5	1.0	1.0	42.2	86.4	1.0	2.0
60	CML312/CML539	1622	69.0	1.3	70.3	84.0	2.5	1.0	1.2	2.2	1.2	172.7	10.3	1.0	27.7	75.2	0.7	4.2
61	ZL130-23/CML288	1456	74.0	0.0	74.0	104.8	3.8	1.0	1.3	1.7	1.0	190.3	8.0	1.3	24.5	77.0	0.0	4.2
62	ZL130-23/CML202	2323	74.3	1.7	76.0	106.2	1.3	1.1	1.0	1.5	1.0	194.7	4.7	1.0	33.8	80.6	0.3	2.3
63	ZL130-23/CML539	2792	72.0	0.7	72.7	89.0	2.3	1.0	1.0	2.5	1.0	170.9	2.0	1.0	28.3	78.7	0.3	3.2
64	CML288/CML202	2653	69.7	0.7	70.3	90.7	2.7	1.3	2.7	2.2	1.2	186.3	2.7	1.0	25.4	80.9	0.7	2.7
65	CML288/CML539	3677	75.7	0.3	76.0	83.4	1.7	1.1	1.5	2.3	1.0	181.0	2.3	1.0	48.9	81.4	0.3	1.7
66	CML202/CML539	3942	71.0	0.7	71.7	102.9	1.3	1.3	1.0	1.8	1.0	193.2	3.0	1.3	34.7	82.1	3.0	1.0
67	MH27	4744	74.7	1.3	76.0	86.9	1.0	1.1	1.0	2.0	1.0	192.3	5.7	1.0	34.2	77	0.7	1.3
68	MH26	6018	74.3	1.7	76.0	111.3	1.0	1.0	1.2	2.0	1.0	199.4	7.0	1.0	41.2	83	0.0	1.2
	<b>Mean</b>	<b>3205.2</b>	<b>72.6</b>	<b>1.2</b>	<b>73.8</b>	<b>92.0</b>	<b>2.1</b>	<b>1.1</b>	<b>1.3</b>	<b>2.2</b>	<b>1.1</b>	<b>180.9</b>	<b>5.0</b>	<b>1.1</b>	<b>34.9</b>	<b>81.0</b>	<b>1.0</b>	<b>2.2</b>
	<b>LSD</b>	<b>1650</b>	<b>0.8</b>	<b>1.4</b>	<b>1.3</b>	<b>328.2</b>	<b>0.8</b>	<b>0.2</b>	<b>0.5</b>	<b>0.8</b>	<b>0.4</b>	<b>38.7</b>	<b>6.7</b>	<b>1.1</b>	<b>10.2</b>	<b>10.5</b>	<b>3.8</b>	<b>1.1</b>
	<b>MSE</b>	<b>4E+06</b>	<b>0.3</b>	<b>0.8</b>	<b>0.6</b>	<b>29.3</b>	<b>0.3</b>	<b>0.0</b>	<b>0.1</b>	<b>0.3</b>	<b>0.1</b>	<b>574.1</b>	<b>17.2</b>	<b>0.5</b>	<b>39.5</b>	<b>42.3</b>	<b>5.6</b>	<b>0.5</b>
	<b>P</b>	<b>0.001</b>	<b>0.001</b>	<b>0.00</b>	<b>0</b>	<b>0.63</b>	<b>0.0</b>	<b>0.17</b>	<b>0</b>	<b>0.4</b>	<b>0.2</b>	<b>0.75</b>	<b>0.00</b>	<b>0.08</b>	<b>0.001</b>	<b>0.05</b>	<b>0.35</b>	<b>0</b>
	<b>CV (%)</b>	<b>31.9</b>	<b>0.7</b>	<b>74.7</b>	<b>0.8</b>	<b>19.7</b>	<b>24.3</b>	<b>12.4</b>	<b>22.8</b>	<b>23.7</b>	<b>21.3</b>	<b>13.2</b>	<b>82.9</b>	<b>0.2</b>	<b>18.0</b>	<b>8.0</b>	<b>230.1</b>	<b>30.8</b>

LSD = Least significant difference, MSE = Mean square error, CV = coefficient of variation, GY = grain yield (kg ha<sup>-1</sup>), AD = days to anthesis (days), ASI = anthesis-silking interval (days), DS = days to silking (days), EH = ear height (cm), LB = leaf blight disease (1-5), EPP = ears per plant (#), GLS = gray leaf spot disease (1-5), GT = grain texture (1-5), MSV = maize streak virus disease (1-5), PH = plant height (cm), RL = root lodging (#), Rust = rust disease (1-5), SWT = 100 seed weight (g), SH = shelling percentage, SL = stem lodging (#), VIG = vigour, # = number.

**Appendix 14 Mean performances of diallel cross progenies across three low pH environments in 2012**

	<b>Pedigree</b>	<b>GY</b>	<b>AD</b>	<b>ASI</b>	<b>DS</b>	<b>EH</b>	<b>EPP</b>	<b>LB</b>	<b>GLS</b>	<b>GT</b>	<b>MSV</b>	<b>PH</b>	<b>RL</b>	<b>RUST</b>	<b>SWT</b>	<b>SH%</b>	<b>SL</b>	<b>VIG</b>
1	CZL999601/CML481	894	83.4	2.9	86.3	46.8	0.5	2.3	1.3	2.2	1.0	110	0.5	2.4	22.7	62.0	2.2	4.3
2	CZL999601/CML359	678	79.3	3.1	82.4	47.3	0.5	2.3	2.5	1.5	1.0	117	0.7	2.5	27.0	66.4	1.8	4.0
3	CZL999601/CML144	853	82.7	2.6	85.2	46.1	0.4	1.3	1.7	2.2	1.0	111	0.7	2.2	18.4	68.2	1.3	5.0
4	CZL999601/CML161	657	79.2	2.4	81.7	38.3	0.5	2.0	2.0	2.1	1.0	97	0.5	2.3	23.6	78.5	1.8	4.7
5	CZL999601/CML172	787	78.8	3.8	82.6	42.5	0.7	2.3	1.9	2.2	1.0	108	0.7	2.2	24.6	74.0	1.3	4.7
6	CZL999601/CML448	953	77.4	3.0	80.4	43.8	0.5	2.7	2.1	2.6	1.0	113	0.5	2.3	18.9	73.6	1.0	5.0
7	CZL999601/CML312	867	81.2	3.0	84.2	30.2	0.5	2.7	2.1	1.9	1.1	97	0.8	1.7	20.9	65.8	1.8	4.7
8	CZL999601/ZL130-23	945	82.9	3.1	86.0	41.5	0.5	2.3	1.8	2.1	1.0	104	0.5	2.3	24.0	70.2	1.5	4.3
9	CZL999601/CML288	821	79.6	2.2	81.8	37.5	0.5	2.7	2.5	2.3	1.2	102	0.5	2.0	23.5	65.4	1.0	5.0
10	CZL999601/CML202	971	79.6	2.6	82.1	40.4	0.5	2.3	1.3	1.9	1.0	104	0.5	2.6	20.4	69.5	2.3	5.0
11	CZL999601/CML539	664	83.9	2.4	86.3	35.6	0.6	2.3	1.6	1.8	1.0	105	0.7	1.9	22.7	61.7	2.0	4.7
12	CML481/CML359	631	80.4	3.7	84.1	37.1	0.5	3.0	2.1	2.3	1.0	106	0.8	2.1	16.3	59.0	2.2	4.7
13	CML481/CML144	670	80.8	2.7	83.4	40.7	0.5	3.0	1.5	1.6	1.0	107	0.5	2.3	22.8	73.0	1.3	4.3
14	CML481/CML161	835	78.7	2.4	81.1	39.5	0.5	3.0	1.8	1.7	1.0	105	0.5	1.9	20.5	73.4	3.0	4.7
15	CML481/CML172	695	82.4	3.9	86.3	34.9	0.4	1.3	1.9	2.1	1.0	98	0.5	2.1	20.9	58.2	1.8	5.0
16	CML481/CML448	752	81.8	2.1	83.9	38.6	0.5	1.7	1.8	2.2	1.0	99	0.5	2.2	23.4	66.2	1.5	4.0
17	CML481/CML312	651	84.1	3.0	87.1	39.9	0.4	1.7	2.3	2.6	1.0	110	0.5	2.2	19.7	59.5	1.8	4.3
18	CML481/ZL130-23	958	83.0	3.2	86.2	44.4	0.5	2.0	2.0	1.9	1.1	110	0.5	2.0	17.2	65.0	1.5	4.3
19	CML481/CML288	999	77.8	3.0	80.8	47.8	0.5	1.3	2.3	1.7	1.0	113	0.5	1.6	21.6	66.8	1.5	4.3
20	CML481/CML202	872	79.3	2.9	82.2	43.6	0.6	2.7	2.1	2.1	1.0	111	0.5	2.3	23.0	73.4	1.2	4.3
21	CML481/CML539	721	80.6	2.8	83.3	41.6	0.5	2.7	1.8	2.3	1.0	105	0.7	1.8	21.0	56.3	1.3	4.7
22	CML359/CML144	643	82.1	3.8	85.9	41.5	0.5	1.0	1.4	1.7	1.0	110	0.5	2.0	21.3	61.6	1.0	5.0
23	CML359/CML161	686	80.3	3.0	83.3	50.7	0.4	1.7	2.1	2.1	1.0	115	0.5	2.0	23.9	62.4	2.2	4.7
24	CML359/CML172	628	81.0	2.7	83.7	41.5	0.7	1.3	2.1	1.9	1.0	104	0.5	2.2	18.8	62.2	0.5	4.7
25	CML359/CML448	1008	81.9	3.4	85.3	32.6	0.8	1.7	1.3	2.2	1.0	93	0.5	2.0	21.8	70.3	1.3	4.7
26	CML359/CML312	685	81.3	3.3	84.7	47.8	0.8	2.0	2.2	2.2	1.2	113	0.7	2.4	19.9	65.1	0.8	4.7
27	CML359/ZL130-23	815	81.9	2.7	84.6	43.1	0.6	2.0	1.3	2.2	1.2	112	0.5	2.0	20.6	66.5	0.5	4.0
28	CML359/CML288	638	81.6	2.3	84.5	35.0	0.7	1.0	1.6	1.8	3.5	99	0.8	2.3	20.8	58.4	1.6	4.7

**Appendix 14 continued**

29	CML359/CML202	569	79.8	3.2	83.0	35.4	0.5	2.0	1.8	2.6	1.0	105	0.7	2.3	16.9	53.7	1.7	4.7
30	CML359/CML539	804	82.3	2.4	84.8	30.8	0.5	1.7	1.3	2.1	1.0	93	0.7	2.0	22.1	72.3	1.7	5.0
31	CML144/CML161	704	78.7	2.3	81.0	47.6	0.5	2.3	2.5	2.0	1.3	102	0.5	2.2	26.7	64.8	1.2	5.0
32	CML144/CML172	821	78.7	3.2	81.9	34.6	0.6	1.7	1.6	2.0	1.0	100	0.5	1.8	26.8	64.4	1.3	4.3
33	CML144/CML448	825	77.6	3.2	80.8	38.2	0.5	2.7	1.5	1.8	1.0	103	0.5	2.3	27.5	72.2	1.7	5.0
34	CML144/CML312	725	80.6	3.0	83.6	41.5	0.6	1.0	1.5	1.8	1.0	102	0.5	2.3	21.5	74.7	1.5	4.3
35	CML144/ZL130-23	827	85.7	2.8	88.4	38.0	0.5	1.7	2.0	1.7	1.0	102	0.5	2.0	21.0	83.2	1.8	4.3
36	CML144/CML288	712	79.7	2.8	82.4	39.4	0.5	1.3	2.2	1.7	1.0	102	0.5	2.1	20.0	63.6	1.7	4.7
37	CML144/CML202	736	81.6	3.1	84.7	44.7	0.4	1.3	2.3	1.7	1.0	114	0.5	1.9	26.2	72.3	3.2	5.0
38	CML144/CML539	821	82.8	2.8	85.6	43.0	0.6	2.3	1.6	2.4	1.0	121	0.8	2.3	22.3	70.9	1.3	4.3
39	CML161/CML172	773	80.3	2.3	82.7	44.3	0.5	3.0	2.5	2.3	2.0	95	0.5	2.0	22.5	71.2	2.0	5.0
40	CML161/CML448	608	79.3	2.3	81.7	34.3	0.7	1.0	1.0	2.4	1.0	107	0.5	1.5	27.2	57.9	1.0	4.0
41	CML161/CML312	435	83.0	3.0	86.0	33.3	0.4	4.0	2.0	2.3	1.5	94	0.5	2.0	22.2	65.2	0.5	5.0
42	CML161/ZL130-23	581	78.0	2.3	80.3	51.0	0.5	3.0	2.0	1.9	1.0	116	1.0	2.3	18.0	80.5	2.5	5.0
43	CML161/CML288	487	84.7	3.7	88.3	36.1	0.6	2.0	2.0	1.6	1.0	105	0.5	2.0	27.6	61.0	2.5	4.0
44	CML161/CML202	458	80.7	2.3	83.0	46.4	0.6	2.0	2.0	2.3	1.0	106	0.5	2.8	20.5	57.5	0.5	4.0
45	CML161/CML539	594	82.7	2.7	85.3	50.1	0.8	4.0	1.5	1.6	1.5	117	0.5	2.5	22.2	68.9	0.5	4.0
46	CML172/CML448	464	79.3	2.7	82.0	44.1	0.4	2.0	2.0	2.6	1.0	114	1.0	3.0	25.6	35.9	3.0	5.0
47	CML172/CML312	468	83.7	2.7	86.3	55.0	0.6	3.0	1.8	2.2	1.0	112	0.5	2.8	23.8	72.8	1.0	4.0
48	CML172/ZL130-23	811	80.3	3.0	83.3	51.4	0.7	2.0	3.0	2.4	1.5	115	0.5	2.8	25.1	70.1	0.5	5.0
49	CML172/CML288	636	81.0	3.7	84.7	57.7	0.6	2.0	2.0	1.6	1.0	123	0.5	2.0	22.8	64.3	2.0	4.0
50	CML172/CML202	478	81.0	2.7	83.7	35.6	0.5	4.0	1.5	2.0	1.0	95	0.5	2.3	20.0	50.0	1.5	5.0
51	CML172/CML539	896	80.3	3.0	83.3	45.0	0.6	3.0	2.0	2.1	1.0	100	0.5	2.5	20.3	65.4	1.0	4.0
52	CML448/CML312	509	79.3	2.7	82.0	38.7	0.4	1.0	2.0	2.4	1.0	101	0.5	2.5	21.0	62.5	0.5	4.0
53	CML448/ZL130-23	554	81.7	2.7	84.3	30.3	0.5	2.0	1.5	2.0	1.0	97	1.0	1.5	25.5	64.9	2.0	5.0
54	CML448/CML288	373	83.0	2.7	85.7	47.3	0.4	1.0	1.5	1.9	1.0	112	0.5	2.0	32.4	69.8	1.0	5.0
55	CML448/CML202	508	81.3	3.3	84.7	44.8	0.5	1.0	2.5	1.0	1.0	110	0.5	2.5	23.8	67.3	0.5	4.0
56	CML448/CML539	426	79.3	3.7	83.0	36.0	0.5	1.0	1.8	1.9	1.0	87	0.5	2.0	12.7	62.5	1.0	4.0
57	CML312/ZL130-23	472	81.0	3.0	84.0	50.3	0.6	2.0	2.5	1.6	1.0	114	1.0	3.0	25.0	63.0	0.5	4.0

**Appendix 14 continued**

58	CML312/CML288	360	85.0	2.3	87.3	38.3	0.7	3.0	1.5	2.2	1.0	103	1.0	2.0	17.1	61.4	1.0	5.0
59	CML312/CML202	589	84.7	3.3	88.0	43.4	0.5	1.0	1.5	1.4	1.0	115	0.5	2.5	26.2	61.1	2.5	5.0
60	CML312/CML539	583	81.7	3.0	84.7	45.3	0.6	1.0	1.8	1.6	1.0	116	0.5	2.3	17.6	70.6	3.5	5.0
61	ZL130-23/CML288	824	79.7	3.0	82.7	45.7	0.4	2.0	1.5	1.9	1.0	105	1.0	2.5	28.7	67.8	1.5	5.0
62	ZL130-23/CML202	266	82.7	3.3	86.0	35.7	0.6	4.0	2.0	1.6	1.0	98	0.5	2.0	25.0	61.6	2.5	5.0
63	ZL130-23/CML539	799	81.0	3.3	84.3	39.0	0.5	2.0	1.5	2.9	1.0	104	0.5	3.0	20.2	58.9	1.5	5.0
64	CML288/CML202	1041	77.7	2.7	80.3	34.1	0.4	2.0	2.3	1.5	1.0	101	0.5	1.8	16.5	65.6	2.5	5.0
65	CML288/CML539	829	80.0	2.3	82.3	28.7	0.4	3.0	2.0	1.9	1.0	95	0.5	2.0	27.4	70.7	3.5	5.0
66	CML202/CML539	847	77.3	3.3	80.7	28.7	0.4	3.0	1.5	2.2	1.0	88	0.5	2.5	17.9	64.6	2.0	5.0
67	MH27	639.5	77.7	1.3	79.0	57.0	1.0	1.7	2.0	2.0	1.0	166.3	0.0	1.0	33.6	59.9	2.2	5.0
68	MH26	879.3	78.7	1.0	79.7	65.0	1.0	1.3	2.0	2.0	1.3	171.0	0.0	1.7	29.9	63.2	3.5	4.5
	<b>Mean</b>	<b>701.6</b>	<b>80.9</b>	<b>2.9</b>	<b>83.8</b>	<b>41.7</b>	<b>0.5</b>	<b>2.1</b>	<b>1.9</b>	<b>2.0</b>	<b>1.1</b>	<b>107</b>	<b>0.6</b>	<b>2.2</b>	<b>22.5</b>	<b>65.6</b>	<b>1.6</b>	<b>4.6</b>
	<b>LSD</b>	<b>293.3</b>	<b>3.1</b>	<b>0.7</b>	<b>0.4</b>	<b>12.6</b>	<b>0.3</b>	<b>0.5</b>	<b>0.8</b>	<b>0.8</b>	<b>0.7</b>	<b>19.3</b>	<b>0.3</b>	<b>0.7</b>	<b>6.9</b>	<b>57.8</b>	<b>1.6</b>	<b>0.6</b>
	<b>MSE</b>	<b>1001</b>	<b>3.7</b>	<b>0.5</b>	<b>4.0</b>	<b>61.4</b>	<b>0.0</b>	<b>0.2</b>	<b>0.2</b>	<b>0.3</b>	<b>0.2</b>	<b>145</b>	<b>0.0</b>	<b>0.2</b>	<b>18.3</b>	<b>1295.</b>	<b>1.0</b>	<b>0.1</b>
	<b>P</b>	<b>0.001</b>	<b>0.00</b>	<b>0.001</b>	<b>0.001</b>	<b>0.001</b>	<b>0.001</b>	<b>0.001</b>	<b>0.001</b>	<b>0.001</b>	<b>0.001</b>	<b>0.001</b>	<b>0.001</b>	<b>0.001</b>	<b>0.001</b>	<b>0.005</b>	<b>0.001</b>	<b>0.001</b>
	<b>CV (%)</b>	<b>45.2</b>	<b>2.4</b>	<b>24.6</b>	<b>2.4</b>	<b>19.0</b>	<b>39.3</b>	<b>26.9</b>	<b>0.5</b>	<b>26.0</b>	<b>42.5</b>	<b>11.4</b>	<b>36.4</b>	<b>19.4</b>	<b>19.0</b>	<b>52.1</b>	<b>63.8</b>	<b>7.7</b>

LSD = Least significant difference, MSE = Mean square error, CV = coefficient of variation, GY = grain yield (kg ha<sup>-1</sup>), AD = days to anthesis (days), ASI = anthesis-silking interval (days), DS = days to silking (days), EH = ear height (cm), EPP = ears per plant (#), LB = leaf blight disease (1-5), GLS = gray leaf spot disease (1-5), GT = grain texture (1-5), MSV = maize streak virus disease (1-5), PH = plant height (cm), RL = root lodging (#), Rust = rust disease (1-5), SWT = 100 seed weight (g), SH = shelling percentage, SL = stem lodging (#), VIG = vigour, # = number.

**Appendix 15 Mean performance of the diallel cross progenies under optimal conditions in 2011/12**

	<b>Pedigree</b>	<b>GY</b>	<b>AD</b>	<b>ASI</b>	<b>DS</b>	<b>EH</b>	<b>LB</b>	<b>EPP</b>	<b>GLS</b>	<b>GT</b>	<b>MSV</b>	<b>PH</b>	<b>RL</b>	<b>RUST</b>	<b>SWT</b>	<b>SH%</b>	<b>SL</b>	<b>VIG</b>
1	CZL999601/CML481	3809	69.0	0.0	69.0	91.1	1.5	1.0	1.2	1.8	1.2	163.5	6.0	1.2	33.2	80.0	0.7	2.0
2	CZL999601/CML359	5210	73.0	0.3	73.3	82.7	2.0	1.0	2.7	2.3	1.0	178.1	5.7	1.8	38.0	82.7	0.3	2.0
3	CZL999601/CML144	6955	74.0	0.7	74.7	89.4	1.0	1.2	1.8	2.8	1.0	173.3	3.0	1.2	42.7	81.9	4.7	1.0
4	CZL999601/CML161	3740	74.0	2.0	76.0	102.8	2.3	1.0	1.0	2.0	1.0	195.9	4.3	1.2	38.4	79.1	0.0	2.2
5	CZL999601/CML172	3624	69.0	1.0	70.0	94.3	2.3	1.0	2.3	2.2	1.0	189.5	6.7	1.0	31.2	87.7	2.3	2.2
6	CZL999601/CML448	2663	70.0	2.0	72.0	89.3	4.0	1.0	1.0	2.2	1.0	174.0	3.0	1.0	33.1	80.5	2.0	3.8
7	CZL999601/CML312	4024	69.3	0.7	70.0	92.7	1.5	1.0	1.2	2.0	1.0	195.2	5.0	1.2	35.5	86.5	2.7	2.7
8	CZL999601/ZL130-23	5099	72.0	0.0	72.0	95.6	1.5	1.2	1.2	1.8	1.0	191.7	8.3	1.0	36.3	83.9	2.3	1.0
9	CZL999601/CML288	3779	74.0	0.0	74.0	90.6	3.7	1.1	1.0	2.3	1.0	169.4	10.3	1.2	36.8	83.4	0.7	2.8
10	CZL999601/CML202	5386	74.0	1.0	75.0	71.3	2.0	1.0	1.0	2.3	1.2	163.8	3.7	1.0	38.6	85.3	1.0	2.3
11	CZL999601/CML539	3939	72.0	0.7	72.7	92.3	1.7	1.0	1.0	2.0	1.2	180.3	4.0	1.0	33.3	77.3	0.0	1.0
12	CML481/CML359	3128	71.7	0.7	72.3	99.2	3.2	1.0	1.3	2.3	1.2	188.0	4.7	1.5	31.7	85.0	9.3	2.8
13	CML481/CML144	3628	74.0	1.3	75.3	96.4	3.7	1.1	1.0	1.8	1.0	179.9	1.3	1.0	28.8	79.1	3.3	2.3
14	CML481/CML161	3566	70.0	2.0	72.0	89.2	2.3	1.2	1.0	2.2	1.5	177.8	7.3	1.0	28.0	84.0	2.7	2.7
15	CML481/CML172	2349	67.3	1.0	68.3	100.6	3.3	1.0	1.2	2.0	1.0	184.8	12.0	1.0	25.2	81.9	0.3	3.0
16	CML481/CML448	4976	76.0	0.3	76.3	70.8	1.3	1.0	1.0	2.3	1.0	124.2	1.3	1.2	38.2	79.9	1.0	1.3
17	CML481/CML312	2509	75.3	0.7	76.0	82.2	3.8	1.0	1.0	1.8	1.5	180.0	16.0	1.2	28.2	81.8	3.7	2.3
18	CML481/ZL130-23	3612	73.3	2.3	75.7	90.8	1.7	1.1	1.2	2.2	1.0	188.0	2.0	1.0	45.2	83.6	0.3	1.0
19	CML481/CML288	4676	72.0	0.3	72.3	92.0	1.5	1.0	1.3	2.2	1.0	181.8	5.7	1.0	43.8	86.8	0.7	1.2
20	CML481/CML202	2702	72.0	1.7	73.7	90.5	2.2	1.2	1.8	2.7	1.0	171.5	1.7	1.2	33.8	87.4	0.3	1.7
21	CML481/CML539	2509	70.0	0.7	70.7	79.3	2.0	1.0	1.0	2.3	1.0	175.6	5.7	1.0	34.9	82.3	0.7	1.7
22	CML359/CML144	2484	72.0	2.0	74.0	90.9	1.5	1.0	1.0	2.0	1.0	190.7	2.7	1.0	34.8	80.9	0.3	2.8
23	CML359/CML161	2535	73.0	2.3	75.3	96.5	1.7	1.0	1.2	2.5	1.0	188.7	2.0	1.2	42.2	70.8	0.3	2.0
24	CML359/CML172	4640	73.3	2.7	76.0	81.1	1.3	1.3	1.3	1.8	1.0	168.9	5.0	1.0	37.5	78.3	1.7	1.0
25	CML359/CML448	3073	76.0	0.0	76.0	93.4	3.0	1.0	1.0	2.2	1.5	185.9	2.7	1.0	39.2	82.7	1.3	2.3
26	CML359/CML312	2344	69.0	2.7	71.7	84.3	3.2	1.2	1.5	2.3	1.0	180.0	10.7	1.0	25.3	82.5	1.0	2.3
27	CML359/ZL130-23	2722	74.0	0.0	74.0	109.7	1.3	1.0	1.0	2.5	1.2	197.3	1.7	1.0	32.9	74.7	0.3	2.8
28	CML359/CML288	3621	70.0	2.0	72.0	81.8	1.5	1.0	1.2	1.8	1.2	173.2	3.7	1.0	38.0	81.4	1.7	2.0

29	CML359/CML202	3170	70.3	1.7	72.0	108.6	2.0	1.0	2.2	2.0	1.0	194.3	3.7	1.0	39.7	81.7	0.3	1.2
30	CML359/CML539	3353	76.0	2.0	78.0	97.4	1.5	1.0	1.2	2.2	1.2	188.6	10.0	1.0	34.0	77.6	1.3	1.8
31	CML144/CML161	2142	71.0	0.0	71.0	101.0	2.0	1.0	1.7	2.7	1.0	187.7	4.3	1.0	32.5	83.1	0.7	2.3
32	CML144/CML172	2379	70.0	1.0	71.0	94.3	1.7	1.1	1.0	2.2	1.0	184.5	3.0	1.2	37.4	90.1	1.3	2.8
33	CML144/CML448	2513	71.3	0.3	71.7	74.1	2.3	1.0	1.7	2.0	1.5	151.3	2.0	1.0	31.8	86.3	0.0	2.3
34	CML144/CML312	3280	74.0	0.3	74.3	86.8	1.7	1.0	1.0	2.2	1.0	172.0	2.7	1.3	46.4	83.1	0.7	2.3
35	CML144/ZL130-23	1835	76.0	0.7	76.7	95.8	1.5	1.2	1.0	1.7	1.2	174.3	10.0	1.2	33.7	74.5	0.0	2.2
36	CML144/CML288	3210	70.0	2.0	72.0	88.5	1.7	1.1	2.3	2.5	1.0	178.6	7.3	1.8	31.5	87.2	1.0	1.5
37	CML144/CML202	4172	70.0	1.0	71.0	91.0	1.5	1.1	2.3	2.3	1.0	184.3	5.7	1.0	38.0	83.4	0.3	1.2
38	CML144/CML539	2513	70.0	1.7	71.7	94.7	1.7	1.1	1.0	2.0	1.0	183.4	2.0	1.0	31.9	83.8	1.3	1.2
39	CML161/CML172	5026	75.0	1.3	76.3	98.1	1.2	1.0	1.3	2.7	1.0	194.8	4.0	1.0	40.7	83.8	0.3	1.0
40	CML161/CML448	757	72.0	3.7	75.7	88.1	4.3	0.9	1.0	2.2	1.0	177.9	8.0	1.0	27.9	70.0	0.0	4.5
41	CML161/CML312	1805	72.0	0.3	72.3	85.5	1.3	1.0	1.0	2.5	1.2	170.7	2.3	1.2	29.8	75.1	0.3	3.0
42	CML161/ZL130-23	2652	75.3	0.7	76.0	85.7	2.7	1.0	1.5	1.8	1.0	167.3	3.7	1.0	28.6	80.7	0.7	1.8
43	CML161/CML288	1381	74.0	0.7	74.7	84.3	1.3	1.0	1.0	2.2	1.0	180.2	3.0	1.0	29.5	79.4	0.0	3.5
44	CML161/CML202	1556	76.0	0.7	76.7	122.2	2.7	1.1	1.0	2.0	1.0	216.9	1.0	1.0	31.5	72.5	0.3	2.8
45	CML161/CML539	4713	74.0	2.0	76.0	88.2	1.5	1.1	1.0	1.8	1.0	174.1	9.7	1.2	34.0	82.3	0.0	2.3
46	CML172/CML448	2514	69.3	0.7	70.0	85.9	2.8	1.1	1.0	1.8	1.2	179.9	12.3	1.2	29.7	74.3	0.3	1.5
47	CML172/CML312	2365	69.7	1.3	71.0	65.2	4.0	1.0	2.0	2.7	1.0	162.9	9.0	1.0	28.9	82.7	0.3	3.7
48	CML172/ZL130-23	5218	74.0	2.0	76.0	85.9	2.0	1.1	1.3	2.3	1.0	180.4	5.0	1.5	37.0	80.7	1.3	2.0
49	CML172/CML288	3251	74.0	0.7	74.7	81.8	1.5	1.1	1.2	1.8	1.5	169.4	6.3	1.2	37.9	79.5	0.7	1.8
50	CML172/CML202	3649	76.0	1.3	77.3	104.2	3.2	1.0	1.5	2.2	1.0	194.3	5.3	1.2	29.0	81.4	1.3	2.8
51	CML172/CML539	2775	78.0	2.0	80.0	95.0	1.3	1.0	1.0	2.0	1.0	173.3	0.3	1.0	45.7	77.6	0.3	1.3
52	CML448/CML312	2448	75.7	1.7	77.3	95.0	1.5	0.9	1.0	2.5	1.0	192.3	4.3	1.0	39.7	68.2	1.3	2.7
53	CML448/ZL130-23	2882	74.0	2.0	76.0	109.2	2.0	1.0	1.2	1.7	1.3	199.4	2.7	1.2	42.6	80.2	0.0	1.2
54	CML448/CML288	2732	78.0	0.7	78.7	104.0	1.5	1.1	1.7	2.7	1.0	191.2	3.3	1.0	39.0	84.9	0.7	1.7
55	CML448/CML202	2239	77.0	0.0	77.0	92.9	1.5	1.1	1.0	2.2	1.0	186.3	2.7	1.0	31.7	75.6	0.3	2.8
56	CML448/CML539	3243	69.0	3.7	72.7	93.6	2.0	1.1	1.0	1.7	1.0	182.0	5.3	1.0	44.8	84.9	0.3	2.2
57	CML312/ZL130-23	2448	76.3	1.0	77.3	86.9	1.7	1.1	1.0	3.0	1.3	178.4	5.3	1.0	32.0	79.4	0.3	2.8
58	CML312/CML288	3737	69.0	3.0	72.0	111.3	2.3	1.0	1.2	2.2	1.2	187.1	6.0	1.2	33.7	87.6	0.0	1.8

59	CML312/CML202	3820	72.0	0.7	72.7	97.6	1.5	1.0	1.0	2.5	1.0	182.5	1.0	1.0	42.2	86.4	1.0	2.0
60	CML312/CML539	1622	69.0	1.3	70.3	84.0	2.5	1.0	1.2	2.2	1.2	172.7	10.3	1.0	27.7	75.2	0.7	4.2
61	ZL130-23/CML288	1456	74.0	0.0	74.0	104.8	3.8	1.0	1.3	1.7	1.0	190.3	8.0	1.3	24.5	77.0	0.0	4.2
62	ZL130-23/CML202	2323	74.3	1.7	76.0	106.2	1.3	1.1	1.0	1.5	1.0	194.7	4.7	1.0	33.8	80.6	0.3	2.3
63	ZL130-23/CML539	2792	72.0	0.7	72.7	89.0	2.3	1.0	1.0	2.5	1.0	170.9	2.0	1.0	28.3	78.7	0.3	3.2
64	CML288/CML202	2653	69.7	0.7	70.3	90.7	2.7	1.3	2.7	2.2	1.2	186.3	2.7	1.0	25.4	80.9	0.7	2.7
65	CML288/CML539	3677	75.7	0.3	76.0	83.4	1.7	1.1	1.5	2.3	1.0	181.0	2.3	1.0	48.9	81.4	0.3	1.7
66	CML202/CML539	3942	71.0	0.7	71.7	102.9	1.3	1.3	1.0	1.8	1.0	193.2	3.0	1.3	34.7	82.1	3.0	1.0
67	MH27	4744	74.7	1.3	76.0	86.9	1.0	1.1	1.0	2.0	1.0	192.3	5.7	1.0	34.2	77	0.7	1.3
68	MH26	6018	74.3	1.7	76.0	111.3	1.0	1.0	1.2	2.0	1.0	199.4	7.0	1.0	41.2	83	0.0	1.2
	<b>Mean</b>	<b>3205.2</b>	<b>72.6</b>	<b>1.2</b>	<b>73.8</b>	<b>92.0</b>	<b>2.1</b>	<b>1.1</b>	<b>1.3</b>	<b>2.2</b>	<b>1.1</b>	<b>180.9</b>	<b>5.0</b>	<b>1.1</b>	<b>34.9</b>	<b>81.0</b>	1.0	2.2
	<b>LSD</b>	<b>1650</b>	<b>0.8</b>	<b>1.4</b>	<b>1.3</b>	<b>328.2</b>	<b>0.8</b>	<b>0.2</b>	<b>0.5</b>	<b>0.8</b>	<b>0.4</b>	<b>38.7</b>	<b>6.7</b>	<b>1.1</b>	<b>10.2</b>	<b>10.5</b>	3.8	1.1
	<b>MSE</b>	<b>4E+06</b>	<b>0.3</b>	<b>0.8</b>	<b>0.6</b>	<b>29.3</b>	<b>0.3</b>	<b>0.0</b>	<b>0.1</b>	<b>0.3</b>	<b>0.1</b>	<b>574.1</b>	<b>17.2</b>	<b>0.5</b>	<b>39.5</b>	<b>42.3</b>	5.6	0.5
	<b>P</b>	<b>0.001</b>	<b>0.001</b>	<b>0.001</b>	<b>0</b>	<b>0.63</b>	<b>0.0</b>	<b>0.17</b>	<b>0</b>	<b>0.36</b>	<b>0.16</b>	<b>0.75</b>	<b>0</b>	<b>0.08</b>	<b>0.001</b>	<b>0.05</b>	0.35	0
	<b>CV (%)</b>	<b>31.9</b>	<b>0.7</b>	<b>74.7</b>	<b>0.8</b>	<b>19.7</b>	<b>24.3</b>	<b>12.4</b>	<b>22.8</b>	<b>23.7</b>	<b>21.3</b>	<b>13.2</b>	<b>82.9</b>	<b>0.2</b>	<b>18.0</b>	<b>8.0</b>	230.1	30.8
	<b>SE</b>	<b>1021</b>	<b>0.5</b>	<b>0.9</b>	<b>1.1</b>	<b>18.1</b>	<b>0.5</b>	<b>0.1</b>	<b>0.3</b>	<b>0.5</b>	<b>0.2</b>	<b>24.0</b>	<b>4.2</b>	<b>25.5</b>	<b>6.3</b>	<b>6.5</b>	2.4	0.7
	<b>MIN</b>	<b>757.0</b>	<b>67.3</b>	<b>0.0</b>	<b>68.3</b>	<b>65.2</b>	<b>1.0</b>	<b>0.9</b>	<b>1.0</b>	<b>1.5</b>	<b>1.0</b>	<b>124.2</b>	<b>0.3</b>	<b>1.0</b>	<b>24.5</b>	<b>68.2</b>	0.0	1.0
	<b>MAX</b>	<b>6955.0</b>	<b>78.0</b>	<b>3.7</b>	<b>80.0</b>	<b>122.2</b>	<b>4.3</b>	<b>1.3</b>	<b>2.7</b>	<b>3.0</b>	<b>1.5</b>	<b>216.9</b>	<b>16.0</b>	<b>1.8</b>	<b>48.9</b>	<b>90.1</b>	9.3	4.5

LSD = Least significant difference, MSE = Mean square error, CV = coefficient of variation, SE = error, MIN = minimum, MAX = maximum, GY = grain yield (kg ha<sup>-1</sup>), AD = days to anthesis (days), ASI = anthesis-silking interval (days), DS = days to silking (days), EH = ear height (cm), LB = leaf blight disease (1-5), EPP = ears per plant (#), GLS = gray leaf spot disease (1-5), GT = grain texture (1-5), MSV = maize streak virus disease (1-5), PH = plant height (cm), RL = root lodging (#), Rust = rust disease (1-5), SWT = 100 seed weight (g), SH = shelling percentage, SL = stem lodging (#), VIG = vigour, # = number.

**Appendix 16 Estimated general combining ability effects for 12 inbred lines for grain yield and agronomic traits at low pH environments in 2011/12**

	Name	GY	AD	ASI	DS	EH	EPP	GLS	GT	LB	MSV	PH	RL	RUST	SWT	SH%	SL	VIG
1	G1 CZL999601	0.12	-0.07	-0.37	0.13	-0.15	0.30	0.01	0.01	0.07	-0.06	1.06	0.02	-0.05	0.95	7.60*	0.05	-0.51
2	G2 CML481	0.03	0.28	0.11	0.24	0.22	0.44	0.02	-0.11	0.03	-0.02	0.93	0.16	-0.20	-1.03	-3.22	-0.01	-0.89
3	G3 CML359	0.03	-0.61	0.16	-0.33	-0.85	1.62*	-0.08	0.09	-0.04	0.06	0.25	-0.18	0.37	-1.81*	-5.18	0.00	3.16*
4	G4 CML144	0.17	0.05	0.16	-0.16	0.00	-0.14	-0.04	-0.17	-0.21	-0.02	0.71	-0.01	-0.22	1.36	1.16	-0.05	-0.65
5	G5 CML161	-0.19	-0.31	-0.23	-0.69	2.00	-0.75	0.08	0.05	0.20	0.19**	0.23	-0.08	-0.15	0.92	0.41	0.05	-0.64
6	G6 CML172	-0.04	-0.18	-0.38	0.52	3.51*	-0.06	0.16	0.10	0.08	0.07	0.79	-0.20	0.06	0.43	4.23	0.02	-0.57
7	G7 CML448	-0.09	-0.67	-0.52	-0.18	-2.28	-0.71	-0.16	0.07	-0.32*	-0.10*	-2.10	-0.25	-0.11	1.51	-0.20	-0.05	-0.67
8	G8 CML312	-0.20	1.69**	0.67	1.10	0.98	-0.70	0.05	0.04	-0.08	-0.02	1.13	-0.21	0.07	-0.97	-0.59	0.07	-0.81
9	G9 ZL130-23	0.03	0.82	0.28	0.60	1.69	0.12	0.05	0.02	0.05	0.02	1.20	0.01	0.05	0.42	-0.10	-0.01	-0.65
10	G10 CML288	0.11	-0.90	0.75	-1.86	-0.43	-0.11	0.11	-0.09	0.05	0.03	-0.83	0.21	0.17	1.39	0.89	0.06	3.24
11	G11 CML202	0.00	-0.42	-0.18	-0.12	-1.91	-0.75	0.04	-0.13	0.07	-0.10*	-0.62	0.34*	0.03	-0.56	-2.98	-0.12	-0.44
12	G12 CML539	0.03	0.32	-0.44	0.73	-2.78	0.74	-0.24	0.14	0.10	-0.03	-2.74	0.19	-0.01	-2.62**	-2.01	-0.01	-0.57

\*\*P<0.01; \*P<0.05; GY = grain yield (kg ha<sup>-1</sup>), AD = days to anthesis (days), ASI = anthesis-silking interval (days), DS = days to silking (days), EH = ear height (cm), EPP = ears per plant (#), GLS = gray leaf spot disease (1-5), GT = grain texture (1-5), LB = leaf blight disease (1-5), MSV = maize streak virus disease (1-5), PH = plant height (cm), RL = root lodging (#), Rust = rust disease (1-5), SWT = 100 seed weight (g), SH = shelling percentage, SL = stem lodging (#), VIG = vigour, # = number.

**Appendix 17 Estimated specific combining ability effects of 12 inbred lines for grain yield and agronomic traits across low pH environments 2011/12**

Cross	Pedigree	GY	AD	ASI	DS	EH	EPP	LB	GLS	GT	MSV	PH	RL	SWT	SH	SL	VIG	Rust	
1	S12	CZL999601/CML481	-0.09	2.45	0.77	1.73	5.68	-1.53	-0.02	-0.66*	0.35	0.00	2.70	0.31	0.56	-6.96	-0.04	0.60	0.41
2	S13	CZL999601/CML359	-0.31	-0.78	-2.28	1.64	7.20	7.76**	0.05	0.69*	-0.53	-0.09	10.37	0.48	2.01	-12.58	-0.05	-3.78	-0.07
3	S14	CZL999601/CML144	-0.03	1.89	-0.39	1.80	5.23	-0.88	-0.28	-0.18	0.04	-0.01	4.25	-0.52	-1.24	-4.42	0.33	1.03	0.18
4	S15	CZL999601/CML161	-0.02	-1.19	-1.33	0.00	-4.54	-0.22	-0.36	0.03	0.09	-0.22*	-9.72	0.05	-0.66	-7.01	0.06	0.69	0.19
5	S16	CZL999601/CML172	-0.13	-1.76	0.48	-0.65	-1.94	-0.73	-0.07	-0.14	-0.08	-0.09	0.94	0.00	1.61	56.17**	-0.07	0.62	-0.10
6	S17	CZL999601/CML448	0.36	-2.61	-1.04	-1.18	5.18	-0.16	0.49	0.36	0.09	0.07	9.28	-0.29	-3.26	-4.19	-0.17	1.05	0.24
7	S18	CZL999601/CML312	0.37	-1.19	0.88	-1.79	-11.74**	-0.22	0.26	0.15	-0.15	0.08	-10.29	0.35	-0.84	-5.34	0.21	0.85	-0.61
8	S19	CZL999601/CZL130-23	0.08	1.35	2.27	-0.40	-1.12	-1.16	-0.04	-0.10	0.12	-0.04	-3.80	-0.20	0.32	-1.89	-0.04	0.36	0.08
9	S110	CZL999601/CML288	-0.24	-0.27	-0.87	-0.39	-2.89	-0.94	0.13	0.50	0.35	0.09	-3.55	-0.90*	-0.49	-6.47	-0.11	-2.86	-0.38
10	S111	CZL999601/CML202	0.30	-0.75	-1.38	-0.02	1.48	-0.22	-0.06	-0.59	0.00	0.07	-1.42	0.46	-0.02	0.35	-0.10	0.82	0.35
11	S23	CML481/CML359	-0.26	-0.01	1.24	-0.25	-3.29	3.39	0.41	0.27	0.04	0.01	-0.72	0.51	-0.61	-2.03	0.35	-2.73	-0.34
12	S24	CML481/CML144	-0.36	-0.34	-0.64	-0.20	-0.47	-1.09	0.59	-0.35	-0.07	-0.05	0.38	-0.16	-0.43	6.73	-0.27	0.74	0.49
13	S25	CML481/CML161	0.27	-2.09	-2.25	-0.22	-3.80	-0.42	0.18	-0.14	-0.42	0.00	-1.26	1.58**	-0.71	7.38	-0.37	1.07	0.01
14	S26	CML481/CML172	0.07	1.56	1.89	1.23	-9.87*	-1.12	-0.54	-0.14	-0.25	0.01	-8.70	0.20	-0.49	-5.47	0.00	1.34	-0.03
15	S27	CML481/CML448	-0.02	1.38	-0.07	-0.18	-0.30	-0.46	0.03	0.10	0.19	0.03	-4.59	0.08	0.50	4.29	-0.10	0.44	0.22
16	S28	CML481/CML312	0.07	1.36	1.51	-0.12	-4.78	-0.50	-0.21	0.39	0.38	-0.04	-6.05	0.05	2.31	-1.81	0.11	-0.76	0.04
17	S29	CML481/CZL130-23	0.34	0.24	2.24	-1.94	2.57	-1.22	-0.17	0.06	-0.46	0.13	4.26	-0.34	-1.01	6.31	0.03	0.91	-0.11
18	S210	CML481/CML288	0.15	-2.39	-3.45	1.39	6.96	5.15**	-0.50	0.24	-0.28	-0.09	7.80	-0.20	-2.97	-1.89	-0.38	-3.15	-0.73
19	S211	CML481/CML202	0.01	-1.32	-2.08	0.54	4.22	-0.38	0.14	0.15	0.38	0.03	5.04	-1.34**	1.90	10.37	0.47	0.54	0.25
20	S34	CML359/CML144	-0.39	1.88	-0.36	3.60	1.37	-2.22	-0.34	-0.33	-0.13	-0.14	3.50	-0.32	-0.86	1.17	-0.12	-2.64	-0.41
21	S35	CML359/CML161	0.21	0.47	0.70	0.13	8.49	-1.61	-0.42	0.21	-0.12	-0.06	9.20	0.25	3.40	2.42	0.45	-2.98	-0.47
22	S36	CML359/CML172	-0.12	1.00	-0.38	0.14	-2.25	4.25*	-0.47	0.13	-0.46	-0.22*	-2.58	-0.47	-4.15	-8.57	-0.35	-3.05	-0.51
23	S37	CML359/CML448	0.23	2.38	0.76	2.29	-5.35	-1.32	0.10	-0.30	-0.02	-0.05	-10.58	0.41	-2.01	11.11	-0.29	-2.95	-0.51
24	S38	CML359/CML312	0.11	-0.53	-2.42	2.23	6.62	-1.22	0.03	0.33	0.17	0.04	6.63	-0.12	-1.16	-4.33	-0.24	-2.81	-0.28
25	S39	CML359/CZL130-23	0.15	0.89	-0.81	0.73	1.14	-2.31	-0.10	-0.59	0.03	0.26	4.89	-0.67	0.09	7.17	-0.32	-3.64	-0.67
26	S310	CML359/CML288	0.12	-7.16**	3.22	-10.78	-4.37	-2.10	1.20**	-0.10	0.35	0.42**	-11.04	0.13	-0.77	-3.43	0.11	30.47**	4.20**

**Appendix 17 continued**

27	S311	CML359/CML202	-0.29	0.02	-0.02	0.11	-2.93	-1.60	-0.12	0.00	0.72*	-0.05	0.27	-0.17	-2.69	-2.68	0.29	-3.18	-0.32
28	S45	CML144/CML161	-0.11	-1.86	-2.30	-0.04	4.52	0.18	0.09	0.59	0.14	0.07	-4.48	-0.42	0.91	-1.06	0.00	1.16	0.28
29	S46	CML144/CML172	0.44	-1.99	-1.04	-0.59	-9.88*	-0.42	-0.13	-0.41	0.09	-0.14	-6.93	0.03	2.98	-5.36	-0.14	0.43	-0.35
30	S47	CML144/CML448	-0.09	-2.62	-1.90	0.00	-0.53	0.13	0.77*	-0.17	-0.13	0.02	-1.04	0.24	3.21	8.08	0.10	1.19	0.32
31	S48	CML144/CML312	0.02	-1.97	-1.09	-0.72	-0.57	0.20	-0.29	-0.38	-0.10	-0.05	-5.16	-0.12	-1.48	2.69	0.15	0.66	0.22
32	S49	CML144/ZL130-23	-0.21	4.00*	4.86	-1.11	-7.83	-0.69	-0.09	0.12	-0.20	-0.09	-12.79	0.16	-1.91	-2.91	0.06	-0.73	-0.09
33	S410	CML144/CML288	0.19	-0.69	0.47	-1.71	-1.98	-0.44	-0.26	0.48	-0.09	0.40**	-7.49	0.13	-3.74	0.08	-0.44	-3.22	-0.38
34	S411	CML144/CML202	0.75	0.08	1.27	-1.33	5.38	0.27	-0.25	0.63	0.03	0.02	12.05	1.52**	3.79	-2.25	-0.06	0.96	-0.47
35	S56	CML161/CML172	0.17	0.04	-0.10	-0.72	-2.21	0.06	0.13	0.38	0.21	0.66**	-11.01	0.43	-1.29	2.03	0.10	1.09	-0.16
36	S57	CML161/CML448	0.06	-0.47	-0.29	-0.69	-6.42	0.87	-0.47	-0.79*	0.24	-0.18	3.88	-0.02	2.33	-6.86	-0.34	0.19	-0.50
37	S58	CML161/CML312	0.00	0.84	2.53	-0.97	-10.68*	0.64	0.79*	0.00	0.26	0.25*	-12.02	-0.55	-0.14	0.91	-0.45	1.32	-0.18
38	S59	CML161/CZL130-23	-0.08	-3.30*	-3.42	-0.47	6.28	-0.10	0.16	0.00	-0.05	-0.29*	9.57	0.73	-5.81*	-2.38	0.63	1.16	0.09
39	S510	CML161/CML288	-0.25	5.09**	6.78	0.66	-6.60	0.17	-0.34	-0.07	-0.27	-0.30*	0.27	0.03	2.85	3.08	0.56	-3.73	-0.28
40	S511	CML161/CML202	-0.18	0.61	-0.63	0.58	5.22	0.85	-0.36	0.01	0.43	-0.18	1.06	-1.11*	-2.34	-4.43	-0.26	-0.05	0.62
41	S67	CML172/CML448	-0.11	-0.61	0.19	-1.57	1.73	-0.03	0.14	0.12	0.52	-0.06	9.99	1.60**	1.22	-22.36*	0.70*	1.12	0.79
42	S68	CML172/CML312	-0.12	1.37	0.34	0.16	9.48	0.15	0.41	-0.34	0.22	-0.13	5.09	0.06	1.96	4.63	-0.42	0.25	0.36
43	S69	CML172/CZL130-23	-0.01	-1.10	-2.27	0.99	5.11	-0.58	-0.22	0.91**	0.24	0.33*	7.68	-0.65	1.81	1.46	-0.34	1.09	0.39
44	S610	CML172/CML288	-0.09	1.29	1.59	1.11	13.56*	-0.42	-0.22	-0.15	-0.32	-0.18	18.05	-0.35	-1.46	-5.39	0.59	-3.80	-0.49
45	S611	CML172/CML202	-0.18	0.81	0.85	-0.96	-6.96	0.22	0.76*	-0.58	0.05	-0.06	-9.82	0.01	-2.26	-15.82	-0.23	0.89	-0.09
46	S78	CML448/CML312	0.23	-2.47	-0.85	-2.14	-1.07	0.77	-0.19	0.24	0.24	0.03	-3.02	-0.39	-1.94	-1.29	-0.35	0.35	0.28
47	S79	CML448/ZLL130-23	-0.06	0.72	0.87	-0.64	-10.10*	-0.05	0.18	-0.26	-0.07	-0.01	-7.76	0.90*	1.17	0.61	0.23	1.19	-0.70
48	S710	CML448/CML208	-0.29	3.78*	-0.60	4.15	9.01	0.18	-0.32	-0.32	0.04	-0.02	9.60	-0.80	7.05	4.55	0.16	-2.70	-0.32
49	S711	CML448/CML202	0.02	1.63	1.66	0.74	7.83	0.90	-0.34	0.75*	-0.92*	0.11	7.73	-0.94*	0.40	5.92	-0.16	-0.01	0.33
50	S89	CML312/ZL130-23	-0.19	-2.30	-3.65	1.41	6.64	-0.08	-0.06	0.53	-0.37	-0.08	6.34	-0.64	3.10	-0.84	0.11	0.33	0.62
51	S810	CML312/CML288	-0.37	3.42*	1.88	0.54	-3.24	0.21	0.45	-0.53	0.40	-0.09	-2.96	-0.34	-5.80*	-3.42	0.04	-2.56	-0.51
52	S811	CML312/CM202	-0.04	2.61	2.81	0.46	3.24	0.75	-0.58	-0.46	-0.56	0.03	9.16	0.03	5.24*	0.11	0.72*	1.12	0.14
53	S910	ZL130-23/CML288	0.27	-1.05	-3.40	2.70	3.38	-0.65	-0.19	-0.53	0.09	-0.13	-0.70	-0.05	4.49	2.47	0.12	-2.72	0.02
54	S911	ZL130-23/CML202	-0.59	1.47	2.86	-0.71	-5.13	-0.08	0.79*	0.04	-0.20	-0.01	-7.57	0.81	2.69	0.17	-0.20	0.96	-0.33
55	S112	CZL999601/CML539	-0.32	2.85	2.88	-0.75	-2.54	-1.70	-0.09	-0.06	-0.27	0.15	1.26	0.28	2.01	-7.66	-0.04	0.62	0.15

**Appendix 17 continued**

56	S212	CML481/CML539	-0.17	-0.83	0.85	-1.98	3.08	-1.81	0.11	0.10	0.14	-0.03	1.16	-0.69	0.96	-16.91	0.20	1.00	-0.03
57	S312	CML359/CML539	0.56	1.83	0.35	0.15	-6.63	-3.02	-0.32	-0.30	-0.07	-0.12	-9.94	-0.02	6.75*	11.74	0.18	-2.71	-0.12
58	S412	CML144/CML539	-0.21	1.62	1.13	0.31	4.74	4.96*	0.19	0.00	0.43	-0.04	17.72	-0.52	-1.23	-2.76	0.40	0.43	-0.04
59	S512	CML161/CML539	-0.07	1.87	0.30	1.73	9.75	-0.43	0.61	-0.21	-0.50	0.26*	14.52	-0.95*	1.46	5.93	-0.37	0.09	0.26
60	S612	CML172/CML539	0.07	-0.60	-1.55	0.86	3.23	-1.38	0.23	0.21	-0.22	-0.12	-2.70	-0.84	0.09	-1.33	0.16	0.02	-0.12
61	S712	CML448/CML539	-0.34	-1.11	1.26	-0.78	0.02	-0.83	-0.37	0.28	-0.19	0.04	-13.48	-0.79	-8.67**	0.13	0.23	0.12	0.04
62	S812	CML312/CML539	-0.07	-1.13	-1.93	0.95	6.10	-0.69	-0.61	0.07	-0.49	-0.03	12.29	1.68**	-1.25	8.69	0.11	1.25	-0.03
63	S912	ZL130-23/CML539	0.30	-0.93	0.46	-0.56	-0.94	6.92**	-0.24	-0.18	0.86*	-0.07	-0.12	-0.04	-4.94	-10.18	-0.30	1.09	-0.07
64	S1011	CML288/CML202	0.23	-1.81	-3.61	1.42	-4.68	0.07	-0.21	0.23	-0.26	-0.02	-2.88	0.61	-5.34*	3.17	-0.27	-2.93	-0.02
65	S1012	CML288/CML539	0.28	-0.21	-2.01	0.90	-9.15	-1.24	0.26	0.26	-0.03	-0.08	-7.09	1.76**	6.19*	7.25	-0.38	-2.80	-0.08
66	S1112	CML202/CML539	-0.17	-4.25	-0.93	-1.57	-8.81	1.53	0.34	0.42	0.31	-0.22	-18.52	0.80	-1.45	-2.09	0.12	4.53	-0.22

\*\*P≤0.01; \*P≤0.05; GY = grain yield (kg ha<sup>-1</sup>), AD = days to anthesis (days), ASI = anthesis-silking interval (days), DS = days to silking (days), EH = ear height (cm), EPP = ears per plant (#), LB = leaf blight disease (1-5), GLS = gray leaf spot disease (1-5), GT = grain texture (1-5), MSV = maize streak virus disease (1-5), PH = plant height (cm), RL = root lodging (#), SWT = 100 seed weight (g), SH = shelling percentage, SL = stem lodging (#), VIG = vigour, Rust = rust disease (1-5), # = number.

**Appendix 18 Estimated general combining ability effects for 12 inbred lines for grain yield and agronomic traits for optimal soil conditions in 2011/12**

	Line	Name	GY	AD	ASI	DS	EH	EPP	GLS	GT	LB	MSV	PH	RL	SWT	SH%	SL	VIG
1	G1	CZL999601	1.31**	-0.87**	-0.45**	-1.32**	-2.01	-1.54	0.12*	0.02	0.07	-0.09	-1.57	0.51	1.94	3.13	0.56	-0.76
2	G2	CML481	0.23	-0.83**	-0.18	-1.02**	-3.00	-1.54	-0.12*	0.00	0.37**	-0.01	-7.53	0.88	-0.66	3.47	1.19	-0.86
3	G3	CML359	0.12	-0.07	0.35*	0.28	1.36	-1.54	0.13*	0.04	-0.07	-0.03	4.32	-0.26	1.55	0.13	0.69	-0.75
4	G4	CML144	0.01	-0.67**	-0.15	-0.82**	0.10	-1.50	0.17**	0.03	-0.26*	-0.08	-2.64	-1.04	1.14	3.63	0.13	-0.85
5	G5	CML161	-0.57**	0.73**	0.32*	1.05**	3.05	1.38	-0.15*	0.06	-0.02	0.02	4.04	-0.52	-3.01*	-4.36*	-0.56	1.22
6	G6	CML172	0.25	-0.33**	0.22	-0.12	-2.58	-1.53	0.10	0.00	0.18	-0.08	-0.76	1.41	0.28	2.10	-0.08	-0.75
7	G7	CML448	-0.51**	0.93**	0.22	1.15**	-1.58	-1.58	-0.17**	-0.03	0.35**	0.01	-4.60	-0.72	2.00	-0.94	-0.38	-0.43
8	G8	CML312	-0.40*	-0.77**	0.08	-0.68	-4.39	1.06	-0.13*	0.20*	0.10	0.07	-0.29	1.53*	-1.33	-1.38	0.09	0.97
9	G9	ZL130-23	-0.31	1.63**	-0.25	1.38**	3.76	4.76**	-0.15*	-0.18	-0.17	0.17*	2.80	-0.06	-2.88*	-5.73**	-0.54	1.81*
10	G10	CML288	-0.02	0.13	-0.25	-0.12	-0.21	-1.54	0.20**	0.03	-0.05	-0.03	1.17	0.13	1.87	3.12	-0.48	-0.66
11	G11	CML202	-0.01	0.33**	-0.15	0.18	6.70*	1.46	0.23**	-0.02	-0.17	-0.01	7.65	-1.99*	-1.52	-0.72	-0.19	0.68
12	G12	CML539	-0.10	-0.23**	0.25	0.02	-1.22	2.11	-0.23**	-0.15	-0.36	0.06	-2.59	0.13	0.63	-2.44	-0.44	0.39

\*\*P<0.01; \*P<0.05; GY = grain yield (kg ha<sup>-1</sup>), AD = days to anthesis (days), ASI = anthesis-silking interval (days), DS = days to silking (days), EH = ear height (cm), EPP = ears per plant (#), GLS = gray leaf spot disease (1-5), GT = grain texture (1-5), LB = leaf blight disease (1-5), MSV = maize streak virus disease (1-5), PH = plant height (cm), RL = root lodging (#), SWT = 100 seed weight (g), SH = shelling percentage, SL = stem lodging (#), VIG = vigour, # = number.

**Appendix 19 Estimated specific combining ability effects of 12 inbred lines for grain yield and agronomic traits at optimal soil conditions 2011/12**

Cross	Pedigree	GY	AD	ASI	DS	EH	EPP	LB	GLS	GT	MSV	PH	RL	SWT	SH%	SL	VIG	
1	S12	CZL999601/CML481	-0.90	-1.94**	-0.53	-2.47**	4.06	1.60	-1.01**	-0.12	-0.34	0.14	-8.35	-0.38	-2.40	-6.36	-2.09	0.84
2	S13	CZL999601/CML359	0.61	1.30**	-0.73	0.56	-8.70	1.64	-0.08	1.13**	0.13	-0.01	-5.61	0.42	0.14	-0.31	-1.92	0.72
3	S14	CZL999601/CML144	2.44**	2.90**	0.10	3.00**	-0.68	1.75	-0.89**	0.26	0.64**	0.04	-3.41	-1.46	5.30	-4.58	2.98*	-0.17
4	S15	CZL999601/CML161	-0.18	1.50**	0.97*	2.46**	9.78	-1.27	0.21	-0.25	-0.23	-0.06	12.51	-0.65	5.17	0.64	-1.01	-1.07
5	S16	CZL999601/CML172	-1.16*	-2.44**	0.07	-2.37**	6.84	1.60	0.01	0.83**	-0.01	0.04	10.85	-0.25	-5.34	2.79	0.84	0.89
6	S17	CZL999601/CML448	-1.33*	-2.70**	1.07*	-1.64**	0.90	1.63	1.51**	-0.24	0.03	-0.04	-0.78	-1.78	-5.14	-1.42	0.81	2.24
7	S18	CZL999601/CML312	-0.08	-1.67**	-0.13	-1.80**	7.05	-0.93	-0.74*	-0.10	-0.36	-0.10	16.08	-2.03	0.55	5.01	1.01	-0.33
8	S19	CZL999601/CZL130-23	0.94	-1.40**	-0.47	-1.87**	1.84	-4.42	-0.48	-0.09	-0.16	-0.21	9.55	2.89	2.85	6.78	1.31	-2.83
9	S110	CZL999601/CML288	-0.73	2.10**	-0.47	1.63**	0.80	1.72	1.57**	-0.60**	0.14	0.00	-11.18	4.70*	-1.30	-2.59	-0.42	1.47
10	S111	CZL999601/CML202	0.86	1.90**	0.43	2.33**	-25.41**	-1.34	0.02	-0.64**	0.19	0.14	-23.20	0.15	3.81	3.15	-0.37	-0.37
11	S23	CML481/CML359	-0.41	-0.07	-0.67	-0.74	8.83	1.68	0.79*	0.03	0.14	0.07	10.25	-0.95	-3.56	1.66	6.44**	1.66
12	S24	CML481/CML144	0.22	2.86**	0.50	3.36**	7.32	1.67	1.48*	-0.34	-0.35	-0.04	9.09	-3.50	-5.98	-7.77	1.01	1.27
13	S25	CML481/CML161	0.70	-2.54	0.70	-1.84	-2.83	-1.14	-0.09	-0.02	-0.05	0.36	0.37	1.99	-2.67	5.21	1.03	-0.47
14	S26	CML481/CML172	-1.35*	-4.14**	-0.20	-4.34	14.13	1.64	0.71*	-0.10	-0.16	-0.04	12.17	4.72*	-8.63*	-3.44	-1.79	1.82
15	S27	CML481/CML448	2.05**	3.26**	-0.87	2.40	-16.60	1.72	-1.46**	0.00	0.21	-0.13	-44.65**	-3.81	2.51	-2.31	-0.82	-0.16
16	S28	CML481/CML312	-0.50	4.30**	-0.40	3.90	-2.42	-0.96	1.29**	-0.04	-0.51	0.31	6.87	8.60**	-4.17	0.00	1.38	-0.56
17	S29	CML481/CZL130-23	0.52	-0.10	1.60**	1.50	-1.97	-4.56	-0.61	0.15	0.19	-0.29	11.78	-3.81	14.40**	6.11	-1.32	-2.73
18	S210	CML481/CML288	1.25*	0.06	-0.40	-0.34	3.23	1.59	-0.89	-0.04	-0.01	-0.09	7.24	-0.33	8.26*	0.45	-1.06	-0.09
19	S211	CML481/CML202	-0.76	-0.14	0.83	0.70	-5.22	-1.20	-0.11	0.43*	0.54*	-0.11	-9.54	-2.21	1.64	4.89	-1.67	-0.94
20	S34	CML359/CML144	-0.83	0.10	0.63	0.73	-2.54	1.59	-0.25	-0.59**	-0.21	-0.03	8.03	-1.03	-2.24	-2.53	-1.49	1.65
21	S35	CML359/CML161	-0.22	-0.30	0.50	0.20	0.11	-1.27	-0.33	-0.10	0.25	-0.13	-0.58	-2.21	9.31*	-4.66	-0.81	-1.25
22	S36	CML359/CML172	1.09*	1.10**	0.93	2.03	-9.66	1.93	-0.86	-0.19	-0.36	-0.03	-15.58	-1.15	1.35	-3.64	0.04	-0.29
23	S37	CML359/CML448	0.26	2.50**	-1.73**	0.76	1.64	1.67	0.64	-0.25	0.01	0.39*	5.19	-1.35	1.26	3.82	0.01	0.72
24	S38	CML359/CML312	-0.59	-2.80**	1.07*	-1.74	-4.72	-0.76	1.06**	0.21	-0.05	-0.17	-4.98	4.40*	-9.26*	4.00	-0.79	-0.68
25	S39	CML359/CZL130-23	-0.27	-0.20	-1.27*	-1.47	12.60	-4.67	-0.51	-0.27	0.49	-0.11	9.26	-3.01	-0.07	0.59	-0.82	-1.02
26	S310	CML359/CML288	0.33	-2.70**	0.73	-1.97	-11.33	1.60	-0.46	-0.45**	-0.38	0.10	-13.28	-1.20	0.25	-1.62	0.44	0.62

27	S311	CML359/CML202	-0.11	-2.57**	0.30	-2.27	8.52	-1.39	0.16	0.51**	-0.16	-0.09	1.41	0.92	5.34	2.53	-1.17	-1.55
28	S45	CML144/CML161	-0.52	-1.70**	-1.33**	-3.04	5.80	-1.30	0.20	0.36*	0.43	-0.08	5.39	0.90	0.06	4.08	0.09	-0.81
29	S46	CML144/CML172	-1.07*	-1.64**	-0.23	-1.87	4.76	1.72	-0.34	-0.55**	-0.01	0.02	6.95	-2.36	1.58	4.70	0.28	1.65
30	S47	CML144/CML448	-0.20	-1.57**	-0.90*	-2.47	-16.41	1.63	0.16	0.38*	-0.15	0.44*	-22.37	-1.23	-5.67	3.86	-0.76	0.83
31	S48	CML144/CML312	0.45	2.80**	-0.77	2.03	-0.96	-1.00	-0.25	-0.32*	-0.21	-0.12	-6.02	-2.81	12.21**	1.08	-0.56	-0.57
32	S49	CML144/ZL130-23	-1.10*	2.40**	-0.10	2.30	-0.07	-4.54	-0.15	-0.30	-0.33	-0.06	-6.78	6.10*	1.11	-3.10	-0.59	-1.58
33	S410	CML144/CML288	0.03	-2.10**	1.23**	-0.87	-3.38	1.66	-0.10	0.68**	0.29	-0.02	-0.84	3.25	-5.83	0.69	0.34	0.23
34	S411	CML144/CML202	0.99	-2.30**	0.13	-2.17	-7.79	-1.29	-0.15	0.65**	0.18	-0.04	-1.62	3.70	3.98	0.81	-0.61	-1.45
35	S56	CML161/CML172	2.14*	1.96**	-0.37	1.60	5.61	-1.35	-1.08**	0.10	0.45	-0.08	10.57	-1.88	9.38*	6.29	-0.04	-2.25
36	S57	CML161/CML448	-1.36*	-2.30**	1.97**	-0.34	-5.42	-1.31	1.92**	0.03	-0.01	-0.16	-2.52	4.25	-5.42	-4.43	-0.07	0.93
37	S58	CML161/CML312	-0.44	-0.60*	-1.23**	-1.84	-5.21	-3.86	-0.83**	0.00	0.09	-0.05	-14.00	-3.66	-0.23	1.10	-0.21	-1.97
38	S59	CML161/CZL130-23	0.34	0.33	-0.57	-0.24	-13.15	-7.57	0.77*	0.51**	-0.20	-0.33	-20.46	-0.75	0.13	11.11	0.76	-3.98*
39	S510	CML161/CML288	-1.22*	0.50	-0.57	-0.07	-10.53	-1.28	-0.68*	-0.34*	-0.07	-0.12	-5.99	-1.60	-3.66	0.90	0.03	0.16
40	S511	CML161/CML202	-1.43*	2.30**	-0.33	1.96	21.48**	25.24**	0.11	-0.37*	-0.44	0.86**	23.01	-1.48	-14.06**	-29.65**	0.24	12.77**
41	S67	CML172/CML448	-0.41	-3.90**	-0.93*	-4.84	-1.93	1.75	0.22	-0.22	-0.29	0.11	4.35	6.65*	-6.92	-6.55	-0.22	-0.11
42	S68	CML172/CML312	-0.73	-1.87**	-0.13	-2.00	-19.81*	-0.97	1.64**	0.75**	0.32	-0.12	-16.96	1.07	-4.41	2.22	-0.69	0.66
43	S69	CML172/CZL130-23	2.09**	0.06	0.87	0.93	-7.26	-4.62	-0.09	0.10	0.36	-0.23	-2.56	-1.35	5.22	4.61	0.94	-1.85
44	S610	CML172/CML288	-0.21	1.56**	-0.47	1.10	-7.46	1.68	-0.71*	-0.42*	-0.35	0.48*	-11.92	-0.20	1.40	-5.47	0.21	0.46
45	S611	CML172/CML202	0.18	3.36**	0.10	3.46	8.03	-1.36	1.07**	-0.12	0.04	-0.04	6.46	0.92	-4.06	0.32	0.59	0.11
46	S78	CML448/CML312	0.17	2.86**	0.20	3.06	8.95	-1.05	-1.03**	0.01	0.19	-0.20	16.28	-1.46	4.72	-9.19	0.61	-0.66
47	S79	CML448/ZLL130-23	0.52	-1.20**	0.87	-0.34	14.97	-4.61	-0.26	0.20	-0.27	0.02	20.28	-1.55	9.16*	7.17	-0.09	-3.00
48	S710	CML448/CML208	0.09	4.30**	-0.47	3.83	13.74	1.74	-0.88**	0.35*	0.52	-0.10	13.65	-1.06	0.82	3.01	0.51	-0.03
49	S711	CML448/CML202	-0.45	3.10**	-1.23*	1.86	-4.21	-1.26	-0.76*	-0.35*	0.07	-0.13	2.34	0.39	-3.11	-2.48	-0.11	-0.20
50	S89	CML312/ZL130-23	-0.06	2.83**	0.00	2.83	-4.48	19.17**	-0.68*	0.00	0.50	0.63*	-5.06	-1.13	-10.29*	-17.23**	-0.22	8.62**
51	S810	CML312/CML288	1.86**	-3.00**	2.00**	-1.00	20.73	-1.04	-0.63*	-0.35	-0.12	0.09	18.97	-3.15	6.13	4.93	-0.62	-2.09
52	S811	CML312/CM202	1.00	-0.20	-0.43	-0.64	3.30	-3.97	-0.51	-0.39*	0.18	-0.19	-5.81	-3.53	10.75**	8.80	0.09	-2.44
53	S910	ZL130-23/CML288	-1.44**	-0.40	-0.67	-1.07	9.27	-4.65	1.97**	0.00	-0.33	-0.27	5.35	2.94	-8.83*	-0.09	0.01	0.23
54	S911	ZL130-23/CML202	-0.55	-0.27	0.90	0.63	3.69	-7.60	-0.41	-0.37*	-0.45	-0.29	3.30	1.72	3.81	7.35	0.06	-2.95
55	S112	CZL999601/CML539	-0.47	0.46	-0.30	0.16	3.52	-1.97	-0.12	-0.17	-0.01	0.07	3.54	-1.63	-3.63	-3.10	-1.12	-1.41
56	S212	CML481/CML539	-0.83**	-1.57**	-0.57	-2.14	-8.52	-2.05	-0.09	0.06	0.34	-0.18	4.77	-0.33	0.61	1.55	-1.09	-0.64

57	S312	CML359/CML539	0.15	3.66**	0.23	3.90	5.25	-2.02	-0.15	-0.02	0.14	0.01	5.88	5.14*	-2.51	0.17	0.08	-0.59
58	S412	CML144/CML539	-0.41	-1.74**	0.73	-1.00	13.95	-1.89	0.29	-0.22	-0.27	-0.11	11.58	-1.58	-4.52	2.75	-0.69	-1.07
59	S512	CML161/CML539	2.17**	0.86	0.27	1.13	-5.64	-4.88	-0.20	0.10	-0.22	-0.21	-8.30	5.07*	2.00	9.39	-0.01	-2.05
60	S612	CML172/CML539	-0.59	5.93**	0.37	6.30	6.75	-2.03	-0.57*	-0.15	0.00	-0.11	-4.33	-6.20*	10.44*	-1.82	-0.16	-1.09
61	S712	CML448/CML539	0.65	-4.34**	2.03**	-2.30	4.35	-1.91	-0.07	0.11	-0.30	-0.19	8.24	0.94	7.80*	8.52	0.14	-0.57
62	S812	CML312/CML539	-1.07*	-2.64**	-0.17	-2.80	-2.44	-4.62	0.68*	0.25	-0.02	-0.09	-5.37	3.69	-5.99	-0.71	0.01	0.02
63	S912	ZL130-23/CML539	-0.99	-2.04**	-1.17*	-3.20	-15.45	28.07**	0.45	0.10	0.19	1.14**	-24.66	-2.06	-17.49**	-23.31**	-0.02	11.09**
64	S1011	CML288/CML202	-0.55	-3.44**	-0.10	-3.54	-7.85	-1.08	0.81*	0.95**	0.01	0.08	-3.50	-0.46	-9.32*	-1.25	0.33	-0.14
65	S1012	CML288/CML539	0.58	3.13**	-0.83	2.30	-7.22	-1.94	0.00	0.25	0.31	-0.15	1.50	-2.91	12.08**	1.02	0.24	-0.84
66	S1112	CML202/CML539	1.08	-1.53**	0.67	-0.87	-7.17	-0.09	0.29	-0.02	-0.63	-0.07	-2.10	2.88	1.30	4.94	3.45*	-1.83

**\*\*P≤0.01; \*P≤0.05; GY = grain yield (kg ha<sup>-1</sup>), AD = days to anthesis (days), ASI = anthesis-silking interval (days), DS = days to silking (days), EH = ear height (cm), EPP = ears per plant (#), LB = leaf blight disease (1-5), GLS = gray leaf spot disease (1-5), GT = grain texture (1-5), MSV = maize streak virus disease (1-5), PH = plant height (cm), RL = root lodging (#), SWT = 100 seed weight (g), SH = shelling percentage, SL = stem lodging (#), VIG = vigour, # = number.**