Investigation of Heterotic Patterns and Genetic Analysis of Downy Mildew Resistance in Mozambican Lowland Maize (*Zea mays* L.) Germplasm

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General Abstract

In Southern Africa and Mozambique, tropical lowland accounts for 22% and 65%, respectively, of area under maize production, but grain yield is compromised by downy mildew disease (DM, which is caused by *Peronosclerospora sorghi* (Weston and Uppal) Shaw), and lack of appropriate varieties, especially hybrids. Among other factors, productivity can be enhanced by deploying DM resistant hybrids, which are higher yielding than open pollinated varieties. Development of a viable hybrid-breeding programme requires knowledge of genetic effects governing yield and DM resistance in inbreds, and effective germplasm management requires heterotic groups and heterotic patterns to be established. In addition, knowledge of farmer-preferred traits is required. Currently, such information is not available to the hybrid-breeding programme in Mozambique. The objectives of this study were, therefore; i) to identify farmers' preferred variety traits and major production constraints, ii) to determine combining ability effects of inbred maize S4 lines for grain yield and DM resistance, iii) to determine heterotic groups and heterotic patterns among the elite inbred maize lines, and iv) to investigate gene effects governing resistance to DM in breeding source inbred maize lines from the breeding programme in Mozambique.

During 2007/08, 142 households were involved in a survey conducted in three districts representing two maize agro-ecological zones in Mozambique. Formal surveys and informal farmer-participatory methods were employed and data subjected to analysis in the SPSS computer programme. Results indicated that there was a low utilization of improved varieties, especially hybrids, with grain yield estimated at 0.7 t ha^{-1}. Farmers were aware of the major production constraints and could discriminate constraints according to their importance for their respective communities. For the lowland environment, farmers identified downy mildew, drought, and cutworm and stem borer damage as the main constraints. In contrast, for the high altitude environments, they ranked ear rot, seed and fertilizer availability, turcicum leaf blight, grey leaf spot diseases and low soil fertility among the major constraints limiting productivity. The most important variety selection criteria were grain yield, short growth cycle, white and flint grain with stress tolerance to drought, low soil fertility, diseases, and grain weevils. These afore mentioned traits, would be priority for the breeding programmes for the lowland and mid altitude environments in Mozambique.

To determine combining ability for downy mildew resistance, heterotic groups and heterotic patterns, two testers (open-pollinated varieties) ZM523 (Z) and Suwan-1 (S), were crossed with 18 lines to generate 36 top crosses for evaluation. Crosses were evaluated at two sites under DM. Preponderance of GCA effects indicated that additive gene effects were more important than non-additive gene effects in governing both grain yield and downy mildew resistance in the new maize lines. Based on specific combining ability (SCA) data, lines for yield were classified into two heterotic groups, S and Z; whereas based on heterosis data, lines were fitted into three heterotic groups (S, Z and SZ).

Further heterotic patterns and gene action for yield were determined by subjecting nine inbred lines and the two testers, S and Z, to an 11 x 11 diallel-mating scheme. The diallel crosses, three hybrid checks and the two testers were evaluated in six environments in Mozambique. Results revealed that non-additive gene effects were predominant for yield components. In addition, high levels of heterosis for yield was observed and three heterotic groups identified (Z, S and S/Z), and five exceptional heterotic patterns among the inbred elite maize lines were observed. Topcrosses with yield levels comparable to single cross hybrids were also identified, and these would be advanced in the testing programme with potential for deployment as alternative cheaper and sustainable technology to conventional hybrids for the poor farming communities in Mozambique.

To determine gene effects for downy mildew resistance in potential breeding lines, two maize populations were derived from crosses between downy mildew susceptible line LP67, and resistant lines DRAC and Suwan-L1. To generate F2 and backcross progenies (BCP1 and BCP2), F1 progenies were self-pollinated and simultaneously crossed to both inbred parents (P1 and P2). All the six generations (P1, P2, F1, F2, BCP1, and BCP2) of the populations were evaluated at two sites under downy mildew infection. A generation mean analysis was performed in SAS. It was revealed that downy mildew resistance was influenced by genes with additive and dominance effects, plus different types of epistatic effects such as additive x additive, and dominance x dominance. Overall results indicated that genes with predominantly non-additive effects controlled resistance in DRAC, whereas resistance in Suwan-L1 was largely influenced by additive gene effects. These findings have serious implications on the effective use of these downy mildew resistance sources in breeding programmes that aim to generate varieties with downy mildew resistance.

Overall, results suggested that inbreeding and selection within heterotic groups, followed by hybridization between inbreds within and across heterotic groups would be effective to generate new hybrids. The breeding programme will consider development of

conventional hybrids, such as single crosses and three way crosses, and top crosses. Implications of the findings of the study and recommendations are discussed.

Declaration

I **Pedro Fato** declares that:

(i)   The research reported in this thesis, except where otherwise indicated, is my original work;

(ii)   This thesis has not been submitted for any degree or examination at any other university;

(ii)   This thesis does not contain other persons' data, pictures, graphs or information unless specifically acknowledged as being sourced from other persons;

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…………………………………………   Date……………………

Pedro Fato (Candidate)

As the candidate's supervisors, we agree to the submission of this thesis

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

Dr John Derera (Supervisor)

Signed

…………………………………………..   Date……………………

Professor Pangirayi Tongoona (Co-supervisor)

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Dedication

This work is dedicated to my loving wife Margarida Morgado Pavista, my children Harry and Hillary, my mother Amanjasse Joack, my late father (FATO MAGUNGE) and my late grandmother (FANIA JOACK).

Table of Contents

List of Abbreviations

Abbreviation	Meaning
ACCI	African Centre for Crop Improvement
ADD	Average degree of dominance
AGRA	Alliance for a Green Revolution in Africa
ANOVA	Analysis of variance
BCP_1 and BCP_2	Back cross to first (P1) and second (P2) parents, respectively, in a generation mean analysis
CARS	Chokwe Agriculture Research Station
CIMMYT	International Maize and Wheat Improvement Center
CIMMYT- A, B and A/B	CIMMYT heterotic group A, B and A/B, respectively
CML	CIMMYT Maize Line
CV (%)	Coefficient of variation given in percentage
DINA	Agriculture Directorate under the Ministry of Agriculture in Mozambique
DM	Downy mildew
DMR	Downy mildew resistance
DMR-SR	Downy mildew and maize streak virus resistance
F	Coefficient of dominance
F_1, F_2	Single cross first generation and first self-pollinated generation, respectively, in a generation mean analysis
FAO	Food and Agriculture Organization of the United Nations
FAOSTAT	Food and Agriculture Organization Statistics
FGD	Focus group discussion
FS	Formal survey
G x E	Genotype by environment interactions
GCA	General combining ability
GCA_L	General combining ability of line in Line x Tester mating design
GCA_T	General combining ability of tester in Line x Tester mating design
GLM	General liner model in SAS computer program
GLS	Grey leaf spot disease (which is caused by *Cercospora zeae-maydis*)
IIAM	Acronym for Mozambican Agrarian Research Institute: Instituto de Investgação Agrária de Moçambique

IITA	International Institute of Tropical Agriculture
INE	Acronym for the National Institute for Statistics in Mozambique: Instituto Nacional de Estatística.
INIA	Acronym for the National Agriculture Research Institute (which is now IIAM in Mozambique, see definition for IIAM above): Instituto Nacional de Investigação Agronómica de Moçambique.
IRMA	Insect Resistant Maize for Africa
KARI	Kenya Agriculture Research Institute
LP	Linha Pura, refers to elite maize inbred lines in the IIAM maize program
LSD	Least significant difference
masl	Altitude measured in metres above sea level
MP	Mid-parent value, mean value of two parents in a cross
MPH	Mid -parent heterosis
MSV	Maize streak virus disease of maize
MT	Mean of tester
NGOs	Non-governmental organizations
OPV	Open pollinated variety
P_1 and P_2	First and second parents in a single cross
PRA	Participatory rural appraisal
QTL	Quantitative trait loci
RY	Relative grain yield
S, Z and S/Z	Heterotic group for genetic materials related to maize populations Suwan-1, ZM523 testers, and those displaying heterosis with both testers in the maize breeding programme in Mozambique.
S_1, S_2, S_3, S_4	First to fourth generation progeny of self-pollination, respectively, to achieve inbreeding in maize
SADC	Southern African Development Community
SC	Single cross hybrid
SCA	Specific combining ability
SEMOC	Mozambican Seed company (Sementes de Moçambique)
SH	Standard heterosis
SOPV	Open Pollinated Synthetic Variety
T- S, T- Z	Testers Suwan-1 and ZM523, respectively

TC	Top cross hybrid, which is a product of a cross between an inbred line and a population
TIA	Acronym for the National agriculture surveys in Mozambique: Trabalho de Inquérito Agrário.
TWC	Three way cross hybrid
U.E.M	Eduardo Mondlane University
UARS	Umbeluzi Agriculture Research Station

Introduction to Thesis

1. Importance of maize in Eastern and Southern Africa

Maize (*Zea mays* L.) is the first staple food crop after cassava in Sub-Saharan Africa (DeVries and Tonnessien, 2001). It is a strategic food crop for the majority of families in southern Africa (Lopes and Larkins, 1996). It is the basic source of food and income for more than 100 million people who depend on small-scale farming in Southern Africa (Bänziger and de Meyer, 2002). In Mozambique, for example, maize is the major staple food for more than 80% of the people living in the rural areas. It is a primary source for daily calorie intake (44%) followed by cassava (36%), and in many cases it is also the principal source of protein intake in Mozambique (SADC/FSTAU, 2003). According to the SADC/FSTAU (2003), the per capita maize consumption in Mozambique is the highest among cereal crops, representing about 54kg per annum, representing 66% of the cereal intake.

There is a sharp contrast between population growth rate, which is about 3% per annum and declining of food production in Southern Africa (Wobil, 1998). Recurring drought, floods and hurricanes in some of the countries and socio-politic problems among other factors compromise maize production. Seed security becomes a priority among Southern and Eastern African countries, where demand for improved seed (based on estimates from nine countries) was about 114.1 and 161.8 million tons, respectively in 2006/07 season (Langyintuo *et al.,* 2009). The seed supply in these countries was estimated to be 63 and 49.4 million tons in both regions, respectively (Langyintuo *et al.,* 2009). During the period 2006 to 2008, cereal production in the Southern African Development Community (SADC) region was dominated by maize as indicated in Table 1.

Table 1: Cereal production in SADC Region in 2006/07 and estimates of 2007/08 season (1000*Metric tons)

	Maize		Wheat		Sorghum/ Millet		Rice		All cereals	
	06/07	07/08	06/07	07/08	06/07	07/08	06/07	07/08	06/07	07/08
South Africa	7,339	11,792	1,905	2,063	200	270	0	0	**9,444**	**14,125**
Other SADC *	11,605	10,797	354	370	2,070	2,069	1,247	1,298	**15,276**	**14,534**
Total	**18,944**	**22,589**	**2,259**	**2,433**	**2,270**	**2,339**	**1,247**	**1,298**	**24,720**	**28,659**

Source: SADC Food security Early Warning System (2008).* All SADC countries except South Africa, DRC and Madagascar.

2. Production of maize in Mozambique

The area covered by maize in Mozambique in 2007 was about 1.5 million hectares accounting for 35% of the total cultivation, producing 1.6 million tons; with the average yield of 1.1 t ha^{-1} (FAOSTAT, 2009). In general the trends of maize area, production and yield from 1961 to 2007 in Mozambique, highlights three major historic moments. During the colonial era (up to 1975), a limited number of commercial farmers using relatively high level of mechanization and inputs mainly grew maize. After independence in 1975, most of the commercial farmers left the country and maize production declined progressively while the area continued to increase. The lowest maize production was observed in 1992 at the end of the civil war. Maize production increase of about 28.5% from 1993 to 1997 was reported (CIMMYT, 1998). However, the growth rate of maize production decreased to 14.5% in 2000; due to a flood followed by drought stress (Aquino *et al*., 2001). The trend in yield increase was mainly due to the increased area under maize production (Figure 1) rather than improvements in yield and agronomic practice. To enhance production grain yields that are above 1.2 t ha^{-1} are required. This strategy would lead to increased production levels on the existing land area. It is suggested, therefore that hybrids, which are more productive than open pollinated varieties, should be developed.
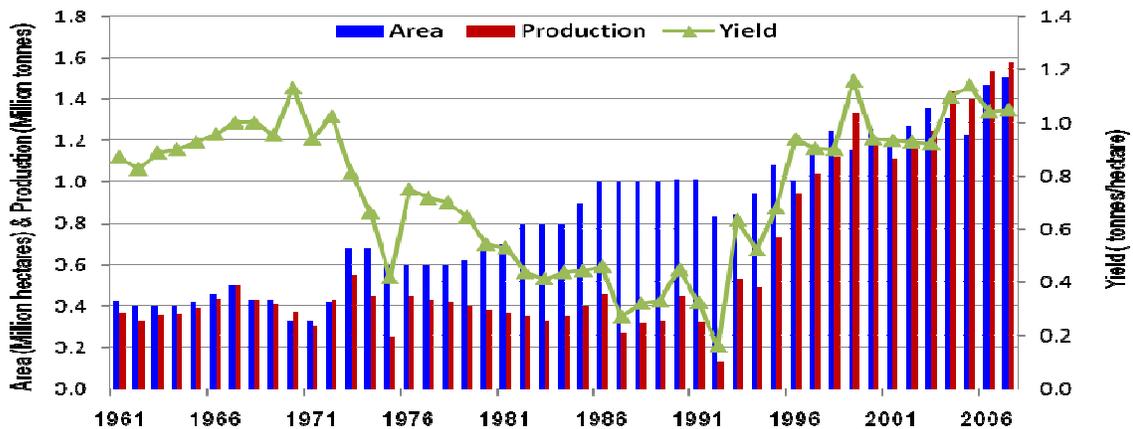
Figure 1 Trend of maize production in Mozambique. **Source: Computed based on Food and Agriculture Organization data, FAOSTAT (2009)**

3. Major production constraints in the lowland environments

In lowland areas, production is limited by several factors such as drought, low soil fertility in sandy soils, and diseases and pests. The main diseases and pests include; downy mildew disease (caused by *Peronosclerospora sorghi*), maize streak virus (MSV), stem and ear rot (*Diplodia spp*. and *Helminthosporium spp.*), stem borer (*Chilo patellus, Busseola fusca* and *Sesamia calamistis*), grain weevils (*Sitophilus zea mais* Motsch.) and the large grain borer (*Prostephanus truncatus*) (FAO, 2004; Fato *et al*., 2008). These constraints when combined with socio-economic factors, such as the unavailability of improved seed and agro-chemicals, lack of labour, marketing related problems, and lack of clear understanding of the farmer's requirements and preferences, make maize grain production extremely difficult in Mozambique. Thus, this study aims to generate stress tolerant germplasm, especially hybrids that are resistant to downy mildew, and to have a clear understanding of socio-economic factors underpinning production in Mozambique.

4. Breeding hybrids for resistance to maize downy mildew

Downy mildew is one of the most important foliar maize diseases in the tropical lowlands worldwide, including Mozambique (Frederiksen *et al.,* 1969; Frederiksen and Renfro, 1977; Williams, 1984; IITA, 1999; Denic *et al.*, 2001; Vivek, 2005). Downy mildew (DM) was reported in African countries, with severe outbreaks from Mozambique, Uganda, Democratic

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Republic of Congo and Nigeria in 1993 (IITA, 1999). A severe situation of DM can cause complete loss of the maize crop in a short time. In Nigeria, for example, yield loss approximating 90% was reported (Anaso *et al*., 1989). The fungus, *Peronosclerospora sorghi*, also infects and causes disease in alternative hosts such as sorghum species, pearl millet, and teosinte (Ullstrup, 1997). A wet, humid environment and mild temperatures favour disease development (IITA, 1999) and these conditions are common in the lowland areas of Mozambique. As a result, DM can reach very high levels in these areas leading to devastating yield losses when susceptible varieties are grown.

The national maize programme of Mozambique under the Institute for Research in Agriculture (Instituto de Investigação Agrária de Moçambique, IIAM) has been working on improving maize cultivars for downy mildew resistance for more than 15 years. Germplasm from the International Maize and Wheat Improvement Center (CIMMYT) maize programme, has been introduced as well as resistance sources to downy mildew from Thailand, Philippines and Nigeria. Some open-pollinated varieties (OPVs) have been developed by the national programme and a local seed company (SEMOC) (Bueno *et al*., 1989, 1991; Chauque *et al*., 2004). However, despite all this effort, no single hybrid with downy mildew resistance has been released in Mozambique. Nonetheless, the demand for maize hybrids has increased in the country (Fato *et al*., 2004; Langyintuo *et al*., 2009). Therefore, this study aims to contribute to the maize-hybrid development programme in Mozambique. Since the maize germplasm used in Mozambique comes from different sources with many backgrounds, understanding the heterotic orientation of this germplasm and identification of appropriate testers to differentiate this germplasm constitutes an important step for hybrid development. Mickelson *et al*. (2001) emphasized the importance of understanding the heterotic relationships between different populations to exploit effectively the exotic germplasm.

5. Farmers preferences

CIMMYT and the International Institute for Tropical Agriculture (IITA) developed many improved varieties, including open pollinated varieties and hybrids, in Eastern and Southern Africa. The majority of these varieties are agronomically superior to the existing private commercial hybrids (Pixley and Banzinger, 2001). Some of these materials have been selected using participatory breeding approaches, such as the mother and baby trials at

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CIMMYT. However, farmers do not grow most of these improved varieties. Participatory rural appraisal (PRA) has been used to assess the level of adoption of improved varieties and to understand the causes of non-adoption of new varieties. Data from PRA can be used to setup a breeding programme that meets quality requirements for farmers and end-users. Therefore, in this study a survey was conducted to investigate farmers' preferences and production constraints in lowland and mid-altitude maize production environments of Mozambique.

6. Research Objectives

The specific objectives of this study were:

(i)   To identify farmers' preferences for maize improved varieties, investigate farmers' perceptions on the major production constraints and investigate farmers' variety selection criteria in two maize ecologies of Mozambique;

(ii)   To determine the combining ability, heterosis and heterotic orientation for downy mildew resistance and yield among the new tropical lowland maize inbred lines in Mozambique using line by tester mating design;

(iii)   To determine gene action, heterosis, heterotic grouping and patterns of lowland tropical Mozambican elite maize germplasm using diallel mating scheme;

(iv)   To determine genetic effects governing downy mildew resistance in two maize populations, using a generation mean analysis.

7. Research Hypotheses

The following hypotheses were tested:

(i)   Farmers in the lowland and high altitude areas of Mozambique are aware of the major production constraints, and have specific preferences for certain agronomic traits; which breeders should consider in developing new cultivars, especially the new maize hybrids;

(ii)   The recently developed Mozambican maize inbred lines have high combining ability effects for grain yield potential and resistance to downy mildew; hence they can be

exploited to make hybrids and synthetic populations that are adapted to the lowland areas in Mozambique;

(iii)   To a large extent, the elite maize lines used in Mozambique belong to different heterotic groups, which can be exploited in developing hybrid varieties;

(iv)   Resistance to downy mildew in these elite maize inbred lines is governed by additive gene action; hence, selection procedures can be used to improve the level of downy mildew resistance in these elite and new inbred lines.

8. The structure of thesis

The foregoing objectives and hypotheses were tested in different chapters as follows:

Chapter 1: Literature review

Chapter 2: Farmers' preferences for maize varieties and their perceptions on the major production constraints in two maize production environments in Mozambique

Chapter 3: Combining ability for downy mildew resistance and grain yield among the tropical lowland maize lines in Mozambique

Chapter 4: Gene action for grain yield, heterosis and heterotic grouping in lowland tropical elite maize germplasm

Chapter 5: Generation mean analysis of downy mildew resistance in African tropical lowland elite maize inbred lines.

Chapter 6: Overview

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CHAPTER 1

Literature Review

1.1 Introduction

This review discusses the major maize research aspects in the context of crop improvement for downy mildew resistance in the maize tropical environments, especially lowland areas. The following aspects are reviewed: (1) major production constraints in Southern African maize growing environments, (2) theories in relation to pathogen-hosts interaction, and (3) genetic studies on maize resistance to downy mildew disease. Technical aspects related to the maize hybrid development programme, such as determination of heterotic groups and patterns, grain yield potential of both hybrids and open-pollinated varieties, genotype stability and genotype x environment interaction (G x E) are also addressed.

1.2 Maize production in lowlands

Maize is one of the most important food crops in Mozambique and in the southern African region. It constitutes the staple food, base of incomes and food security, mainly for smallholder farmers who grow the crop in diverse agro-ecological conditions (Denic *et al*., 2008). According to Bänziger *et al*. (2004), the maize mega-environments in the Southern African region comprises mid-altitude environments (75%), lowland tropical environments (21.9%), and highlands (3.1%). The features of each maize mega-environment, ranging from foliar diseases, temperature, rainfall, soil and areas in Southern Africa are presented in Table 1.1. The tropical lowland environments include humid and dry areas, with maximum temperatures greater than 30°C, annual precipitation below 700 mm and are associated with high risks of drought. In Mozambique, this environment is extended from 0 to 400 m.a.s.l (meters above sea level). It is the most important but challenging environment for maize production, being the most stressed maize growing environment characterized by drought stress, sandy soils, low soil fertility, and pests and diseases. Much of the existing and available maize germplasm is not adapted to this area, which represents more than 65% of the total cultivated land in Mozambique (Denic, 1994; Bänziger *et al*., 2004). Therefore,

there is need to generate new maize varieties especially hybrids and top crosses for potential deployment in this very important agro-ecology.

Table 1.1 Classification of locations based on Southern African Development Community (SADC) Maize Mega-environment

Agro-ecological Zone	Mega-environment	Prevalent Foliar diseases	Average maximum temperature (^{0}C)	Precipitation (mm)	Subsoil pH (water)	Area in SADC (ha)	(%)
A	Mid-altitude	Gray leaf spot Turcicum leaf blight	24 – 27	> 700	< 5.75	46,282	18.2
B	Mid-altitude	Rust (*P. sorghi*)	24 – 27	> 700	> 5.76	28,826	11.4
C	Mid-altitude		24 – 30	< 700		48,291	19
D	Mid-altitude		27 – 30	> 700	< 5.75	17,166	6.8
E	Mid-altitude		27 – 30	< 700	> 5.76	49,589	19.6
F	Lowland	Downy mildew Rust (*P. polysora*) *Helminthosporium maydis*	> 30	> 700		17,146	6.8
G	Lowland	Downy mildew	> 30	< 700		38,403	15.1
H	highland		< 24			7,897	3.1
Total						**253,600**	**100**

Source: Banzinger et al. (2004)

1.3 Biotic constraints

Bellon (2001) listed the following diseases among the major maize biotic constraints in the tropics: maize streak virus (MSV), downy mildew (DM) (*Peronosclerospora sorghi*), turcicum leaf blight (*Exserohilum turcicum)*, gray leaf spot (GLS) (*Cercospora zeae-maydis*)*,* rusts (*Puccinia* spp); ear rot (*Fusarium* and *Diplodia* spp), blandea leaf and sheath blights and corn stunt. Pests including borers (*Chilo, Sesamia spp. and S.W. corn borer*); grain weevil (*Sitophilus zea mais* Motsch.); fall armyworm (*Spodoptera frugiperda*) and weeds (*Striga* spp) were also listed among the constraints.

Some of the biotic stresses are economically important worldwide and others have local importance. Maize downy mildew disease, for example, is a major disease in the tropics,

especially in Asia (Bellon, 2001). It also constitutes one of the most destructive foliar diseases of maize and sorghum in the tropical lowlands worldwide, including Mozambique (Frederiksen *et al.,* 1969; Frederiksen and Renfro, 1977; Williams, 1984; IITA, 1999; Denic *et al.*, 2001; Vivek, 2005). Downy mildew has been reported in several African countries with differences on maize yield suppression. It can wipe out maize crops within a relatively short time in epidemic situations. Severe DM epidemics have been reported in Mozambique, Uganda, Democratic Republic of Congo and Nigeria (IITA, 1999). A wet, humid environment and mild temperatures are the conditions that favour spore germination, infection growth and disease dispersal throughout the field (IITA, 1999). These conditions are prevalent in the lowland areas in Mozambique hence high disease epidemics have been experienced especially when susceptible varieties are grown without chemical treatment.

The other economically important disease for all Mozambican agro-ecological environments is MSV (Nunes *et al*., 1985). Maize streak virus disease is considered one of the major diseases covering at least 66% of the maize area in Africa (Diallo *et al*., 2005). In Nigeria, for example, before DM became economically important MSV was the most destructive disease in the 1970s and was responsible for significant maize yield reduction (Ikene and Amusa, 2004). However, CIMMYT and IITA have developed varieties with genetic resistance to MSV disease resulting in many varieties with MSV resistance being released over the years. In both West Africa and the Eastern and Southern Africa regions considerable germplasm with resistance to MSV have been developed for use by national research programmes as sources of resistance (Maredia *et al.*, 1998) in breeding varieties with acceptable level of resistance.

The most important diseases for Mozambican mid-altitude and highland areas are grey leaf spot, turcicum leaf blight, rusts, and ear and stem rots (Nunes *et al*., 1985; Segeren *et al*., 1994). The most serious pests are principally the stem borer species (*Chilo partellus*, *Busseola fusca* (Fuller) and *Sesamia calamistis* Hampson), and storage pests, mainly weevils (*Sithophilus* spp). More recently, the large grain borer (*Prostephanus truncatus*) has also become a problem for grain storage in most maize growing areas (FAO, 2004). In southern Mozambique, especially in years of prolonged rainfall, maize yield losses due to pests, particularly rats and insect pests in local maize storage were reported to be as high as 40% per annum (FAO, 2004). Weevils have been responsible for more than 80% of

grain damage and more than 60% of grain weight loss under smallholder farmer's storage systems (Fato *et al*., 2008).

1.4 Breeding for resistance to maize downy mildew

Efforts to breed for resistance to downy mildew in Africa started in Nigeria as a collaborative programme between the National Maize Research Programme and International Institute of Tropical Agriculture (IITA) in the mid 1970s (IITA, 1999; Ajala *et al*., 2003). Screening methods for multiple disease resistance using artificial infection were employed and downy mildew resistant varieties with resistance to other important diseases, such as maize streak virus (MSV) were developed (Fajemisin *et al.,* 1985). Although the occurrence of maize DM disease was reported from several other countries in the Southern African region, it is a serious problem in Mozambique, the only country where an epidemic situation was reported (IITA, 1999). The dispersal of DM seems to increase due to favourable climatic conditions particularly in the south of Mozambique. This situation is exacerbated by the unavailability of suitable improved varieties that are adapted to the region. Therefore, there is need to breed for downy mildew resistance in these materials. Most of the germplasm developed by the CIMMYT programme in Harare, Zimbabwe does not have resistance to this disease.

1.4.1 Causal organisms and host range

Maize DM disease caused by *Peronosclerospora sorghi* (Kulk.), Weston and Uppal (Shaw), is one of the known downy mildew diseases attacking maize. There are many different types of DM, each of which is caused by a specific fungal pathogen from three genera namely; *Peronosclerospora*, *Sclerophthora* and *Sclerospora.* These fungi are obligate parasites and are systemic in the hosts (Ullstrup, 1997). Ullstrup (1997) and Nair et al. (2001) listed the following as different DM diseases:

- Philippine downy mildew (*Peronosclerospora philippinensis* Weston);
- Javanese downy mildew (*Peronosclerospora* maydis (Raciborki) Butle;
- Sugaracea/Sugarcane downy mildew (*Peronosclerospora sacchari* Miyaki);
- Spontaneoum downy mildew (*Peronosclerospora spontanea* Weston (from wild sugarcane*, Saccharum spontaneum* L.) ;
- Rajasthan downy mildew (RDM) (*Peronosclerospora heteropogoni*);

- Crazy top (*Sclerophthora macrospora* (Sacc.) Thirum. Shaw, Naras.
- Brown stripe downy mildew (*Sclerophthora rayssiae* var. *zeae* Payak and Renfro; and
- Green ear disease (*Sclerospora graminicola* (Sacc.) Schroet (after the symptom on pearl millet (*Pennisetum glaucum* R.Br).

Peronosclerospora sorghi, apart from infecting maize, also causes disease in sorghum species (*Sorghum bicolor* (L.) Moench), pearl millet (*Pennisentum glacum* (L.) R. Br), and teosinte (*Zea mexicana*) (Ullstrup, 1997). *P. heteropogoni* on the other hand, does not infect sorghum, but can infect the wild grass *(Hetropogon contortus).* Although the occurrence of *P. sorghi* in the wild sorghum species has been reported, it is not known whether specialized host races exist within the species (Ullstrup, 1997).

Although there are no molecular studies confirming the probable prevalent downy mildew species in Mozambique, genetic variability studies among maize, sorghum, and wild sorghum isolates from five Eastern and Southern Africa countries including Mozambique did not show significant evidence of biotypic specialization (Bock *et al.*, 2000). The morphology of isolates was typical of *Peronosclerospora sorghi,* suggesting that this species is the most prevalent in Africa, including Mozambique (Bock *et al.*, 2000). The features of the seven downy mildew fungi are summarised in Table 1.2.

Table 1.2 Major maize downy mildew pathogens, their host range, geographic distribution and grain yield losses

Pathogen (Disease name)	Geographic distribution	Host range	Maize yield loss
Peronosclerospora sorghi (Sorghum downy mildew)	North, Central, and Southern America, Asia, Africa, Europe and Australia.	Cultivated and wild sorghum, Johnson grass, teosinte, wild grasses (*Panicum, Pennisetum, Andropogon* species).	Causes about 90% yield loss in Nigeria
P. maydis (Java downy mildew)	Indonesia and Australia.	Teosinte, wild grasses (*Pennisetum, Tripsacum* species).	Causes about 40% yield losses in Indonesia.
P. philippinensis (Philippine downy mildew)	The Philippines, China, India, Indonesia, Nepal, Pakistan, Thailand, and USA.	Oats, teosinte, cultivated and wild sugarcane, cultivated and wild sorghum.	About 40 to 60% yield losses and reaching 100% on sweet corn in Philippines
P. heteropogoni (Rajasthan downy mildew)	North India State	*Euchlaena mexicana* (Teosinte), *Heteropogon contortus* (Spear grass), H. melanocarpus (Farm grass),	More than 60% yield losses in Rajasthan
P. sacchari (Sugarcane downy mildew)	Australia, Fiji, Taiwan, Japan, Nepal, New Guinea, India, the Philippines, and Thailand.	Sugarcane, teosinte, sorghum and wild grasses.	About 30 to 60% yield losses in Australia and Asia.
Sclerospora graminicola (Graminicola downy mildew or green ear)	USA and Israel.	Wild grasses, millet.	Minor disease in the USA and Israel.
Sclerophthora macrospore (crazy top)	Americas, Eastern and Southern Europe, Africa and Asia.	Oats, wheat, sorghum, rice, finger millet, various grasses.	Rare in tropical areas and with extensive yield losses in localized areas.
Scleropthora rayssiae var. *zeae* (Brown stripe downy mildew)	India, Nepal, Pakistan, Thailand, Bangladesh, and USA.	Several species of crabgrass *(Digitaria* species).	20 and 60% yield loss in India and more than 70% loss also reported.

Adapted from CIMMYT Maize Doctor: Thakur and Mathur (2002); Rathore *et al.* (2002); Jeger *et al*. (1998); Frederiksen and Renfro (1977).

1.4.2 Origin and dispersion of downy mildew disease

The origin of *P. sorghi* downy mildew is still controversial although it is thought to have been from the African and Asian continents. Studies in *P. sorghi* variability using isolates from Ethiopia, Nigeria, USA (Texas), Honduras, Brazil, Argentina, and India showed high variability for African and Indian isolates compared to the American and other Asian isolates (Barbosa *et al.,* 2005). This fact supports the hypothesis that *P. sorghi* probably originated from Africa and India and was introduced to other parts of the world. Researchers such as Spence and Dick (2002) had earlier on recognised two centres of diversity for many *Peronosclerospora* species. One centre was India, where species such as *Peronosclerospora dicbanthiicola, P. heteropogoni and P. westonii* occur and the other in eastern Melanesia and Australia with predominance of *P. globosa, P. maydis, P. miscanthii, P. nobi, P. sachari* and *P. spontanea.* However, the origin for *P. sorghi* and *P. philippinensis,* which are widely distributed, is still uncertain (Spence and Dick, 2002); hence more studies are required in this area.

1.4.3 Pathogenic variability

The knowledge of pathogen genetic variability is very important for crop improvement for resistance to diseases. Most of the plant pathogens have capacity to develop new strains that, in many cases, result in breakdown of resistance in the varieties previously considered resistant. However, the knowledge about existence of pathotypes within a group of pathogens only becomes useful for breeders and pathologists in organisms that have limited variability and stable pathotypes (Roelfs, 1984). Based on the genetic variability profile of the pathogen, breeders can design a breeding programme oriented for developing new maize varieties with more resistance that is appropriate. The information on genetic variability within *Peronosclerospora sorghi* downy mildew, worldwide is scarce. The first report on pathogenic variability of *P. sorghi* in sorghum came from the USA in late 1970s. Later on three distinct *P. sorghi* pathotypes were identified but these did not produce spores (Barbosa *et al*., 2006). Other reports on *P. sorghi* pathotypes in sorghum were reported from Brazil (Fernandes and Schaffert, 1983), Honduras (Craig and Odvody, 1992), and Zimbabwe (de Milliano and Veld, 1990). Other studies using isolates from different regions also showed the existence of two pathotypes of this pathogen in Africa (Adenle and Cardwell, 2000). One strain can attack both maize and sorghum crops, while the other

attacks specifically maize (Anaso *et al.,* 1987; Olanya and Fajemisin, 1993). The maize or sorghum strain was reported from Botswana, Kenya, North Nigeria, Rwanda, Tanzania, Uganda, Zambia and Zimbabwe (Bock, 1995; de Milliano, 1992; Bigirwa *et al*., 2000); while the maize strain was reported from the Democratic Republic of Congo (DRC) (Adenle and Cardwell, 2000), Mozambique (Plumb- Dhindsa and Mondjane, 1984) and Southern Nigeria (Olanya and Fajemisin, 1993).

1.4.4 Biology, life cycle and epidemiology

The *Peronosclerospora sorghi* life cycle has asexual and sexual development phases. The asexual phase consists of development of conidia, while the sexual phase produces oospores (Weston and Uppal, 1932). The conidia are produced in erect conidiophores, which grow out through stomata on fresh leaves at early stages of the maize plant development. They are responsible for systemic and polycyclic increase and secondary spread of the disease in the field and epidemic infections throughout the season (Rajasab *et al.,* 1979; Jeger *et al*., 1998; Bock and Jeger, 2002). Wind plays a major role for dispersing conidia in the field (Bock *et al*., 1997; Bock and Jeger, 2002). Denic (1996) observed that prevailing wind direction was a determinant factor for uniform distribution of inoculum throughout the nursery screening field when artificial infestation was applied.

Amongst the most important environmental conditions affecting asexual spore production are light intensity, darkness, relative humidity and temperature. Schmitt and Freytag (1974) suggested that under artificial infestation, prior to conidia production, host plants of DM must be exposed to a minimum of four hours of high light intensity. Shetty and Safeeulla (1981) found that maximum conidia production from systemically infected sorghum leaves occurred at 20^0C, in the dark and less than 100% relative humidity. Bonde *et al.* (1985), studying the USA sorghum downy mildew isolates on maize found optimal temperature for sporulation to be between 15 and 23^0C. Some studies under field conditions revealed a high relationship between the process of conidia production and temperature, relative humidity and leaf wetness. Bock *et al.* (1998a), studying isolates of DM from Zimbabwe and other semi-arid regions of Southern Africa, found that conidia were produced between midnight and five o'clock in the morning when temperatures were around 20^0C. Shenoi and Ramalingam (1979) reported similar results for studies conducted in India (

The sexual phase of *P. sorghi* is related to the development of oospores and these constitute the primary inoculum source. The host infection occurs through the seed and soil (Adenle and Cardwell, 2000; Thakur and Mathur, 2002). According to Adenle and Cardwell (2000), there is strong evidence that oospores of *P. sorghi* present in the seed are from crazy tops of diseased plants not removed from the field before maturity. These oospores can survive and remain infective in seeds with less than 10% moisture content, for periods between eight months and ten years in storage.

1.4.5 Geographical distribution and economic importance

Maize downy mildew disease occurrence was reported from more than 44 countries in Africa, Asia, Americas (North, Central, and South), Australia, Europe, and Mildest (Jeger *et al.*, 1998). In Africa, *P. sorghi* has a wide distribution, occurring at different altitudes and in different agro-ecological environments (William, 1984). Apart from Mozambique, *P. sorghi* was reported from Angola, Benin, Botswana, Burundi, DRC, Egypt, Ethiopia, Ghana, Kenya, Malawi, Mauritania Nigeria, Rwanda, Somalia, South Africa, Sudan, Swaziland, Tanzania, Uganda, Zambia and Zimbabwe (Plumb-Dhindsa and Mondjane, 1984; Williams, 1984; Frison and Sadio, 1987; De Milliano, 1992; Bock *et al.*, 1998b; Jeger *et al.*, 1998; IITA, 1999; Bock *et al.*, 2000; Adenle and Cardwell, 2000).

Downy mildew occurrence was reported from the early 1980s. Plumb-Dhinda and Mondjane (1984) and Nunes *et al*. (1985) reported occurrence of DM from the Southern region of Mozambique comprising three provinces: Maputo, Gaza and Inhambane. More comprehensive disease survey conducted between 1994 and 1995 across the country revealed that DM infection was more frequent in heavy soils, where maize was grown under irrigation conditions in the South, and in the humid zones countrywide (Segeren, 1995). Later on, the disease was also becoming a huge problem in some areas of Manica and Sofala provinces in the Centre (Denic, 1996). Presently the disease incidence seems to be increasing in the South and spreading to new areas in the Centre and North. For example, DM occurrence was observed for the first time in some areas in Angónia (1330 m.a.s.l), suggesting that the disease is now covering all the maize growing environments of Mozambique (*Personal observation*). Therefore, it is prudent to deploy varieties with some acceptable level of downy mildew resistance in all production areas.

Downy mildew is considered one of the most destructive diseases in Asia, particularly, in the Philippines, Indonesia, Thailand, and India (Ullstrup, 1997; Nair *et al*., 2001). In Africa, epidemic situations were reported from Mozambique, Uganda, DRC, and Nigeria, where vast areas of maize crop were wiped out by downy mildew within a relatively short time in 1993 (IITA, 1999). Downy mildew is the principal foliar disease limiting maize production in the lowland areas of southern and central parts of Mozambique. Grain yield loss associated with the disease is unknown, but can be higher than 50%, based on the level of incidence observed in some areas of Maputo and Gaza provinces in 2008.

1.4.6 Disease control by genetic resistance

Downy mildew can be controlled by cultural practices, or by treating seed with systemic fungicide, such as ridomil. However, seed treated by ridomil is generally too expensive for resource poor farmers, who predominantly grow maize for subsistence without cash income in Mozambique and in other areas of Sub-Saharan Africa. Thus, use of genetic resistant cultivars provides the most efficient and economic control method (Bellon, 2001; Denic *et al*., 2001). Development of maize cultivars resistant to downy mildew disease may be done through transferring genes for resistance to adapted genotypes (Ullstrup, 1997). Studies on the inheritance of resistance of maize to this disease, although not exhaustive, indicate that resistance is dominant to intermediate gene effect. The literature also suggests that a few or many genes might be involved in the inheritance (Bocholt and Frederiksen, 1972; Frederiksen and Ullstup, 1975; Lal and Singh, 1984; Agrama *et al*., 1999; Kim *et al*., 2003).

Use of molecular markers appears promising for the better understanding of the nature of inheritance of resistance to this disease in maize. Nair *et al*. (2001) detected important polymorphic loci in two Indian genotypes (CM139 and CM117) related to resistance to the maize downy mildew. These preliminary results have suggested the possibility of using marker-assisted selection for downy mildew resistance in maize. Further, quantitative trait loci (QTL) with significant effects on resistance to the five Asian downy mildew diseases, including for maize, were identified for resistance to *P.sorghi* (George *et al.*, 2003; Nair *et al*., 2005), A strong and stable QTL was found on chromosome six of maize (George *et al.*, 2003; Nair *et al*., 2005).

1.4.7 Symptomatology

The primary infection of downy mildew starts in tender leaves with small, pale yellow spots, with undefined borders on the upper leaf surface. This is then followed by discoloration of the upper leaf surface, starting from the base and gradually progressing to the upper extreme of the leaf (Figure 1.1-A). Infection results in plants with stiff, narrow, erect with yellow leaves and inflorescence that are distorted resulting in abnormal cobs being formed. Generally, the infected plant does not produce cobs resulting in 100% single plant yield loss, and overall, yield is compromised when many plants are infected at a young stage if susceptible varieties are grown. In the cool and humid conditions, the low surface of the infected leaf is covered with a white layer of conidia and conidiophores (Figure 1.1-B). Generally, the upper plant structure is replaced by a mass of twisted leaves sometimes called 'crazy top' (Figure 1-C; Lal and Singh, 1984; IITA, 1999; Ajala *et al*. 2003).

Figure 1.1 Symptomatology of downy mildew on maize: A- progress of yellow spots with indefinite borders on the leaf surface in the early stages; B- advanced stage showing spores at surface of leaves; C- more advanced formation of crazy top

1.4.8 Sources of resistance

Sources of resistance to downy mildew among cultivated maize were reported by IITA (IITA, 1999; Ikene and Amusa, 2004) and CIMMYT- Asian Regional Maize Program (Yen and Prassana, 2001; Nair *et al.,* 2001; CIMMYT, 2004; Yen *et al.,* 2004). CIMMYT (2004)

reported five DM resistant maize populations (Pop 100, Pop 145, Pop 345 and MDR-DMR) with levels of resistance varying from 90% to 100%. Kalpana et al. (2009) also reported similar levels of resistance to DM from lines KV-10 and NAI- 129. Yen *et al.* (2004) identified five maize lines resistant to both *Peronosclerospora sorghi* and *P. heteropogoni* downy mildews in Asia. Lines such as NAI 116, Nei 9008 and C3S3B-46-1-1-1-1-2 B were reported to be resistant to many downy mildews and were used in Asian regional maize programme (Sudha *et al.*, 2004). CIMMYT maize lines CML425 to CML433 were described as resistant to DM by Pratt and Gordon (2006) while some national programmes have also reported important sources of genetic resistance among locally adapted germplasm (IITA, 1999; Yen and Prassana, 2001; Denic *et al.*, 2001; Ikene and Amusa, 2004; Sudha *et al.,* 2004). These existing DM resistant germplasm can be used in breeding programmes. Kim *et al.* (2003) reported effective resistance to DM after two to four cycles of S_1 recurrent selection on six lowland tropical maize populations from CIMMYT-Mexico (Pop 22, Pop 28 and Pop 43) and IITA-Nigeria germplasm (TZL Comp 4, DMRESR-W and DMRLSR-W). In Nigeria, scientists were able to develop resistant maize cultivars with levels of resistance between 90 and 95% using germplasm introduced from Thailand and the Philippines (Ikene and Amusa, 2004). However, care should be taken on the use of different sources of resistance due to variability in pathogen virulence, as there have been reports of susceptibility in lines characterised as resistant in one region, when tested in another region. For example, Sudha *et al.* (2004) reported a susceptible reaction in India from lines KUI1411 and KUI1414b that were resistant to DM in Thailand. In another study, line MO17Ht described as resistant to *P. sorghi* DM in Texas was reportedly susceptible in Thailand (Singburaudom and Renfro, 1982). This demonstrates the dangers of relying on introduced sources of resistance without local verification. Kamala *et al.* (2002) reported potential sources of resistant genes from wild and weed sorghum, which can also be exploited by genetic engineering tools to develop sorghum or maize DM resistant varieties.

1.4.9 Screening methods for resistance and rating scales

Various methods were suggested for screening maize for downy mildew resistance. The most widely used methods are those based on spreader rows (Williams, 1984; Cardwell *et al.*, 1994; Denic, 1996), which resulted in heavy disease infection in nurseries. The method involves planting experimental material between spreader row plots 14 days after planting

infected pre-germinated seed. For the spreader rows, seed of a susceptible variety is pre-germinated and then inoculated using fresh infected leaves, and after inoculation, seed is maintained at 20^0C over night. The pre-germinated inoculated seed is planted at both ends of each experimental plot row. Methods combining spreader-rows with direct spray of seedlings or spreader-row plants have been suggested (Yamada and Aday, 1977; Neeley, 2001).

According to Denic *et al.* (2001), double the plant density in nurseries is maintained for five weeks after emergence and then, plants with DM are rouged out. Disease severity can be assessed using diagrammatic percentage visual rating scale of 1% to 50% (James, 1971). This disease rating scale is based on the area of discoloration in the leaf (Fig. 1-A). Many other rating scales were reported for example, 1-5 or 0- 9, where 1 and 0 represent low infection and 5 and 9 high infection levels (IITA, 1999). Disease incidence is also assessed by estimating percentage of diseased plants per plot (Denic *et al*., 2001; Ajala *et al*., 2003).

1.4.10 Progress and challenges in breeding for downy mildew resistance in maize

The most improved cultivars worldwide are adapted to the most productive areas (covering 15% to 20% of total cultivated area) where DM is not economically important (Denic *et al.,* 2001). The National Maize programme in Mozambique has been working on genetic improvement for resistance on maize for more than two decades using resistant source germplasm from Thailand, Philippines and Nigeria. Some open pollinated varieties were developed by national maize programme and the seed company SEMOC (Chauque *et al.,* 2004). Despite all these efforts, the programme in Mozambique released only one hybrid (Lhuvukani) with some resistance to this disease in 2008. However, the demand for improved maize varieties especially hybrids tends to be on the increase among farmers in the country (Fato *et al*., 2004). Therefore, there is a call to develop new hybrid varieties with higher levels of resistance to downy mildew.

1.4.11 Inheritance of downy mildew resistance and combining ability in maize

A better understanding of the gene action involved in the inheritance of major traits is important for developing effective breeding strategies without sacrificing yield potential.

Robinson *et al.* (1949) and Falconer and Mackay (1996) classified genetic components for quantitative traits as additive, dominance and epistasis. Additive variance expresses the proportion of a trait that can be transmitted from parents to offspring, thus reflecting the degree of resemblance between progenies and their parents (Falconer and Mackay, 1996). Additive variance can be expressed by narrow sense heritability. When the value of narrow sense heritability is high for a given trait, it means that there is high probability that the trait will be transmitted from parents to the progeny. Non-additive gene action includes all types of variation that cannot be explained by the additive model (Falconer and Mackay, 1996). This includes different forms of dominance and epistasis. In general, this kind of gene action may not be transmitted from parents to offspring.

Studies of combining ability of maize genotypes for DMR revealed the presence of both additive and non-additive gene effects, with additive effects being more important in determining disease reaction. The inheritance for DMR in maize was reported to be monogenic dominant, recessive or polygenic additive depending on the lines involved in the studies (Frederiksen and Ullstup, 1975; Jinahyon, 1973). Orángel and Borges (1987), studying inheritance of DM using crosses between the resistant lines and susceptible (48-s-28) observed intermediate disease reaction, suggesting a polygenic system for resistance to *P. sorghi* in maize. Jinahyon (1973), observed the same results in the study using open pollinated maize varieties. However, Frederiksen and Ullstrup (1975) observed that resistance was dominant in some crosses and recessive in others. Many studies on resistance of maize to *P. sorghi* revealed that the resistance was controlled polygenically, but the phenotypic expression varied with level of infection with some kind of threshold reaction (Lal and Singh, 1984).

1.5 Heterosis, heterotic groups and heterotic patterns in maize

1.5.1 Implications of significant heterosis

Heterosis is defined as the superiority of the hybrid progeny over its inbred parents. Most maize breeding programmes focused on generating new high yielding varieties through exploitation of heterosis in hybrids and open pollinated synthetic varieties. However, effective exploitation of heterosis has not been achieved due to limited knowledge of the mechanisms on which heterosis is based. The genetic basis of heterosis in hybrids has

been partly explained by the high frequency of genes with dominance and over-dominance effects, and is based on some physiological mechanisms (Williams, 1959; Monma and Tsunoda, 1979; Echarte *et al*., 2004; Tollenaar *et al*., 2004; Melani and Carena, 2005; Frascaroli *et al*., 2008)). Melani and Carena (2005), for example, suggested that heterosis for grain yield in maize hybrids was attributable to differences in allelic frequencies between parents from different (heterotic) populations. Generally, differences in allelic frequencies of heterotic populations are explained by diverse geographical origins of germplasm, natural selection or level of parental germplasm improvement. Therefore, in a practical breeding programme, heterosis is exploited by generating lines within specific heterotic groups (Barata and Carena, 2006). Large specific combining ability, which is reflected by high yield levels in hybrids, is then achieved by crossing lines from the different heterotic groups (Hallauer *et al*., 1988; Melchinger, 1999; Barata and Carena, 2006). However, full expression of heterosis for grain yield, which is a quantitative trait, in hybrids is influenced by many factors such as physiological processes, environmental main effects and interaction between genotypic effects with the environments (William, 1959; Tollenaar *et al*., 2004), suggesting that hybrids should be tested in multi-location environments to confirm heterosis levels. Significance of heterosis therefore implies that hybrid varieties can be developed to enhance grain yield, whereas lack of its significance would imply that open pollinated varieties should be deployed.

1.5.2   Heterotic grouping and patterns

In breeding programmes, heterotic patterns are defined as a pair of heterotic groups that shows high heterosis in their crosses (Carena and Hallauer, 2001). Knowledge of genetic diversity of the germplasm is essential for designing a maize hybrid oriented breeding programme. Inbred lines selected from divergent populations are preferable because of their difference in heterotic orientation. Generally, hybrids created by crossing these divergent inbred lines result in high hybrid vigor or expression of heterosis (Hallauer and Miranda, 1988). Thus, based on differences in gene frequency of the parental genetic materials employed to make crosses, germplasm can be distributed into different heterotic groups. Each breeding programme can create its own maize heterotic grouping system that is convenient for use by the programme. The system can be based on broad classification like that used by CIMMYT to the narrow systems, with four to nine heterotic groups, used in

successful breeding programmes in Zimbabwe and South Africa (Gevers and Whythe, 1987; Olver, 1988; CIMMYT, 2001; Mickelson *et al.*, 2001; Derera, 2005).

Derera (2005) reported at least nine maize heterotic groups (Table 1.3) used in breeding programmes in Eastern and Southern Africa and. Most of the public and national breeding programmes (NARs) in Sub-Saharan Africa use CIMMYT heterotic group classification. This is because a greater proportion of germplasm used in the public sector originates from CIMMYT and it is easy to use a system that deals with only three heterotic groups (A, B, and A/B). The most comprehensive heterotic group classification on CIMMYT lowland germplasm gene pool was done by combining field evaluation and SSR molecular markers (Xia *et a*l., 2004). Xia *et al*. (2004) used as testers lines CML247 and CML254 and found four heterotic groups (A, B, C and D).

Table 1.3 Main heterotic groups of maize inbred lines in use in Southern Africa.

Heterotic group	Population of derivation	Examples of public lines	References
SC	Southern Cross	SC5522	Mickelson *et al*. (2001)
N3	Salisbury White	N3-2-3-3	Mickelson *et al*. (2001)
K	K64R/M162W	K64R, M162W	Mickelson et al. (2001)
P	Natal Potchefstroom Pearl Elite Selection (NPP ES)	NAW5867	Gevers and Whythe (1987); Olver (1998)
I	NYHT/TY	A26, I137TN	Gevers and Whythe (1987)
M	21A2. Jellicorse	M37W	Gevers and Whythe (1987)
F	F2934T/Teko Yellow	F2834T	Gevers and Whythe (1987)
CIMMYT- A	Tuxpeno, Kitale, BSSS, N3 (more dent type)	CML442, CML202 CML247, CML254	CIMMYT (2001); Xia *et al*. (2004)
CIMMYT- B	ETO, Ecuador 573, Lancaster, SC (more flint type)	CML444, CML395	CIMMYT (2001)

Source: Derera (2005)

Some developing countries established potential patterns, such as Tuxpeno x ETO, Tuson x Tuxpeno, Cuba flint x Tuxpeno, Suwan-1 x Tuxpeno (Vasal *et al.*, 1999). Thus, different programmes have developed their own convenient system for grouping their maize germplasm.

1.5.3 Methods used to determine heterotic groups

Several methods have been employed in the heterotic grouping of maize lines. Among them, are pedigree analysis methods, quantitative genetic analysis and molecular marker methods (Zhang *et al*., 2002). The pedigree analysis methods are based on the origin of the germplasm. According to Zhang *et al.* (2002), the Reid and Lancaster groups, for example, were identified through pedigree and geographic analysis of inbred lines used in the Corn Belt. Researchers like Wu (1983) employed the same procedure to establish new heterotic groups and patterns among Chinese and exotic maize germplasm.

Genetic based methods rely on differences in gene frequency of the parental genetic materials employed to make crosses (Barata and Carena, 2006). Thus, based on differences in genetic background, germplasm can be distributed into different heterotic groups and relationships among groups can be used to define heterotic patterns. Within these methods, inbred lines are assigned into different groups based on average heterosis (difference between the mean of single-cross progeny and the mean of the parents) or based on SCA estimates (Baker, 1978; Revilla, 2002; Soengas *et al*., 2003; Melani and Carena, 2005; Dhliwayo *et al*., 2009). Based on the heterosis classification, lines that display significant heterosis in their crosses are assigned to the different groups. While based on SCA effects lines that display negative SCA effects for grain yield when crossed together are considered to belong to the same heterotic group (Vasal *et al.*, 1992). These lines are expected to have similar gene frequency.

The SCA effect based classification is considered more reliable than heterosis based classification (Betrán *et al.,* 2003). This is because heterosis can be affected by environment, which can have differential effect on parental inbreds and hybrids. Thus, use of SCA based methods is more recommended because SCA effects have better predictive value for F_1 grain yield than heterosis, and it is not affected by parental inbred performance (Betrán *et al.,* 2003). Cluster analysis based on SCA data generated in diallel, North

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Carolina design II and line x tester analysis are the most used methods for establishing heterotic groups (Vasal *et al.*, 1999; Zhang *et al.*, 2002). In some cases, GCA effects were also used as criteria for heterotic definition. Fan *et al*. (2008) found GCA effects to be more important and reliable than SCA effects for heterotic patterns classification between temperate and tropical germplasm.

Molecular genetic markers represent powerful tools to delimit heterotic groups and to assign inbred lines into existing heterotic groups (Melchinger, 1999) and for diversity analysis (Legesse *et al.*, 2007). In some cases with a single method, it is not possible to discriminate lines into their appropriate heterotic groups; therefore, some authors (Parentoni et al., 2001; Menkir et al., 2004; Dhliwayo et al., 2009; Fan et al., 2009) have suggested combinations of methods. Fan *et al.* (2009), for example, classified maize inbred lines into heterotic groups using a factorial mating design that combined heterotic groups, SCA and GCA effects to assign inbred lines into heterotic groups. Parentoni *et al.* (2001) used yield SCA data to classify 28 OPVs into four heterotic groups and the consistence of lines in different groups was confirmed by mid parent heterosis data. Combining molecular markers based classification with SCA effects classification were also reported (Menkir *et al.*, 2004; Dhliwayo *et al.*, 2009).

For the case of developing hybrids, it is important to organize germplasm into separate groups. Serious problems that can affect breeding progress can arise when germplasm mix up occurs in breeding programmes. The maize CIMMYT populations and pools, for example, were formed by mixing germplasm from different sources. This mixed genetic constitution of the germplasm was thought to be the major limitation in assigning the CIMMYT germplasm into genetically diverse and complementary heterotic groups for the hybrid-breeding programme (Vasal *et al.*, 1999). The germplasm was eventually categorized based on their yield performance, heterosis and combining ability into three different heterotic groups (A, B, and A/B) (Vasal *et. al.,* 1999).

1.5.4 Conventional vs. molecular tools for determining heterotic groups and patterns in maize

Heterosis has been reported to be expressed for many traits including seed germination in maize. For example, Meyer *et al.* (2007) reported the association of heterosis in embryos

with genes that affect transduction and regulatory processes. However, there is an over-emphasis on the use of molecular tools to determine heterotic groups, which are based on genetic distances in maize breeding, than on the use of heterosis.

Results from molecular approaches have not always been consistent in associating hybrid performance with genetic distances. Therefore molecular markers may not be useful in predicting heterosis among inbred maize lines or defining effective heterotic groups for use in a breeding programme. Analysis of testcross data indicated that high levels of heterosis could be obtained even between parent-inbred lines from the same group. Although the SSR markers were almost effective in grouping lines in clusters used at CIMMYT and IITA, Dhliwayo *et al*. (2009) reported that genetic distances among inbred lines from CIMMYT and IITA were not significantly correlated with hybrid yield, SCA effects or mid parent heterosis. Barata and Carena (2006) also previously reported similar inconsistencies in the classification of North Dakota lines based on molecular and testcross data. Barata and Carena (2006) concluded that clusters of genetically similar genotypes were not identified accurately and reliably with the use of SSR markers. Osorno and Carena (2008) also reported that the best maize crosses were not always found between lines from different heterotic groups, suggesting that in general there was not a strong association between Euclidean distances among genotypes and combining ability data. Although the use of AFLP and SSR markers was effective in revealing genetic diversity and placing 40 lines into groups and subgroups that were associated with pedigree information, Menkir *et al*. (2004) observed that heterotic groups formed using testcross data were different from those formed using molecular data. Qi *et al*. (2010) investigated reliability of using genetic distances to predict hybrid performance and the results indicated moderate association ($r \leq 0.47$) of genetic distances with yield, mid- and better-parent heterosis, and SCA data.

Based on results from a survey of the literature, Barata and Carena (2006) concluded that the evaluation of testcrosses would provide more convincing data than SSR marker analysis for classifying unrelated inbred maize lines. Betran *et al.* (2003) suggested that the effects of genotype by environmental interactions on hybrid performance could explain the lack of highly significant associations between genetic distances and heterosis data.   This, therefore, indicated that testcross data from multi-environment trials was required to predict hybrid performance. The use of conventional methods such as the diallel mating scheme to determine heterotic groups and heterotic patterns is therefore still widely used and effective

in both tropical and temperate programmes (Melani and Carena, 2001; dos Santos *et al.*, 2001; Osorno and Carena, 2008). The line x tester mating design has also been successfully used to classify maize lines according to their heterosis with well-known testers (Fan *et al.*, 2008). Fan *et al.* (2009) found the use of SCA and GCA data to classify inbreds into heterotic groups to increase breeding efficiency by more than 17% compared to the use of SSR markers and SCA data alone.

Overall, the literature survey suggests that molecular tools would be more appropriate for establishing genetic diversity that is crucial in managing germplasm, whereas testcross data that is more reliable should be used to establish heterotic groups and patterns, or to predict hybrid performance in multi-location testing. The SSR markers were appropriately used to determine genetic diversity among 24 landraces, 23 open pollinated varieties and 261 inbred lines from CIMMYT (Warburton et al., 2008). Therefore, in the current study conventional methods that employ diallel and line x tester mating designs were used to identify heterotic groups and heterotic patterns among inbred maize lines from the breeding programme in Mozambique. Detailed reviews of the diallel and line x tester mating schemes have been presented by Christie and Shattuck (1992), Dabholkar (1992), and Kearsey and Pooni (1996).

1.5.5 Line x tester mating design

The line x tester method proposed by Kempthorne (1957) can accommodate large numbers of genotypes and is therefore appropriate for testing early generation lines that are usually many. It is also useful for determining the performance of early generation inbred lines to produce desirable hybrid combinations. The method can also provide information about the utility of lines for use as parents in a hybridization programmes (Dabholkar, 1992). All inbred lines are mated to the testers and single crosses are evaluated to provide information about general combining ability effects (GCA) of the lines and testers, and specific combining ability effects (SCA) which are equivalent to line x tester interaction effects (Dabholkar, 1992; Fan *et al.*, 2010). Mid-parent and high-parent heterosis can be calculated from the means of lines and testers (Hallauer and Miranda, 1998).

In general, the selection of testers has been controversial for many years. Some authors defended the use of high performance testers while others defended the use of low performance testers. There are also studies that did not find any correlation between performances of testers with ranking of inbred lines in relation to their GCA (Sharma *et al.,* 1967). In general, the selection of a tester is based on (i) the genetic base of the tester (broad or narrow based); (ii) low performance or susceptibility of the tester to the major traits under investigation (i.e., drought or downy mildew susceptibility), and (iii) heterotic background of the testers. Use of broad-based population as a tester was suggested by several studies; especially at early generation stage of lines and aiming to generate improved synthetics (Narro *et al*., 2003). Lonnquist (1949) successfully used an OPV as a tester and developed contrasting synthetics (high and low yielding) based on the performance of topcrosses. The narrow-based testers such as inbred lines and single crosses are considered the best alternative in a hybrid- oriented breeding programme that aims at generating superior single cross, three-way and double cross hybrids (Castellanos *et al*., 1998). However, Russell (1961) considered an ideal tester as the one showing maximum genetic variability among the lines in the study. Use of many testers was considered as an advantage in genetic studies oriented for heterotic grouping of lines based on their heterosis, SCA and GCA effects, mainly when the lines under study were exotic (Fen *et al*., 2010). In the current study, two testers, one with DM resistance and the other susceptible were used to classify inbred lines into heterotic groups. The two testers also differed in their level of adaptation to the tropical lowland environment.

1.5.6 Diallel mating design

Kang (1994) defined diallel as a mating design that involves all possible combinations among a given number of parental genotypes. In maize, parental genotypes involved in a diallel can be inbred lines or heterozygous populations (open pollinated populations) depending on the objective of the study. Generally, the number of parents is limited when compared to the other mating designs, such as the line x tester. Diallel mating designs can provide genetic information, such as variance components of GCA and SCA, genetic effects for fixed set of parents or heritability for a population when parents are randomly chosen (Baker, 1978; Kang, 1994). Information provided by the diallel can also be used to measure

hybrid performance and devise breeding methods and strategies in the process of developing new genotypes (Baker, 1978; Kang, 1994; Zhang *et al.*, 2005, Qi *et al.*, 2010).

According to Griffing (1956), depending on whether parents and reciprocals are included or excluded in a particular design, there are four diallel techniques proposed for determining the combining ability of lines and study gene action. Analysis of components of variance and genetic estimates can be performed based on fixed effects model (model-1) or random effects model (model-2) depending on whether parents were a fixed set or randomly chosen, respectively (Christie and Shattuck, 1992). In maize all the four methods and models are extensively used. In the current study, a diallel cross analysis was used to study gene action and to identify heterotic patterns among the inbred combinations involving tropical lowland lines from Mozambique.

1.6 Implications of significant additive and non-additive gene effects

Genetic variance components and their practical implications in breeding programmes are summarised by Parvez *et al.* (2007). Significance of additive and non-additive variance components can be used to make an inference on gene effects that are governing the traits. Genetic components are partitioned into additive and non-additive gene effects. Non-additive effects are subdivided into dominance and epistatic gene effects.

Additive genetic variance, which measures a breeding value of a genotype, is associated with the average effect of individual genes. In a hybrid oriented breeding programme, significant additive gene effects for a trait of interest suggest that the trait can be fixed in a line through six to eight generations of self-pollination with selection. Significance of additive gene effects therefore suggests that the population can be improved by appropriate selection procedures to increase frequency of the favourable alleles in the population of interest.

Dominance variance is associated with interactions between alleles at segregating loci and measure breeding behaviour of alleles in heterozygote. Relative dominance is the ratio of dominance variance to additive genetic variance. Values greater than unit indicate presence of over-dominance, value equal to one unity indicate complete dominance, while values less than unit indicate predominance of genes displaying partial dominance. Knowledge of

the levels of dominance is therefore crucial for making appropriate decision of breeding strategy to be addressed. Significance of dominance gene effects has been reported to be the basis for heterosis for grain yield in maize (Parvez *et al*., 2007). Therefore, it can be exploited in hybrid breeding programmes, by crossing complementary inbred lines.

Epistasis, which is associated with the non-allelic interaction of genes at two or more segregating loci, is partitioned into additive x additive, additive x dominance and dominance x dominance gene effects. Implications of significant epistasis for breeding, therefore, depend on the type of epistasis that is predominant. Additive x additive interaction effects are fixable and can be exploited by selection in intra-population improvement, and self-pollination with selection of lines to develop inbreds in a hybrid oriented breeding programme. On the other hand, the additive x dominance and dominance x dominance epistatic gene effects cannot be fixed hence they cannot be fully exploited in intra-population improvement but can be exploited to develop hybrids by crossing complementary lines in a hybrid development programme.

Many studies have been conducted for estimating different components of variance in maize; especially for grain yield, and most of them found that additive genetic variance was larger than dominance variance (Lamkey and Eduards, 1998). Generally, when open pollinated populations are involved in the study, the additive effects are more important than dominance or epistatic effects (Lamkey and Edwards, 1998). However, studies involving inbred lines, generally non-additive effects (dominance and epitasis) are more important than additive effects (Lamkey and Edwards, 1998). In many cases, lack of significance for epistatic components is not easy to estimate statistically. However, in the studies of most polygenic traits the presence of epistasis is generally detected. It implies that assuming additive–dominance model, the estimates of linear components of means can be biased due to epitasis effects (Viana, 2000; Parvez *et al.,* 2006).

Several methods have been used to study gene action and effects in maize. These include the diallel, other factorial mating such as the line x tester, North Carolina Design I, II and III mating schemes. In the current study, the diallel, line x tester mating and generation mean analyses were used to study gene effects for grain yield, agronomic traits and downy mildew resistance. Diallel and line x tester mating schemes have been reviewed (see 1.5 above),

but the actual application of generation mean analysis in studying gene effects is discussed below (see 1.7).

1.7 Generation mean analysis

Generation mean analysis partitions genetic effects into additive, dominance and epistasis (Cheverud and Routman, 1995). Generation mean analysis is a powerful statistical procedure for detection of epistasis using several basic generations from a cross between two inbred lines (Parvez *et al.,* 2006). Two-generation mean models were proposed, one involving diallel among a group of inbred lines or population and another involves the cross between two inbred lines and generations derived from such cross.

The first model generally uses different diallel models of Griffing (1956), Eberhart and Gardner (1966), and Gardner and Eberhart (1966) to estimate GCA and SCA, but not epistasis. The result from this model makes inference to the set of parents involved in the crosses (Lamkey and Eduards, 1998). The second model of generation mean analysis is generally used to study the inheritance of specific traits (e.g. determining the number of genes involved in the resistance for DM in maize). The huge disadvantage of this second model is that all analyses are based on the F_2 means that can be influenced by effects of heterosis. Probably, this method is more useful for crops or traits that do not exhibit heterosis.

Melchinger (1987) proposed another model for calculating generation means. In this model, two lines from the same heterotic group (P_1 and P_2) are used to create F_1, F_2, BCP_1, and BCP_2 generations. Another line from divergent heterotic group is used as parental tester (PT), which is used to topcross all other generations and their means are generated based on each segregating population. The advantage of Melchinger's model is that the generation mean of testcross can be calculated in the presence of linkage and absence of epistasis, as well as in the presence of epistasis but absence of linkage. Probably this model is as useful as the previous two due to the large number of generations and crosses to be included in the study. This requires more resources in terms of time and labour constraints. However, this approach is capable of partitioning epistasis into additive x additive (aa), additive x dominance (ad) and dominance x dominance (dd) effects.

In general, significant epistatic effects were reported from many studies when generation mean analysis was used. When epistasis estimates were obtained by analysis of variance (covariance of relatives) approach, generally non-significant epistatic effects were reported (Lamkey and Lee, 1993). Studies involving open pollinated varieties have generally shown additive effects to be more important than dominance or epistatic effects. While studies with elite inbred lines generally found dominance and epistatic effects to be more important than additive effects for yield in maize (Lamkey and Lee, 1993).

The reports on generation mean analysis for downy mildew resistance on maize are limited in literature. Bellum *et al*. (1992) used generation mean analysis to study inheritance of *P. sorghi* in sorghum and reported resistance to be controlled by about two genes. In one of the lines, there were recessive alleles in homozygous condition, while the other line had dominant alleles in the homozygous condition conferring resistance for DM. Several studies on generation mean analysis have been reported for different traits in different crops (Gellner and Sechler, 1986; Chungu *et al*., 1996; Olatinwo *et al*., 1999; Carson, 2001; Checa *et al*., 2006).

1.8 Yield and Genotype x Environment Interaction (G x E)

Evans and Fischer (1999) defined grain yield in different ways depending on the conditions under which it is estimated. In general, grain yield is defined as the grain mass with specific moisture content. Grain yield potential is defined as yield that is achievable when a variety is grown without any stress and in the environment where it is well adapted. Further, they defined "actual yield" as the grain yield that is achievable when the variety is grown under actual prevailing conditions, which include predominant constraints such as biotic and abiotic stress factors. Thus, grain yield reflects combined genetic effects of the variety and variety x environment interaction effects. Table 1.4 summarizes the gap between yield potential and actual yield that has been reported in different environments in SSA and other regions. For example, in the tropical lowland environment into which Mozambique is largely categorised, there is a huge gap between the potential and actual yield (Table 1.4). This gap is attributed to the variable environmental conditions as well as abiotic factors and biotic factors that include DM.

Table 1.4 Maize yield potential and actual yield sub-Saharan Africa and other regions

Country/ Region	Yield potential (t ha^{-1})	Yield actual (t ha^{-1})	Yield gap (Potential –Actual)	Reference
USA	14.5 - 20.9	7	7.5 - 12.9	Tollenaar and Lee (2002)
USA (Nebraska under irrigation)	18	-	-	Duvick and Cassman (1999)
Highland/Transitional zone (SSA)	5	0.5	4.5	Pingali and Pandey (2001)
Mid-altitude zone (SSA)	7	2.5	4.5	Pingali and Pandey (2001)
Tropical Lowland (SSA)	4.5	0.7	3.8	Pingali and Pandey (2001)
Eastern and Southern Africa Small scale farmers in	-	1.3	-	Bänziger and Diallo (2002)
Eastern and Southern Africa Researchers	-	4 - 14	-	Bänziger and Diallo (2002)

SSA: Sub-Saharan Africa

Genotype by environment interaction is one of the major factors limiting response to selection and efficiency of breeding programmes, mainly when selection is performed under favourable conditions and production is done in a different environment (Ceccarelli *et al*., 1996). There are two responses to environments by genotypes. In one case, the genotypes may change in ranks and in the other; the magnitude of the difference may change without necessarily changing the ranks. The former, also known as the cross over G x E is the most important in crop improvement because it entails a change in the composition of selection of each environments thereby impeding progress (Fox *et al*., 1997). For the non-crossover type, varieties are ranked consistently across environments resulting in similar selection in all environments. Therefore, the crossover type G x E demonstrate specific adaptation by genotypes, that is, instability across environments whereas the non-crossover type represents stability of performance across the environments (Fox *et al*., 1997).

Unfavourable environments represent the majority of famers' maize growing environments in southern Africa, where genotype by environment interaction effects result from factors

related to temperature, rainfall, crop growing period, within-season drought, soil acidity and socio-economic factors that limit the access of farmers to timely acquisition of appropriate inputs (Bänziger *et al.,* 2005). This largely explains the yield gap in many regions presented in Table 1.4. Crop improvement to adapt the genotypes to these conditions is, therefore, an important activity that can raise the actual yields attainable in the region. A large proportion of yield improvement in maize may be attributed to the capacity of newer hybrids to better tolerate stress conditions (Tollenaar and Whu, 1999). For example, Duvick (1984) showed that differences in grain yield between old and newer hybrids were a function of plant population density and tolerance to stresses.

1.9 Use of hybrids and open pollinated varieties (OPV)

Several studies reported superiority of hybrids, ranging between 10% and 25%, to OPVs (Duvick, 1984, Pixely and Bazinger, 2001; Pixley, 2006). Hybrid varieties significantly improved maize yield without expanding production area in the USA (Duvick, 2001). It is argued that hybrid varieties are most appropriate in developed countries, such as the USA, with well-established seed industry. Use of hybrids in the US resulted in a four-fold yield increase despite a 20% decline in area under production over 50 years (Pixely, 2006). Despite the increased hybrid seed utilization among the USA farmers, there are still some limited circumstances where OPVs are still desirable (Kutka and Smith, 2007). Therefore, hybrids have not permanently replaced OPVs even in developed countries; they still exist alongside the OPVs in most regions. However, OPVs are predominant in countries where the seed industry is not well developed to ensure accessibility of seed to farmers.

The use of hybrid seeds in the SADC region started in the early 1960s, but there has not been a significant improvement in grain production in the region. In many African countries, including Mozambique, improved OPVs are more important than hybrids; because the predominant maize environment in these areas are the tropical lowlands and hybrid seeds have not been available for these regions until recently (Maredia *et al*., 1998). Due to high price and poor infrastructure, improved seeds are often not available to the communities in remote areas in SSA and other developing regions. Based on the relative advantages of different types of cultivars from hybrids to OPVs in the farming communities in Southern Africa (Table 1.5), it seems that topcrosses with cheaper seeds than hybrid seeds might be

a good transition point from growing OPVs to conventional hybrids in developing regions. This is because farmers can replant the seed for at least three seasons and the yield level is higher than that of landraces. They will gradually shift to hybrid seeds as their economic conditions improve over time.

Table 1.5 Comparison of variety type across generations over five sites for grain yield in Southern Africa

Variety type	Generation of seed planted	Mean yield (t ha^{-1})	Yield loss by replanting second-generation seed (%)
Hybrid	F_1	6.12 A	
Hybrid	F_2	4.14 E	32.4
OPV	F_2	4.66 C	
OPV	F_3	4.43 CD	4.9
Topcross	F_1	5.08 B	
Topcross	F_2	4.28 DE	15.8
LSD		0.22	

Source: Pixley and Banziger (2001). Means followed by the same letter are not significantly different (P >0.05).

Adoption of improved varieties that include both OPVs and hybrids of slightly less than 50% of seed volumes has been reported in SSA (Maredia *et al.,* 1998; Rashid *et al.,* 2001). The use of improved seed varieties is still very variable in different countries, which is explained by many factors ranging from economic to poor institutional capacity (Rashid *et al.*, 2001; Langyintuo *et al.*, 2009). In Southern Africa, for example, there are different levels of adoption of improved cultivars among countries (Table 1.6).

Table 1.6 Estimated maize seed supply and demand in 2006/07 in Southern Africa

Country	Maize area (1990-2007) average in Millions ha)	Estimated seed demand (1000 tons)[1]	Improved seed marketed in 2006/07 (1000 tons)[2]		Adoption rate 2006/07 (as percent of maize area)
			OPV	Hybrid	
Angola	0.8	19.3	0.8	0.2	5.0
Malawi	1.4	35.3	5.4	2.5	22.0
Mozambique	1.2	30.3	3.1	0.2	11.0
Zambia	0.6	14.1	0.5	9.7	73.0
Zimbabwe	1.4	34.4	2.2	25.9	80.0
Region	**4.6**	**114.1**	**11.2**	**38.3**	**38.0**

Source: Adapted from Langyintuo *et al*. (2009). [1]Estimate based on area and planting rate of 25 kg ha^{-1}. [2]Recorded amount of seed produced and sold in 2006/2007 season.

1.10 Summary

This review of the literature established that:

- The tropical lowland environment is an important agro- ecological zone for maize production in Southern Africa, particular Mozambique where it extends from 0 to 400 m.a.s.l., and represents more than 65% of cultivated area.

- Drought stress, low soil fertility, diseases, pests, and limited access to seed of improved varieties in tropical lowland environments are the major limiting factors to maize production.

- Maize downy mildew disease caused by *Peronosclerospora sorghi* is one among the known downy mildew diseases widely distributed in Africa attacking maize, sorghum and millets.

- The occurrence of *P. sorghi* downy mildew was also reported from several countries in Asia, northern Central and Southern America, Australia, Europe and Middle East.

- Apart from *P. sorghi* downy mildew, there are other fungal species from the three genera *Peronosclerospora, Sclerophthora and Sclerospora* causing yield losses on cereals especially maize worldwide.

- There is limited information on the origin of the pathogen, disease dispersion, pathogen variability, epidemiology, geographic distribution, and yield losses, particularly for Africa.

- Most research on the pathogen was done in Asia, the USA and Brazil. Available information was discussed, in some cases it was extrapolated to African tropical environments.

- Several methods can be employed to control downy mildew, but use of genetic resistant cultivars was identified as the most efficient and economic particularly for subsistence farmers in Sub-Saharan Africa.

- Few studies were done on gene action and inheritance of resistance to downy mildew in maize, most of the studies revealed presence of both additive and non-additive gene effects, with additive effects being more important in determining disease reaction in maize. The inheritance of DMR in maize was reported to be either monogenic dominant, recessive or polygenic additive depending on the maize lines involved in the studies.

- Spreader rows based screening methods and combined methods of spreader rows with direct spray of inoculum on seedlings or on spreader row plants were the most suggested for screening maize for downy mildew resistance.

- Considerable maize germplasm, including populations and inbred lines with resistance to downy mildew have been reported at CIMMYT, IITA and some national breeding programmes. This germplasm can be used as sources of resistance in breeding programmes in the environment where they show good adaptation.

- A breeding programme for downy mildew resistance in maize in Africa was reported in Nigeria where sources of resistance from Asia were used to deploy DM resistant varieties.

- The reviewed literature on combining ability, heterosis, heterotic grouping and heterotic patterns on maize revealed that:
  - Among several mating designs, line x tester and diallel are the most used schemes for combining ability and heterosis studies in maize although they do not separate epistasis from dominance effects.
  - Generation mean analysis is an important tool to estimate additive, dominance effects and additive x additive, additive x dominance and dominance x dominance epistatic interactions. It therefore makes up for that limitation in the line x tester and diallel mating schemes. Although it has not been widely used to study gene effects for DMR, it has been widely used to study other traits in maize and other crops.

- o Heterosis is the most important feature that contributed to increased maize yield over the years worldwide through the deployment of hybrid cultivars.
- o Heterosis and SCA data were the widely used parameters for assigning lines into heterotic groups and defining their relationships.
- o Molecular markers combined with SCA effects were also suggested as important tools for maize heterotic group classification. However, it has been shown that molecular tools when used alone will not be adequate in predicting hybrid performance, because materials with small genetic distances sometimes display huge heterosis. It has also been indicated that testcross data that is collected over many environments is more reliable for predicting performance and grouping maize lines according to heterotic groups.

- Genotypes x environment interaction effects affect yield and stability of cultivars across environments. In Africa where diversified maize growing environments exist, G x E has huge implication in breeding programmes and its quantification is viewed as key in interpreting data from multi-location trials.

- The study also reveals that use of hybrid seed is still low due to restricted access to improved seed varieties in developing countries. A survey of the literature has also indicated that the use of hybrid seeds provides an opportunity to enhance productivity in developing regions.

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CHAPTER 2

Farmers' Perceptions of maize varieties and Production Constraints in the Lowland and Mid-altitude Environments in Mozambique

Abstract

Despite the high importance of maize in Mozambique, the yields are low particularly in smallholder farmer growing environments due to various constraints. The present study was designated to identify farmer's preferences for maize improved varieties, investigate farmers' perceptions on the major production constraints and investigate farmers' variety selection criteria. A survey was conducted in three districts representing two maize growing environments in Mozambique during the 2007/08 season. A total of 142 households, comprising 59 males and 83 females were involved in both formal interviews and informal focus group discussions. Data from the literature and all key players involved in community base development were also considered. Twenty seven percent of the households interviewed planted improved seed in 2007/08 season. From this proportion, only 12% was hybrid seed. Unavailability of basic inputs such as improved seed and fertilizer suppliers at community level, lack of knowledge among farmers about improved varieties; high seed price compared to the low price of grain in the market, among others factors contributed to low use of improved varieties especially hybrids. Famers were aware of the major biotic, biotic and socio-economic constraints that contributed to low maize grain yield, which was estimated at 0.7 t ha^{-1} across the three districts sampled. The most important constraints were downy mildew, drought, cutworm and stem borer in lowland environments. Ear rot, low inputs such as seed and fertilizer availability, turcicum leaf blight and gray leaf spot diseases and low soil fertility where major constraints in the high altitude environments. The most important seed selection criteria were grain yield, short maturing cycle, white grain and hardness of kernel endosperm, tolerance to drought and low soil fertility, resistance to the local diseases, and post harvest pests. The white and flint grain were preferred to the yellow and dent grain, respectively. Results indicated a challenge to the breeding programme in Mozambique and a call for improvement in the seed and other essential input distribution systems to enhance maize productivity in both low and mid-altitude environments.

2.1 Introduction

Maize is the predominant food crop in many countries in Sub-Sahara Africa. Historically, maize constitutes the basic staple food, food security and social stability for the majority of households in the region. In Southern Africa, maize plays an important role in the economies of many countries, as a source of income, thus it is used as one measure for assessing the level of poverty among households, particularly in the rural areas (Walker *et al.*, 2006). Therefore, socio-economic stability can only be secured in this region if adequate maize is produced.

In Mozambique for example, maize is cultivated generally from the most suitable environments to the poor and marginal areas, especially by smallholder farmers occupying more than 95% of the maize area and contributing more than 90% to the total annual production. In these smallholder agro-systems, maize is grown as a mixed crop or intercropped with legumes in small plots (Bias, 2000). Production is under traditional management practices and depends mostly on rainfall due to the lack of irrigation systems. In these agro-systems, like in many other southern African countries, maize production and productivity are very low. Average yields are between 0.2 and 1.2 t ha^{-1} (DINA, 1995; INE, 2003). These growing environments for maize are also affected by several other abiotic, biotic and socio-economic constraints.

Among the abiotic factors, drought and low soil fertility are the most important in many maize agro-ecologies. Disease, pests and weeds are among biotic factors limiting maize production. These factors are exacerbated by various socio-economic constraints that influence negatively on maize production in Mozambique, resulting in low adoption of new technologies among smallholder farmers. According to Billing (1999), the major aspects that determine the level of technology adoption among smallholder farmers include; existence of high demand for inputs such as seed and fertilizer among farmers, sufficient income in order to make cash payment for the inputs, availability of active input suppliers in the local markets and good network between communities and service providers.

The level of adoption of improved varieties, especially maize hybrids among farmers in the Southern Africa Development Community (SADC) countries varies between 5% and 80% (Wobil, 1998). Angola, Mozambique, Tanzania, Malawi and Lesotho are countries in the

region where adoption rate of hybrid seed is between 5% and 30%. However, in the region there are countries with a long history and tradition of using maize hybrid seed even in the smallholder sector, such as Zambia, South Africa, Zimbabwe and Swaziland, where hybrid adoption levels vary between 60% and 80% (Wobil, 1998).

Participatory rural appraisal (PRA) is one of the methods used worldwide as a multidisciplinary approach for establishing the farmers' views and knowledge on different agricultural resource management options to ensure household food security and improvement in their welfare (Chambar, 1999, Blaney and Thibault, 2003). Various studies have reported the use of PRA methodology to identify major production constraints and to investigate farmers' preferences for specific varieties or traits in different crops including maize (Langyintuo *el al*., 2005, Doward *et al*., 2007, Langyintuo *el al*., 2009). Generally, information obtained from farmers can help to set the breeding objectives by incorporating the farmers' preferences that will contribute to adoption of new varieties. Therefore, the objectives of this study were to: (i) investigate farmers' perceptions of maize improved varieties, especially hybrids, (ii) investigate farmers' perceptions on the major production constraints and (iii) investigate farmers' maize-seed selection criteria in two maize production environments in Mozambique.

2.2 Materials and Methods

2.2.1 The study area

Mozambique is administratively organized into 11 provinces grouped in three regions North (Cabo Delgado, Niassa and Nampula); Centre (Zambézia, Tete, Manica and Sofala) and South (Inhambane, Gaza, Maputo and Maputo City). There are 128 districts and each district comprises localities, administrative posts and villages. The participatory rural appraisal was carried out in three districts, Manhiça, Chókwè and Angónia located in Maputo, Gaza and Tete provinces, respectively, and representing two of the five maize agro-ecological zones. Manhiça and Chókwè are located in the lowland environments (< 400 m.a.s.l) and Angónia in high altitude environments (> 800 m.a.s.l) (Bokde, 1980). The Mozambican high altitude zone corresponds to the SADC mid-altitude maize mega-environment (Bänziger *et al.,* 2004). The characterization of the areas is summarized in Table 2.1-A. Manhiça and Chókwè districts constitute moderate to high potential for maize

production under rainfed and under irrigation conditions, respectively. Angónia represents high to very high potential for maize production under rainfed conditions and irrigation conditions, respectively. In Manhiça district, the survey was conducted at Ilha Josina Machel in flood plains of Incomati River. This area constitutes the fertile part of Manhiça district. At Chókwè district, the survey was conducted around Chókwè town and Macarretane, in the Limpopo River basin. In Angónia district the survey was conducted in two localities, Domué and Ulongué (Table 2.1-A).

Table 2.1-A. agro-climatic information for the sampled districts in Mozambique

District	Agroecological zone	Maize crop	Altitude (m)	Latitude	Longitude	Precipitation (mm)
Manhiça	Zone-I	Non suitable to suitable	50	$25^{o}24'$	$32^{o}48'$	807
Chókwè	Zone-III	Non suitable to suitable	34	$24^{o}32'$	$33^{o}00'$	600
Angónia	Zone-X	Suitable to highly suitable	1330	$14^{o}22'$	$34^{o} 36'$	725 - 1449

Source: Data from Instituto de Investgação Agrária de Moçambique (IIAM).

2.2.2 Selection of farmers and data collection

One-hundred and forty-two farmers were involved in both formal household survey (114 people) and the informal focus group discussion (28 people). Farmers were identified through local extension officers. A minimum of 5 to 6 female and male household heads per village were randomly selected for formal interview following the Cleveland *et al*. (1999) procedure. The actual numbers of households sampled in each locality or village and district are indicated in Table 2.1-B. Two breeders, one extension officer and one primary school teacher were involved in primary data collection at Manhiça and Chókwè districts during February 2008. At Angónia, the survey was conducted in April 2008 and one breeder, two extension officers and one technician were involved in collecting data. The collected data included the general information on household structures, assets, sources of income; major crops cultivated by farmers in the area and their cropping patterns; maize production practices (planting methods, cropping systems, rotation, use of chemical fertilizers, pesticides and herbicides); identification of farmers' maize production constraints.

Special attention was given to downy mildew awareness and disease management. Finally, the knowledge about maize varieties grown by farmers, with emphasis on varieties on the national list of varieties, which includes open pollinated varieties (OPVs) and hybrids, were investigated. In Mozambique since 1990 more than 10 national surveys (TIA) covering more than 50% of the districts have been conducted to assess agriculture production and incomes of the households in the country. The results from these surveys, in some cases were used as secondary data to support the primary data (Walker *et al.*, 2006). All the quantitative data collected in the survey were subjected to analysis in SPSS 16.0 statistics package. Correlations were performed in SAS computer programme.

Table 2.1-B. Population and household data for the sampled districts in Mozambique

District	Locality or village		Sampling method	Population			Number of household sampled		
				Male	Female	Total	Male	Female	Total
Manhiça	**Ilha Josina Machel**		**FS**	**85,419**	**107,219**	**192,638**	**7**	**7**	**14**
Chókwè						**190,000**	**17**	**35**	**52**
	Chókwè		FS				4	22	26
	Macarretane		FS				4	7	11
	Macarretane		FGD				9	6	15
Angónia				**156,332**	**174,046**	**330,378**	**35**	**41**	**76**
	Domué:	Dzimeza	FS				9	9	18
		Dzimeza	FGD				4	9	13
		Dzatumbe	FS				8	10	18
	Ulongué:	Chiphole	FS				6	5	11
		Chindeke	FS				8	8	16
Informal group discussion			FGD				13	15	28
Formal survey			FS				46	68	114
Overall							**59**	**83**	**142**

FS = formal survey, FGD = focus group discussion, population data are from Ministério de Administração Estatatal de Moçambique (2005).

2.3 Results and Discussion

2.3.1 General information about households

The information about distribution of households and gender is presented in Table 2.1. In general, there was gender balance. The number of males involved in the study was 59 and females 83. This difference expresses the prevalent proportion between males and females, in Mozambique and in particular in each district. In terms of age the survey involved people between 20 and 89 years old. The number of people per family was variable. Classes of 1-2 and 11-21 people per family were less frequent and the most frequent classes were 3-4 and 5-6 people per family (Figure 2.1). Thus, most households had 3-6 people. Only a few households had more than seven members. This might have an implication on the availability of household labour to work on the farmer.
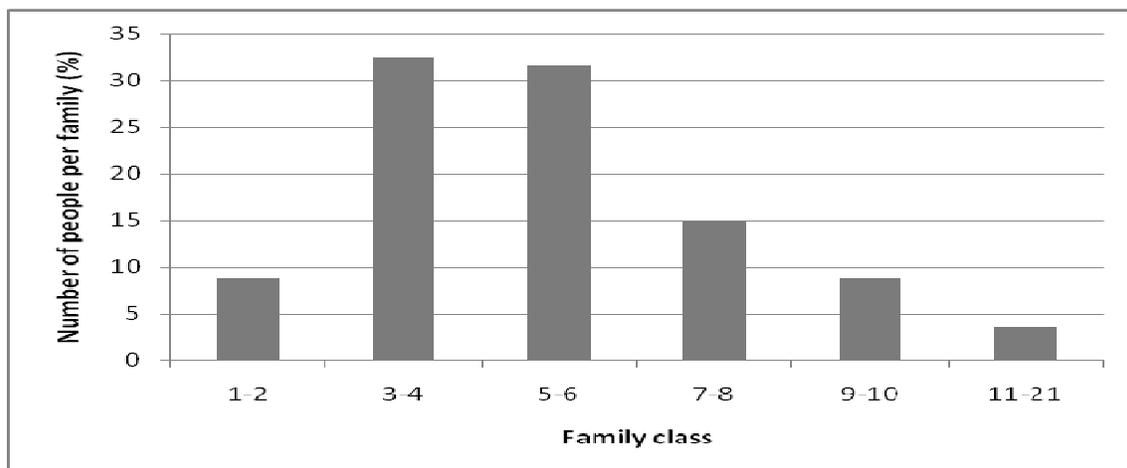
Figure 2.1 Number of people per family

There were seven classes related to the size of land per family in each district (Table 2.2). The total field area was more than 6 hectares (ha) per family at Manhiça, between 2 and 3 ha in Chókwè and between 3 and 4 ha in Angónia. Chókwè had about 8% of the households with land less than 1 ha, while there was no family with less than one hectare at Manhiça and Angónia. These results represent the level of pressure on land use in the three districts. At Ilha Josina Machel at Manhiça, land availability is not a problem; the problem is unequal distribution of land among households. There was evidence of emergent commercial farmers that have larger land acreages than the majority poor small-

scale farmers. There were 43% of households at Ilha Josina Machel without fallow land, 28% with 2 ha of fallow land and 7% with 1 ha, 3 ha, 4 ha and 290 ha, respectively. The fallow land per household in other districts was insignificant. However, this suggests that land is becoming a problem at Chókwè and Angónia where several conflicts of use of land between commercial and small-scale farmers were reported (Ministério da Admistração Estatal, 2005).

Table 2.2 Size of land per household in three districts

Land size (ha)	Manhiça (%) (n = 14)	Chókwè (%) (n = 37)	Angónia (%) (n = 63)	Total (%) (n = 114)
< 1	0.0	5.4	0.0	1.8
1-2	7.1	37.8	6.3	16.7
2-3	14.3	29.7	17.5	21.1
3-4	7.1	8.1	28.6	19.3
4-5	21.4	10.8	19.0	16.7
5-6	7.1	2.7	11.1	7.9
> 6	42.9	5.4	17.5	16.7

2.3.2 Crop production

Maize was the most important food crop grown by the farmers in the three districts. All interviewed households were maize growers (Table 2.3). This result confirms the predominance of maize as a food security crop and major staple food in Mozambique. Common bean was the second most grown crop by farmers in the three districts followed by cassava at Manhiça, vegetables in Chókwè, and groundnut and soybean in Angónia. In general, apart from maize and beans, groundnuts, cowpeas, and sweet potato were important in all the districts. Some crops were specific to an agro-ecological environment, for example, potato, soybean and tobacco were predominant in the high altitude environments. Vegetables were produced mostly in the lowland areas mainly due to the availability of irrigation systems.

The relative importance of the crops grown by farmers across the three districts was estimated based on the number of growers of each crop and is presented in Fig. 2.2. Maize accounted for 26% of the total number of the farmers in the three districts, beans 18% and

groundnut at 11% followed beans. The remaining crops varied from 4% to 10%, and were potatoes and soybean, respectively (Fig 2.2). Bambara groundnut, pigeon pea, chickpea, fruit culture and pearl millet, together accounted for 3% of household growers. Cassava, the second most important staple food in Mozambique, accounted for 5%. Maputo and Gaza are cassava-growing provinces, but usually cassava is grown in dry areas of the interior and the survey was conducted in the areas along the Limpopo and Incomati basins, where cassava is not a predominant crop. At Angónia cassava is grown as a secondary crop. The few farmers that grow cassava use mainly the tubers as a form of payment for labour in the maize fields.

Table 2.3 Crops grown by farmers in each district and over the three districts

| Crop | Farmers growing crops (%) | | | |
	Manhiça (n = 14)	Chókwè (n = 37)	Angónia (n = 63)	Total (n =114)
Maize	100.0	100.0	100.0	100.0
Beans	71.4	48.6	84.1	71.0
Groundnut	35.7	5.4	69.8	44.7
Soybean	0.0	0.0	69.8	38.6
Cowpea	42.9	27.0	25.4	28.1
Sweet potato	35.7	8.1	28.6	22.8
Vegetables	35.7	37.8	7.9	21.0
Tobacco	0.0	0.0	34.9	19.3
Cassava	50.0	5.4	17.5	17.6
Potato	0.0	0.0	30.2	16.7
Others[†]	0.0	2.7	19.2	11.4

[†]Others = bambara groundnut in Chokwe and bambara groundnut, pigeon pea, chickpea, fruit culture and pearl millet in Angónia.
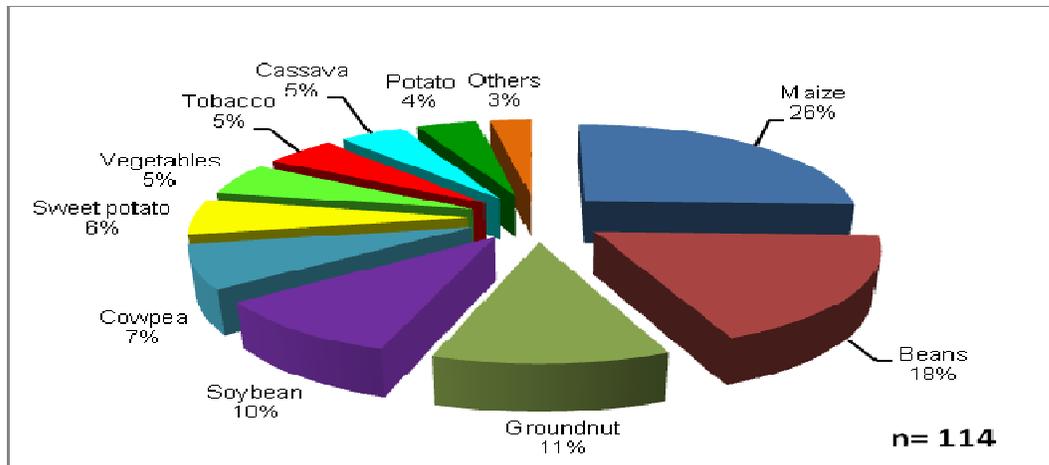
Figure 2.2 Number of farmers growing a crop (s) over the three districts

2.3.3 Cultivated areas and maize yield estimates

The production of maize, by the small-scale farmers in Mozambique is characterized by high yield variations due to various factors. Fluctuation of precipitation in many growing environments particularly in the Southern region, use of non-improved seed varieties and lack of basic inputs such as fertilizer and pesticides, pests and disease pressure all contribute to low yields in most of these maize agro-ecologies. In this study, yield estimates by locality varied between 0.6 and 1.0 t ha^{-1} at Ulongué in Angónia and Macarretane in Chókwè, respectively (Table 2.4). As a result, the national yield average is about 1 t ha^{-1} (FAOSTAT, 2009).

Table 2.4 Mean number of fields and estimates of maize area and grain yield per household within and across districts

District	Locality	field /household (#)	Cultivated land (ha)	Production (t)	Grain yield (t ha^{-1})
Manhiça	Ilha Josina Machel	2.8	8.49	5.99	0.705
Chókwè	Chókwè	1.73	2.54	2.06	0.811
	Macarretane	2.45	1.82	1.88	1.035
Angónia	Domué	2.4	3.76	2.64	0.703
	Ulongué	2.7	3.93	2.27	0.579
Total		**2.39**	**3.9**	**2.75**	**0.71**

2.3.4 Cropping systems and crop management

In the three districts, maize was grown in intercropping systems, where the second crop varied from region to region, and it was grown in mixed cropping systems. In the mixed cropping system, several crops are grown with maize in the same field (Ministério da Admistração Estatal, 2005). Results showed that the intercropping system was predominant in Manhiça and Angónia followed by the mixed cropping system. In contrast, farmers in Chókwè grew maize mostly as a single crop (Figure 2.3). However, in Mozambique, no breeding programme incorporates farmers' growing systems in the research objectives. It seems that the use of mixed cropping systems was one of the contributing factors to low yields in Angónia where farmers have the tradition of growing hybrids (Table 2.4). Chókwè, on the other hand, had a greater chance of getting profit from improved varieties especially hybrids because there was a high proportion of single crop based farming system than intercropping and mixed cropping systems. However, there were many other factors contributing negatively to maize yields at famers' level apart from the growing systems. This suggested that apart from seed technology, appropriate crop management techniques are still lacking among the small-scale farmers in Mozambique. Therefore, research programmes should pay particular attention to crop management in the process of developing new varieties. According to Cook (2006), the intercropping systems, if well exploited, can enhance productivity and sustainability of agriculture in small-scale farming systems.
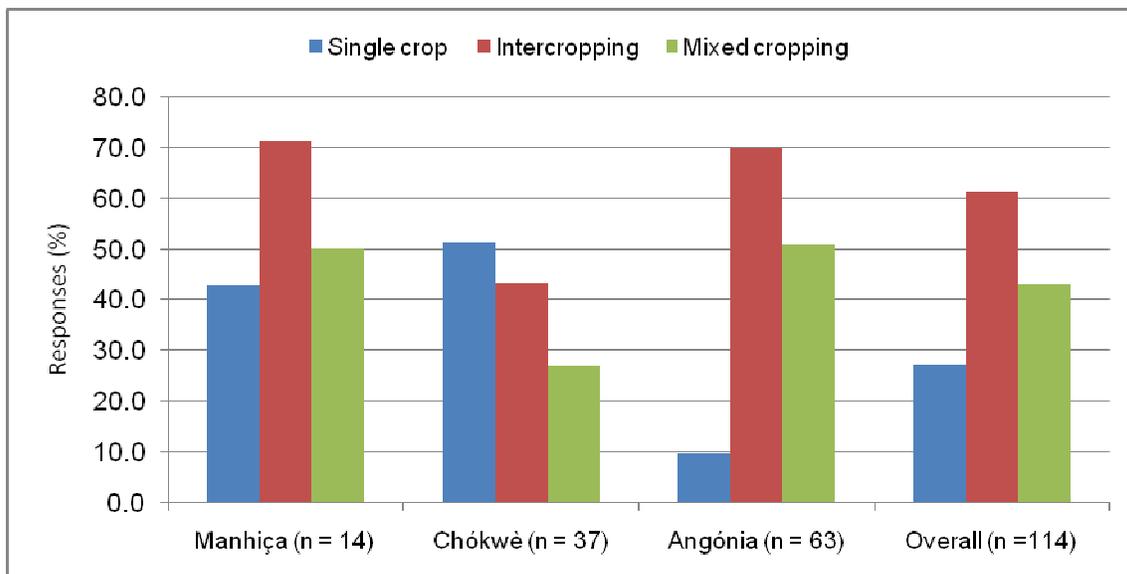
Figure 2.3 Maize production systems in each district and overall districts

63

In general, maize was intercropped with legumes, particularly beans in the three districts. From 114 households involved in the formal survey, 66 grew maize in the intercropping systems. From this number, 64% used beans as the first choice crop for intercropping with maize and the remaining 36% used other crops (Figure 2.4-A). Among the farmers that grew maize in intercropping system there were those that chose more than one crop for the intercropping. The second crop was used as an alternative when the first choice crop, for example beans was not available. Groundnut was the most popular second choice crop representing 64% of this group (Figure 2.4-B).
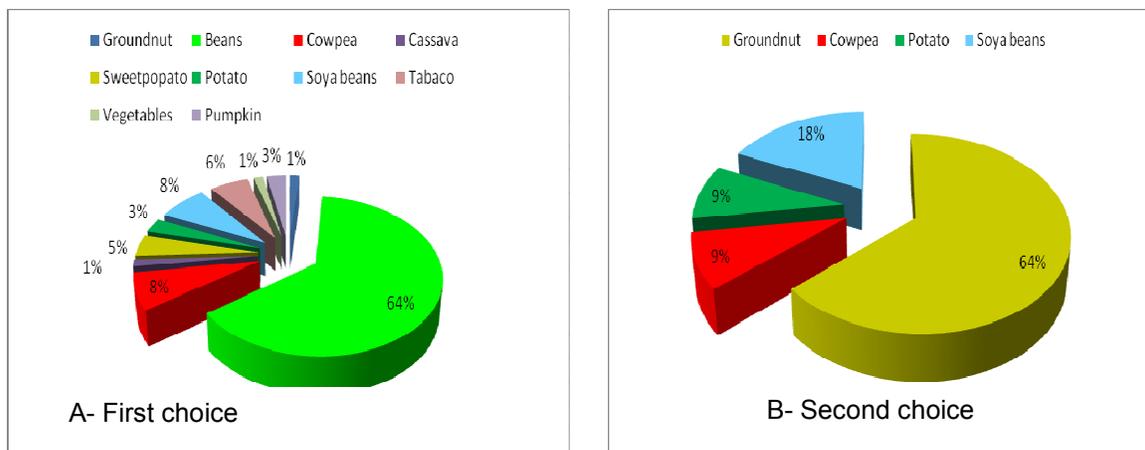
Figure 2.4 Crops used in the intercropping system with maize across the three districts

Plant population (number of plants per unit area) is one of the most important elements that influence the performance of a variety. Usually small-scale farmers plant at low density, but many plants per hill and most of them plant maize in zigzag arrangement. This study found that about 90% of the 114 interviewed households did not plant maize in rows and only 4% of them planted only one plant per hill and 43% two plants per hill and about 52% planted three or more plants per hill. The use of low plant population especially when a few seeds are planted may lead to patchy crop establishment, particularly if there is a problem with germination due to poor seed quality. Consequently, yields are compromised because of less than optimal plant population density.

2.3.5 Source of seed planted by farmers in 2007/08 seasons

During the 2007/08 season, 64% of interviewed farmers used their saved seed, 15% and 11% obtained seed from neighbouring farmers and local grain markets, respectively (Table 2.5). These results are consistent with the previous studies by Langyintuo *et al. (*2009) and Wobil (1988) that recognized the existence of two major categories of seed provision in Africa, that is, formal and informal. The formal seed system is dominated by seed companies and it generally relies on seed laws. Farmers, community based organizations and non-government organizations (NGOs), on the other hand, perform the informal system of seed provision, mainly with limited resources and quality control. In Africa, including Southern African countries, the most important source of seed for the majority of farmers in the informal sector, includes farmers' saved seeds, seed exchanged among farmers and grain purchased from the local market (Wobil, 1998; Libombo and Uaiene, 1999; Badstue *et al., 2007,* Rubyogo *et al.,* 2007). According to Libombo and Uaiene (1999), in Mozambique for example, only 5 to 10% of the seed used by small-scale farmers is certified. Most of this seed reached the farmers through emergency distribution programmes and not through the normal seed commercialization schemes. The use of poor quality seed might partly explain the low grain yield. For example, seed borne diseases can infect farmers' saved seed and thereby compromising the germination and vigour.

Table 2.5 Source of maize seed planted by farmers in five localities across the three districts in Mozambique

District	Locality or village	Source of seed grown by farmers in five localities at three districts (%)							
		Own seed	Other farmer	Extension services	NGO/ Seed Companies	Input suppliers	Grain market	Research	Other sources
Manhiça	Ilha Josina Machel (n= 14)	71.4	7.1	0.0	0.0	0.0	28.6	0.0	0.0
Chókwè	Chókwè (n= 26)	42.3	11.5	23.1	3.8	0.0	23.1	3.8	0.0
	Macarretane (n= 11)	27.3	0.0	9.1	0.0	18.2	54.5	0.0	0.0
Angónia	Domué (n= 36)	69.4	19.4	0.0	11.1	16.7	5.6	0.0	0.0
	Ulongué (n= 27)	88.9	7.4	7.4	7.4	11.1	0.0	0.0	0.0
	Total (n= 114)	**64.0**	**11.4**	**7.9**	**6.1**	**9.6**	**15.8**	**0.9**	**0.0**

2.3.6 Type of maize seed used by farmers in 2007/08 seasons

The results showed that about 74% of interviewed farmers use non- improved seed and 15% improved OPVs and only 12% use hybrid seed (Table 2.6). The limited use of improved varieties is a result of only a few seed companies operating in Mozambique. Until the early 1990s, the importation of hybrid seed in Mozambique was not encouraged by government policy. The government strategy was to adopt and promote the open pollinated varieties (OPVs), which can be continually recycled by farmers without purchasing new seed every year (Billing *et al.,* 1999). As result, the hybrid varieties were not promoted which made the country very unattractive to the seed industry. Consequently, Mozambique has the lowest adoption rate of new varieties after Angola in the SADC region (11%) (Langyintuo *et al*., 2009). However, by the 2000s the Mozambican government imposed reforms in the seed industry. From one national seed company existing in the early 1990s, presently there are 12 national seed companies, two regional/ multi-national seed companies and two community-based organizations (CBOs) (Langyintuo *et al*., 2009). Despite the relative increase in the number of seed providers in the country, there is still a huge gap between maize seed supply and demand. The seed suppliers do not cover all the agro-ecologies and areas. During the 2006/07 season for example, demand for improved seed was about 30,300 tonnes and seed suppliers provided only about 10% (Langyintuo et al., 2009). Thus, Mozambique is one among the SADC countries with greater opportunity for maize-seed industry development and therefore, active intervention of breeding programmes is crucial. The use of agrochemicals among farmers was limited. Only 43.9% were using fertilizer and 21% insecticide, mainly for vegetables (Table 2.6).

Table 2.6 Type of seed, fertilizer and insecticides used by farmers during 2007/08 season

District	Locality or village	% of households				
		Hybrid	OPV	Landrace	Fertilizer	Insecticide
Manhiça	Ilha Josina Machel (n= 14)	0.0	71.5	100	7.1	7.1
Chókwè	Chókwè (n= 26)	0.0	30.8	65.4	53.8	57.7
	Macarretane (n= 11)	9.1	36.4	63.6	72.7	45.5
Angónia	Domué (n= 36)	25	2.8	61.1	27.8	5.6
	Ulongué (n= 27)	14.8	14.8	92.6	63.0	7.4
Total	**(n = 114)**	**12.3**	**15.8**	**74.6**	**43.9**	**21.1**

2.3.7 Maize seed prices

In this survey, causes for the low demand of improved seed among farmers varied from place to place. High seed prices seem to be one of the limitations for farmers to buy certified seed in all districts. For Ilha Josina Machel village, apart from the price, the major bottleneck seemed to be unavailability of improved seed (Table 2.7). This unavailability of seed was translated to a lack of knowledge among the majority of farmers about seed, as well as the long distance to obtain it. The Chókwè situation showed clearly the negative impact seed prices had. The certified seed was available in the local seed shops. However, most farmers preferred to buy grain from the local grain market. Apart from price, there was limited information and promotion of improved varieties. A high percentage of farmers in Macarretane, within Chókwè district, were aware of the existence of improved varieties on the market and they grew some maize hybrid seed. The major problem was the unavailability of seed and complementary inputs mainly fertilizer at local market. This problem was mainly due to the obsolete irrigation system that did not encourage farmers to invest in good quality seed.

The price of certified seed declared by farmers varied from \$1.0 kg^{-1} at Ilha Josina Machel at Manhiça district to \$1.59 kg^{-1} in Domue at Angónia (Table 2.7). The seed price that farmers declared that they could afford if varieties were available in the local market varied between \$0.55 kg^{-1} at Chókwè and \$1.15 kg^{-1} at Domué. The price for improved seed at Chókwè during the time of this survey was \$1.20 kg^{-1} to \$1.40 kg^{-1} for open pollinated varieties; \$1.68 kg^{-1} and \$2.48 kg^{-1} for three-way cross and single cross hybrids, respectively.

Table 2.7 Actual and affordable seed price of predominantly open- pollinated seed by locality across districts

District	Locality or village	Actual seed price ($US kg^{-1})	Affordable seed price ($US kg^{-1})	Mean price ($US kg^{-1})
Manhiça	Ilha Josina Machel	1.00	0.96	0.98
Chokwe	Chokwe	0.94	0.55	0.74
	Macarretane	0.91	0.64	0.78
Angónia	Domué	1.59	1.15	1.37
	Ulongué	1.40	1.00	1.20
Grand mean		**1.17**	**0.86**	**1.01**

3.3.8 Farmers knowledge of improved maize varieties

The level of using improved varieties among farmers in the three districts is still low. Among the selected names of improved varieties present on the market, Matuba (OPV) was the most popular followed by PAN67, a three-way cross hybrid from PANNAR (Table 2.8). However, Matuba was most common in the lowland environments than high altitude. While in medium to high altitude environments farmers were more familiar with hybrids, such as PAN67 and SC513, which are recommended for mid- and high altitude environments. Although Matuba was grown throughout the country, it is an early maturing variety that is recommended for lowland environments. These results indicated the need to deploy hybrids that are adapted to the lowland. The list of varieties that are grown by farmers is presented in Table 2.8.

Table 2.8 The level of using selected improved maize varieties by farmers at least once in the last two seasons (%).

Variety name	Type[≠]	Ilha Josina Machel (n = 14)	Chókwè (n = 37)	Angónia (n =63)	Across (n= 114)
PAN67	hybrid	7.1	8.1	30.2	20.2
PAN407	hybrid	0.0	0.0	0.0	0.0
SC513	hybrid	0.0	0.0	4.8	2.6
SC401	hybrid	0.0	0.0	0.0	0.0
SC501	hybrid	0.0	0.0	0.0	0.0
Matuba	OPV	28.6	56.8	4.8	24.6
Manica	OPV	0.0	0.0	6.3	3.5
Sussuma	OPV	0.0	0.0	14.3	7.9
Semoc-1	OPV	0.0	0.0	4.8	2.6
Mocuba	OPV	0.0	0.0	0.0	0.0
Angónia	OPV	0.0	0.0	1.6	0.9
Milange	OPV	0.0	0.0	0.0	0.0
Djandza	OPV	0.0	0.0	0.0	0.0
Changalane	OPV	0.0	2.7	0.0	0.9
Chinaca	OPV	0.0	0.0	0.0	0.0
Tsangano	OPV	0.0	0.0	4.8	2.6
Others	OPV	0.0	0.0	1.6	0.9
Local	Local varieties	71.4	13.5	66.7	50.0

[≠]OPV = Open pollinated variety

2.3.9 Famers' preferences for specific variety traits

In general, it seemed that the criteria of selecting maize seed by farmers in the five localities was similar. Highly positive and significant correlations were observed among localities except between Ulongué and Chókwè, which was significant but weak. The highest correlation ranking was between the two localities of Angónia (Table 2.9). These suggested that farmers from the same agro-environment had more common preferences for seed traits than those from different agro-environments. This was because they were subjected to the same culture and environmental effects. The positive relationship between localities for variety ranking traits indicated that, in general, farmers had some common preferences,

which constituted their "ideal" variety in the two ecologies. The farmers' ranking of the desired variety traits is presented in Table 2.10.

Table 2.9 Correlations between farmers ranking of maize seed desirable traits over localities

Localities/ District	Manhiça Ilha Josina Machel	Chókwè		Angónia	
		Chókwè	Macarretane	Domué	Ulongué
Ilha Josina Machel		0.7835**	0.7564**	0.8135**	0.7263**
Chókwè			0.7865**	0.6587**	0.5489*
Macarretane				0.7429**	0.5925**
Domué					0.9038**
Ulongué					

Grain yield was the most important trait considered on maize seed selection across five localities in the three districts. However, there were some slight differences, with farmers in Ulongué for example, considering the maturing cycle as the most important trait followed by grain yield. At both localities of Chókwè, the colour of grain endosperm was the number one trait considered by farmers when selecting maize seed, and white grain was more preferred than yellow. In general, the top-10 traits were related to the mega-maize production constraints in Mozambique (Table 2.10). It is therefore, recommended that the research programme in Mozambique should focus on developing new hybrids that incorporate these preferred traits for deployment in both agro-ecologies.

Table 2.10 Famers ranking of desirable traits in maize varieties in the districts in Mozambique

Trait	Manhiça		Chókwè				Angónia				Across	
	Ilha Josina Machel (%)	Rank	Chókwè (%)	Rank	Macarretane (%)	Rank	Domué (%)	Rank	Ulongué (%)	Rank	%	Rank
Grain yield	78.6	1	38.5	4	36.4	2	61.1	1	48.1	2	52.6	1
Maturing cycle	50.0	3	30.8	5	36.4	3	41.7	2	59.3	1	43.9	2
Color of endosperm	14.3	6	69.2	1	72.7	1	27.8	5	18.5	8	37.7	3
Tolerance to drought	64.3	2	42.3	3	27.3	4	25.0	8	18.5	10	32.5	4
Resistance to diseases	35.7	4	7.7	9	9.1	8	33.3	4	22.2	6	22.8	5
Storability	7.1	8	3.8	12	0.0	14	41.7	3	29.6	4	21.9	6
Husk cover	14.3	5	19.2	6	27.3	5	27.8	6	18.5	9	21.9	7
Resistance to the bird attack	0.0	14	50.0	2	0.0	11	16.7	12	11.1	13	19.3	8
Tolerance to low soil fertility	0.0	9	3.8	13	9.1	10	27.8	7	33.3	3	18.4	9
Resistance to post harvest pests	7.1	7	11.5	7	0.0	12	19.4	10	29.6	5	16.7	10
Cob size	0.0	10	11.5	8	9.1	7	19.4	9	22.2	7	14.9	11
Intercropping	0.0	19	0.0	19	0.0	19	19.4	11	14.8	12	9.6	12
Processing	0.0	15	7.7	11	0.0	13	5.6	15	18.5	11	7.9	13
The price in the market	0.0	18	0.0	18	18.2	6	8.3	13	7.4	14	6.1	14
Kennel size	0.0	13	7.7	10	9.1	9	8.3	14	0.0	18	5.3	15
Taste	0.0	16	0.0	16	0.0	17	5.6	16	7.4	15	3.5	16
Tolerance to lodging	0.0	12	0.0	15	0.0	16	2.8	18	7.4	16	2.6	17
Good taste for brew	0.0	20	0.0	20	0.0	20	0.0	20	3.7	17	0.9	18
Number of ears per plant	0.0	11	0.0	14	0.0	15	2.8	17	0.0	19	0.9	19
Nutritional value	0.0	17	0.0	17	0.0	18	0.0	19	0.0	20	0.0	20

2.3.10 Assessment of major maize production constraints

There were differences in ranking of major maize production constraints among the three districts. A strong and positive correlation was observed between Chókwè and Manhiça both in lowland environments. No significant correlations were observed between both districts from lowland and Angónia (Table 2.11). Results indicated that, in general, farmers in the costal lowland have different perceptions of the major production constraints. This clearly indicated that a different strategy is required to deal with the challenges that identified in the two mega-environments. This also has implications on plant breeding intervention and suggests that two maize sub-programmes be designed for the mid-altitude and lowland environments.

Table 2.11 Correlations between district rankings of major maize production constraints.

Districts	Manhiça	Chókwè	Angónia
Manhiça		0.8113**	-0.2059
Chókwè			0.0368
Angónia			

The average ranks of the production constraints are shown in Table 2.12. Downy mildew ranked first in both districts from the lowland environments, Chókwè and Manhiça. Cutworm was ranked second and fourth in Manhiça and Chókwè, respectively. Ear rot, turcicum leaf blight and gray leaf spot (GLS) diseases and excessive rain were the most important constraints for high altitude environments of Angónia and ranked first, third and ninth positions, respectively. However, some constraints were common to both environments and these included; diseases and pests (maize streak virus, birds, termites stem borer, and drought) and some socio-economic constraints, such as lack of financial support, deficient market and unavailability of inputs, especially improved seed and fertilizer (Table 2.12). Results also demonstrated the need for the incorporation of MSV resistance in all the cultivars targeted for release in both the lowland and mid-altitude environments, while downy

mildew resistance should be emphasized in the lowland maize breeding programme. However, government and other key players, such as non-governmental organizations should find ways to address the socio-economic conditions that include infrastructural challenges to facilitate production of new improved varieties and access to the supporting inputs. This problem is common in all countries in sub-Saharan Africa and thus requires serious attention.

Table 2.12 Production constraint ranking by district and across districts according to the importance

Constraint	Manhiça		Chókwè		Angónia		Across	
	%	Rank	%	Rank	%	Rank	%	Rank
Stem Borer	50.0	4	83.8	2	28.6	6	49.1	4.0
Drought	71.4	3	73.0	3	28.6	7	48.2	4.3
Inputs	28.6	7	37.8	5	57.1	2	47.4	4.7
Downy mildew	100.0	1	100.0	1	3.2	16	46.5	6.0
Cut worm	85.7	2	40.5	4	4.8	14	26.3	6.7
Birds	42.9	5	10.8	8	19.0	8	19.3	7.0
Founds	35.7	6	32.4	6	17.5	10	24.6	7.3
Turcicum leaf blight and gray leaf spot	0.0	11	0.0	13	47.6	3	26.3	9.0
Low soil fertility	0.0	16	13.5	7	41.3	4	27.2	9.0
Rates	0.0	14	10.8	9	34.9	5	22.8	9.3
Maize streak virus (MSV)	14.3	8	5.4	10	15.9	11	12.3	9.7
Ear rot	0.0	13	0.0	15	87.3	1	48.2	9.7
Termites	14.3	9	2.7	12	12.7	12	9.6	11.0
Market	14.3	10	5.4	11	4.8	15	6.1	12.0
Maize head smut	0.0	12	0.0	14	4.8	13	2.6	13.0
Excessive rain fall	0.0	17	0.0	17	19.0	9	10.5	14.3
Striga	0.0	15	0.0	16	3.2	17	1.8	16.0

2.3.11 Farmers' management perspectives of downy mildew control

In the lowland environments where downy mildew (DM) is an important problem, farmers recognized diseases as problem and adopted some measures of control according to their perspectives. Removing infected plants from the field was the most adopted management practice for DM control in both Manhiça and Chókwè districts (Table 2.13). The second measure was to leave affected plants in the fields and farmers did this because they knew that infected plants did not give cobs, however, they were not aware that spores from diseased plants could spread throughout the field and infect other healthy plants (Fig 2.5). Most farmers recognized that when they planted early, the level of infection by DM was low. However, in many cases their planting date depended on the first rains, which have not been predictable in the last few years. Some farmers attempted to spray with any chemical they found and recognized that this was not effective. About 13.5% of the interviewed households attempted to control DM chemically (Table 2.13). Three percent of the interviewed farmers across the districts referred to other measures, which included traditional ceremonies when the disease reached epidemic levels. The study did not establish, however, whether these control measures were effective, but the low yields experienced in the area suggested that a more viable and sustainable strategy such as deploying resistant varieties should be seriously considered.

Table 2.13 Farmers' perspective of downy mildew management and control in lowland environments

Downy mildew control measure	Household responses (%)		
	Manhiça (n=14)	Chókwè (n=37)	Across
No measure adopted to control DM	14.3	16.2	15.7
Remove infected plants from field	78.6	62.2	66.7
Chemical control	0.0	13.5	9.8
Remove and binding infected plants	0.0	5.4	3.9
Early planting	0.0	0.0	0.0
Others	7.1	2.7	3.9

Figure 2.5 Farmers field infected by downy mildew in Chókwè in 2008.

2.4 Conclusions

From this study the following conclusions were drawn:

1. There was a low rate of improved seed utilization, especially hybrids among small-scale farmers. Only about 27% of the households planted improved seed in 2007/08 season. From this proportion, only 12% was hybrid seed while 15% were OPVs with low yield potential. Several factors appeared to influence the low adoption of new technologies, including improved varieties and fertilizer in the three districts. Among them were; (i) unavailability of basic inputs suppliers at community level, (ii) lack of knowledge among farmers about improved varieties; (iii) high prices of improved seed that farmers did not afford due to low cash incomes. There are other several socio- economic factors influencing negatively on community development, which reflected the actual level of development of each district and the country in general. These challenges need addressing to provide an enabling environment for crop

production. Varieties that are more productive and fertilizer among other inputs should be made available to the farmers.

2. Farmers were aware of the major biotic, abiotic and socio-economic constraints that contributed negatively to low maize productivity in their particular agro-ecology. Farmers were also capable of discriminating different constraints according to importance for their communities. Downy mildew, drought, cutworm and stem borer were the most important constraints for lowland environments, while input availability (seed and fertilizer), ear rot, turcicum leaf blight and gray leaf spot diseases and low soil fertility were most important in high altitude environments. Different intervention approaches are, therefore, required for the low and mid-altitude ecologies.

3. In general, farmers need maize seed from varieties that can overcome the prevalent production constraints and meet their consumption needs and preferences. The most important variety selection criteria were grain yield, maturing cycle (short maturing), white grain and hardness of endosperm (preferably white and flint), tolerance to drought or low soil fertility and resistance to the local diseases and post harvest pests. These desired traits would be considered in setting priority objectives for the breeding programmes, for the lowland and mid altitude environments in Mozambique.

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CHAPTER 3

Combining ability for Downy Mildew Resistance and Heterotic Orientation of Tropical Lowland Maize Inbred Lines

Abstract

Downy mildew (DM) is the most destructive disease of maize in tropical lowland environments. Productivity can be enhanced by growing resistant hybrids. However, development of a viable hybrid programme requires knowledge of combining ability and heterosis among inbred lines adapted to lowland environment. The objective of this study was to determine combining ability, heterosis and heterotic orientation of S_4 maize inbred lines. Thirty-six top-crosses were generated by crossing 18 S_4 inbred lines with two open pollinated synthetic tester varieties. The 36 top crosses, four check hybrids and two testers ZM523 (Z) and Suwan-1 (S) were evaluated in a 6 x 7 α-lattice design with two replications across three environments in Mozambique. General combining ability (GCA) effects were significant for DM resistance and grain yield suggesting that genes with additive effects were important in controlling the traits. The SCA effects were not significant for DM but were significant for grain yield. Based on SCA data, ten lines were grouped with the S and eight lines with the Z tester. However, based on heterosis with the respective testers, the lines were divided into three groups S, Z and SZ. Lines 2, 30 and 42 displayed heterosis with both testers for grain yield hence they were allocated to SZ-group; lines 8, 10, 25, 45, 48 exhibited positive heterosis with Suwan-1 and were therefore classified in Z-group, and line19 exhibited heterosis with ZM523 and was fitted in S-group. The remaining eight lines did not show positive heterosis with any tester hence they could not be classified based on heterosis data. However, they displayed heterosis values that were more negative in crosses with one tester than the other tester; hence their inclination could be established. Line 42 displayed the highest level of heterosis with Suwan-1 (32%) and ZM523 (29%) and out-performed all standard check varieties qualifying it as a potential candidate for further testing. Generally, there was consistency of heterotic grouping of the lines using SCA and heterosis data.

Keywords: combining ability, down mildew resistance, heterotic patterns, heterosis, lowland tropical maize

3.1 Introduction

Downy mildew (DM) disease, which is caused by *Peronosclerospora sorghi* [Weston and Uppal (Shaw)] (Ullstrup, 1997) is one of the most destructive diseases of maize in many tropical and subtropical regions worldwide (Frederiksen *et al.,* 1969; Frederiksen and Renfro, 1977; Williams, 1984). In Africa, DM has been reported in several countries, especially in the tropical-lowland environments below 800 m.a.s.l (Plumb-Dhindsa and Mondjane, 1984; De Milliano, 1992; Jeger *et al*., 1998; IITA, 1999; Adenle and Cardwell, 2000; Bock *et al*., 2000; Bock and Jeger, 2002). Therefore, the disease is very important in the African region and has the potential to impact negatively on food security especially in the small-scale farming communities that dominate production (accounting for 80% of grain production) in this region but lack access to alternative control methods. For these farmers, host plant resistance to the pathogen is the most practical control measure; hence, development of DM resistant varieties is suggested. Currently such varieties are scarcely available in most tropical lowland environments.

Downy mildew distribution and damage is high. In Mozambique, for example, it was first reported on maize in the 1980s in the Southern lowlands (Plumb-Dhindsa and Mondjane, 1984; Nunes *et al*., 1985; Segeren *et al*., 1994; Segeren, 1995), but it has since spread throughout the country. Denic (1996) reported the occurrence of DM in the five provinces, namely in southern region of Maputo, Gaza and Inhambane provinces; and some parts of Manica and the central regions of Sofala province. Annual grain yield losses associated with DM have not been quantified in Mozambique and the African region, but can be high with up to 50% systemic incidences being observed in farmers' fields during 2007/2008 growing season in areas like Chókwè district in Mozambique. Anaso (1989) reported a strong relationship between grain yield losses and downy mildew disease, and systemically infected plants in endemic areas in Nigeria where systemic infection in the farmers' fields was estimated to exceed 90%. According to the International Institute of Tropical Agriculture (1999), downy mildew can destroy a maize crop in a relatively short period. This may result in huge grain yield losses in epidemic situations. Grain yield losses reaching 100% caused by different downy mildew pathogens, including *P. sorghi*, when susceptible varieties were grown under favourable weather conditions for the disease were reported in Asia, South and North America, West Africa, Australia and Europe (Bonde, 1982). High levels of sporulation occur when temperatures are around 20°C and relative humidity greater than 95% (Thakur and Mathur, 2002), and these conditions are characteristic of the tropical-lowland

environments in Mozambique and the southern African region. The disease increases when farmers plant maize throughout the year in tropical lowlands due to continual availability of large quantities of DM inoculum. Further, the use of unimproved seed from the local markets by farmers, as is the case in Mozambique, has been cited as a potential source of primary infection in southern Nigeria (Olanya, 1993). The use of farm saved seed is common in all developing countries hence the need for an effective control of downy mildew disease.

Maize hybrids which combine high resistance to DM disease and adaptation to the lowland environments, currently not available in sub-Saharan Africa, are viewed as the most practical and cost-effective way of controlling the disease in maize, especially for small-scale farmers (Cardwell *et al*., 1997). Denic *et al*. (2008) recommended use of adapted lowland tropical germplasm, with downy mildew resistance (DMR) background. The national programme in Mozambique (IIAM) and a private seed company (SEMOC) have been developing open pollinated source germplasm with some DM resistance using introduced resistance sources from Thailand, Philippines and Nigeria (Bueno, 1989, 1991; Chauque *et al*., 2004). However, no hybrid orientated breeding programme for DM resistant maize is in place regardless of the high demand for hybrid maize countrywide (Fato *et al*., 2004). Therefore, there is need to develop DM resistant hybrid maize varieties for deployment in tropical lowland environments.

Pertinent to a hybrid oriented breeding programme is the knowledge of general combining ability (GCA) of the lines and specific combining ability (SCA) of their crosses, associated with the efficient exploitation of heterosis and heterotic patterns. Kashif and Khaliq (2003) consider identification of parental source germplasm with good combining ability as the pre-requisite for success of any breeding programme aimed at hybrid development. Further, heterosis is the quantitative measure of superiority of F_1-hybrids over their parents (Stuber, 1994). Paschold *et al.* (2009) define more precisely heterosis as the phenomenon that describes the superiority of highly heterozygous F_1-hybrids in relation to the average (mid-parent) performance of their genetically distinct homozygous parents. The manifestation of heterosis on maize has been reported since early 1900s based on the findings of Shull (1908) and East (1909). The level of heterosis manifestation in F_1-hybrid is strongly associated with genetic diversity of the parental lines. Crosses between inbred lines from groups with divergent genetic backgrounds are expected to exhibit high level of heterosis

than those among lines from the genetically more related groups (Hallauer, 1999; Barata and Carena, 2010). These crosses may also be valuable for high yielding OPV synthetic development (Kutka and Smith, 2007).

The information on both GCA and SCA effects and heterosis and heterotic patterns can be obtained using many mating schemes. With established testers and for a hybrid-breeding programme, the line x tester mating design, proposed by Kempthorne (1957), is the simplest but effective and most commonly used mating scheme for generating the information. Generally, it is used in early stages of the inbreeding process (S_2 or S_3 generations) to reduce the number of inbred lines to be carried to the next generations. At this stage, a large number of lines can be crossed to one or more testers to generate topcross progenies. These progenies are then evaluated for grain yield in different environments. Genter and Alexander (1966) suggested the use of the topcross procedure, combined with the early progeny testing for yield potential, as the best way of selecting lines with high yield potential in hybrid combination and good combining ability. Both broad genetic base materials (populations) and narrow genetic base genotypes such as inbred lines or single cross hybrids have been used as testers in discriminating maize lines according to yield performance. Line x tester method has also been largely used for determining heterotic patterns among maize germplasm, by crossing the given germplasm to two or more genetically different testers (Menkir *et al*., 2004; Fan *et al*., 2008, 2010). Therefore, this design was used in the current study and the resultant experimental hybrids were evaluated in the target tropical lowland environments. The objective was to determine the GCA and SCA effects and heterosis of the new S_4 maize inbred lines with testers (ZM523, Z; Suwan-1, S) for grain yield and DM resistance in tropical lowland environments. The information generated was critical for assigning the inbred lines into heterotic groups.

3.2 Materials and Methods

3.2.1 Germplasm

Eighteen S_4 inbred lines were crossed with two open pollinated synthetic varieties (SOPVs), which were adopted as testers, in accordance with a line x tester mating scheme generating 36 top crosses. The S_4 inbred lines were developed in Mozambique from eight populations introduced from the International Maize and Wheat Improvement Center (CIMMYT)

programme in Harare, Zimbabwe (Table 3.1). Two broad-based SOPVs, ZM523 and Suwan-1-synthetic were designated as Z and S testers, respectively. Tester Z is a white semi-dent grain synthetic population developed by CIMMYT-Zimbabwe by crossing at least six maize inbred lines from heterotic group A (in the CIMMYT system) to at least six other inbred lines from group B (Vivek[1], personal communication). The tester S is a yellow flint grain synthetic population that was derived from the population Suwan-1-Synthetic with DM resistance that was obtained from the International Institute for Tropical Agriculture (IITA) in Nigeria.

Table 3.1 Pedigrees of maize inbred lines and heterotic groups of testers used to generate topcrosses in a line x tester mating scheme

Parents		Generation	Maturity period$^{¥}$
Line Code	Pedigree		
1	(P501SRC0/P502SRC0)FS-12-1-1-3	S_4	Early
2	(P501SRC0/P502SRC0)FS-12-3-1-1	S_4	Early
3	(P501SRC0/P502SRC0)FS-14-1-2-2	S_4	Early
5	(P501SRC0/P502SRC0)FS-31-1-3-1	S_4	Intermediate
8	$DRAC_1F_1$FS-13-1-3-1	S_4	Early
10	$DRAC_1F_1$FS-15-2-1-1	S_4	Early
13	$INTB_1C_1F_1$FS-12-1-4-1	S_4	Intermediate
19	$INTB_1C_1F_1$FS-30-1-1-4	S_4	Intermediate
25	Suwan8075DMR-28-2-1-1	S_4	Early
30	$TSEGRIMB_2$-3-1-1-1	S_4	Early
32	$TSEGRIMB_2$-3-1-5-1	S_4	Intermediate
42	ZM421-96-1-2-1	S_4	Early
45	ZM521-3-2-1-1	S_4	Early
47	ZM521-11-1-3-1	S_4	Early
48	ZM521-20-1-1-1	S_4	Late
51	ZM521-29-2-1-4	S_4	Early
55	ZM621-8-1-2-1	S_4	Late
57	ZM621-15-3-1-2	S_4	Late
Testers			
T1	ZM523	SOPV†	Early
T2	Suwan-1-synt.	SOPV	Early

†SOPV = Open-pollinated- synthetic variety; $^{¥}$Early =Anthesis date (60 to 65 days); Intermediate = anthesis date (65 to 70 days); Late = anthesis date more than 70 days

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[1] B. Vivek, Maize Breeder, CIMMYT-Zimbabwe until 2009.

3.2.2 Experimental sites

The experiment was carried out at Umbeluzi Agriculture Research Station (UARS, $26^{\circ}03'S$; $32^{\circ}23'E$, 12 m.a.s.l) and Chókwè Agriculture Research Station (CARS, $24^{\circ} 31' S$; $33^{\circ} 0' E$, 40 m.a.s.l). The sites represent the tropical-lowland environments in Mozambique according to the cereal mega-environment classification (0 - 400 m.a.s.l) and represent the DM hot spot. The annual average temperature for both sites is $23^{0}C$, with minimum of $17^{0}C$ and maximum of $29^{0}C$ and $30^{0}C$, at UARS and CARS, respectively. The annual rainfall amounts for UARS and CARS were 700 mm and 600 mm, respectively. At both sites, the wet period is between November and March, and dry period from April to October. Three experimental environments defined by a combination of site, season and level of stress were: (i) at UARS during 2006/07 where downy mildew was artificially inoculated under rainfed conditions (E-1), (ii) at CARS during off-season in 2007 under artificial inoculation and reduced irrigation (E-2), and (iii) at CARS during 2007 under artificial inoculation and full irrigation (E-3).

3.2.3 Experimental design and field management

The 36 top-crosses, two testers, and four check varieties comprising three experimental hybrids (two three-way and one double cross) and one commercial three-way cross hybrid were evaluated. The experiment was laid out as a 6 x 7 row-column alpha lattice (0.1) design with two replications in all environments. Two-row plots of 5 m were used for E-1 and 8 m for E-2 and E-3. Two to three seeds were planted per station at 0.25 m and 0.80 m intra and inter-row spacings, respectively. The plants were thinned to one plant per station, achieving a population of about 50,000 plants ha^{-1}, 42 plants per plot in E-1 and 66 plants per plot in E-2 and E-3. A compound fertilizer (12%N: 24%P: 12K) and urea (46%N) were applied as deep (basal) at planting time and as a top-dressing at four and eight weeks after plant emergence, respectively, to achieve 170 kg N, 33 kg P and 44 kg K ha^{-1} in all environments. An insecticide, Supermethrine, was applied at two to four weeks and six to eight weeks in all environments to control stem borer. Weed control was achieved by hand weeding from 3-4 weeks after plant emergence until 2-3 weeks after flowering. In general, three to four weedings were performed during the growing period. The number of weeding operations varied from site to site and according to the growing season, with more weedings in summer due to more rains.

3.2.4 Disease Inoculation and data collection

Artificial epiphytotic of *P. sorghi* downy mildew was created at UARS and CARS using the method of spreader rows suggested by Williams (1984) and Cardwell *et al.* (1994) and modified by Cardwell *et al.* (1997) and Denic (1996). Seeds of a susceptible maize variety were pre-germinated for three days. The seeds were then inoculated on the 4th day by placing freshly collected DM infected maize leaves over the seed that had been previously washed in tap water to remove old conidia. The leaves and seed were incubated overnight at 21^0C in the dark, in a seed cold room. After incubation, the pre-geminated, inoculated seed was planted in the field in the irrigation channels at both ends of each experimental plot row. There were two plantings of the spreader rows, the first at 0.50 m intra row spacing and the second, one week later in between the initial plantings using non-infected seed from the same susceptible maize variety giving a resultant intra row spacing of 0.25 m. The role of the second planting of spreader rows was to enhance the level of infestation because some plants, which originated directly from infected seed, may be weak (Denic, 1996). The experimental material was planted two weeks after the first planting of spreader rows. Disease incidence was assessed by counting the number of plants showing the symptoms of downy mildew in a plot at four, six and eight weeks after emergence of experimental materials. The level of DM resistance of the genotypes was calculated as the percentage of diseased plants over the total number of plants per plot. Days to anthesis (the number of days when 50% of plants in a plot were shedding pollen), silking date (the number of days when 50% of plants in a plot had emerged silks), were measured. Grain texture was rated using a score of 1 to 5 (1 = flint and 5 = dent) and plant and ear aspects were scored using a scale of 1 to 5 (1 = good and 5 = worst appeal). The plant aspect is a qualitative measure that includes the plant architecture; vigour and overall plant health related aspects. Ear aspect assesses also the architecture of the cob in relation to form, size and husk cover. Grain (kg plot^{-1}) yield was measured and converted to t ha^{-1} and adjusted to 12.5% grain moisture content.

3.2.5 Data analysis

The analysis of variance (ANOVA) across environments for all data was performed using GLM procedures in SAS computer package, version 9.1 following a linear model:

$$Y_{ijk} = \mu + r(e_k) + e_k + l_i + t_j + (l \times t)_{ij} + (l \times e)_{ik} + (t \times e)_{jk} + (l \times t \times e)_{eijk} + \epsilon_{ijk}$$

Where: Y_{ijk} is measured trait of the genotype of ith line crossed to j^{th} tester evaluated in r replications across k environments; μ is grand mean; $r(e_k)$ = effect of replication nested within the e_k environments; l and t represent average effects of lines and of testers, respectively, which is equivalent to GCA effects of lines and testers, respectively; l x t = line x tester interaction effects that is equivalent to the SCA effects of the crosses; e_k is the environmental main effects; l x e, t x e and l x t x e are the interactions of the lines, testers and the lines x testers with the environments, and e_{ijk} = random experimental error.

The GCA of lines (GCA_l) and testers (GCA_t), and SCA of crosses (SCA) and their standard errors were estimated according to Dabholkar (1992). Standard heterosis (SH) was calculated using the following formula: SH = ((F_1- MT)/ MT) * 100, where: F_1 = F_1 hybrid mean performance; MT = Mean of S, Z, best hybrid or trial mean.

3.3 Results

3.3.1 Variation among topcrosses

The topcross mean squares were significant (P≤ 0.05) for silking date and highly significant (P≤ 0.01) for all the traits except plant aspect scores (Table 3.2). Environment and lines exhibited highly significant differences (P≤ 0.01) for all traits except plant aspect scores. Testers showed highly significant differences (P≤ 0.01) for DM scores, grain yield, number of plants and ears per plot and grain texture, and were significantly different (P≤ 0.05) for the other traits except for ear and plant aspect scores. Line x tester interaction effects were highly significant (P≤ 0.01) for number of plants and ears per plot, and significant (P≤ 0.05) for grain yield and did not show any significant differences for the other traits. Environment x topcross interactions effects were highly significant (P≤ 0.05) for DM scores, grain yield, number of plants and ears per plot and and were significantly different (P≤ 0.05) for anthesis

date and for the other traits were not significant. Environment x line interaction effects were highly significant (P≤ 0.01) for number of plants and number of ears per plot and significant (P≤ 0.05) for grain yield, anthesis and silking dates. Environment x tester interaction effects were highly significant (P≤ 0.01) for DM incidence and significant (P≤ 0.01) for grain yield. Environment x line x tester interaction effects were highly significant (P≤ 0.01) for the number of plants and ears per plot, and significant (P≤ 0.05) for DM scores and grain yield. Grain yield data showed significant correlations with downy mildew scores (-0.473, P≤0.01), number of ears per plot (0.676, P≤0.01), and number of plants per plot (0.661, P≤0.01). Downy mildew scores also showed significant negative correlations with the yield components such as number of plants per plot (-0.532, P≤0.01 **) and number of ears per plot (-0.612, P≤0.05).

Table 3.2 Mean squares for downy mildew rating scores, grain yield, and secondary traits of 36 topcrosses over three environments.

Source of variation	DF	Downy mildew scores [†]	Grain Yield (t ha^{-1})	Anthesis date (days)	Silking date (days)	Grain texture (score)[¥]	Ear aspect (score)[¥]	Plant aspect (score)[¥]
Env.	2	12091.89**	39.45**	14504.48**	14954.45**	4.18**	10.44**	0.69
Block (Rep x Env.)	46	176.14	2.61	8.31	8.79	0.60	0.57	1.00
Topcrosses	35	701.48**	5.07 **	12.27**	10.14*	1.50**	0.64**	1.26**
Line	17	317.17**	5.73**	15.59**	14.25**	1.29**	0.68**	1.78
Tester	1	16137.95**	28.87**	40.45*	44.46*	17.24**	1.21	0.36
Line x Tester	17	101.17	2.30*	4.90	3.60	0.80	0.33	0.48
Env. x Topcross	70	203.52**	2.46 **	4.22*	3.13	0.36	0.26	0.50
Env.x Line	34	93.69	2.07*	5.37*	4.02*	0.41	0.34	0.46
Env.x Tester	2	2001.24**	5.61*	0.39	1.85	0.07	0.43	0.10
Env. x Line x Tester	34	172.78*	2.02*	3.46	2.68	0.34	0.21	0.55
Error	**96**	**97.87**	**1.23**	**3.02**	**2.96**	**0.36**	**0.24**	**0.66**
Overall Mean		20.00	3.55	62.60	64.50	3.01	2.31	2.60
R^2 (%)		90.64	83.72	99.10	99.15	78.86	0.81	0.70

*, ** Data is significant at P ≤ 0.05 and P ≤ 0.01, respectively.
[†] Downy mildew rating scores (% of diseased plants over a total number of plants in plot),
[¥] Scores for grain texture (1= flint and 5=dent type) and for ear and plant aspect (1=good and 5=worst).

3.3.2 General combining ability effects

The results of GCA effects of 18 lines and the respective two testers are presented in Table 3.3. For DM, grain texture, ear aspect, plant aspect scores, anthesis, and silking dates, negative values of GCA and SCA are desirable. For grain yield, positive values are desirable. Significant negative GCA effects were displayed by lines 2, 19 and 42 (P≤ 0.05) and line 3 (P≤ 0.01) for DM scores. For grain yield, positive significant GCA effects values were shown by lines 2 and 45 (P≤ 0.05) and lines 19 and 42 (P≤ 0.01). For anthesis date significant negative GCA effects were shown by lines 1, 8 and 30 (P≤ 0.05) and line 45 (P≤ 0.01), while for silking date significant and negative values were displayed by lines 3, 30, and 47 (P≤ 0.05) and line 45 (P≤ 0.01). For the other agronomic traits, lines showed different trends (Table 3. 3). For grain texture, negative and significant GCA effects were displayed by lines 8, 10 and 47 (P≤ 0.05) and lines 30 and 45 (P≤ 0.01). In relation to the variation in scores for ear and plant aspects, significant negative GCA effects were shown by lines 45 and 57 for ear aspect and 55 for plant aspect (P≤ 0.05) and line 25 for both traits and lines 8 and 42 for plant aspect (P≤ 0.01). The two testers showed divergent trends on the GCA effects (Table 3.3). The tester ZM523 showed significant positive values for DM scores, anthesis and silking dates, grain texture (P≤ 0.01) and ear and plant aspects (P≤ 0.05). Further, this tester showed significant negative values for grain yield (P≤ 0.01). Tester Suwan-1 displayed significant negative GCA effects for DM scores, anthesis and silking dates and grain texture (P≤ 0.01), plant aspect and ear aspects (P≤ 0.05). The same tester showed positive significant GCA effects for grain yield (P≤ 0.01).

3.3.3 Specific combining ability effects

Among the topcrosses, no significant SCA effects were found for ear and plant aspects (Table 3.4). For the other traits, different crosses showed significant and desirable SCA effects either negative or positive depending on the trait. These crosses were 5 x Suwan-1 (P≤ 0.05) for DM scores; 1 x Suwan-1 for grain yield, silking date (P≤ 0.05); 45 x Suwan-1 for grain yield (P≤ 0.05); 45 x ZM523 and 55 x Suwan-1 (P≤ 0.05) and 13 x ZM523 (P≤ 0.01) for grain texture; 32 x Suwan-1 for anthesis date (P≤ 0.05). The same crosses involving the opposite tester displayed undesirable significant SCA effects (Table 3.4).

Table 3.3 General combining ability estimates of inbred lines for downy mildew rating scores, grain yield and secondary traits over three environments

Lines	Downy mildew (score)†	Grain yield (tha^{-1})	Anthesis date (days)	Silking date (days)	Grain texture (score)$^\maltese$	Ear aspect (score)$^\maltese$	Plant aspect (score)$^\maltese$
1	11.80**	-0.50	-1.00*	-0.40	0.50**	-0.10	0.40
2	-6.48*	0.83*	-0.20	-0.40	0.20	-0.10	0.30
3	-9.79**	-0.40	-0.50	-0.90*	0.30*	0.20	0.10
5	7.86**	-0.79*	1.50**	1.10*	0.50**	0.10	0.70**
8	-0.50	0.30	-1.10*	-0.50	-0.30*	-0.20	-0.70**
10	0.80	0.30	-0.50	-0.10	-0.40*	-0.20	0.10
13	-0.20	0.00	1.70**	0.90*	0.10	0.40**	0.30
19	-5.25*	1.09**	-0.20	-0.10	0.30*	-0.20	-0.10
25	-3.10	0.50	-0.50	0.00	0.10	-0.50**	-0.70**
30	-3.00	0.40	-0.90*	-1.30*	-0.70**	0.40**	-0.10
32	-1.40	-0.30	0.20	-0.20	0.00	-0.10	0.20
42	-7.27*	1.66**	-0.40	0.80	0.30*	-0.10	-0.60**
45	-1.40	0.69*	-2.50**	-2.70**	-0.60**	-0.20*	-0.10
47	8.50**	-1.34**	-0.80	-1.20*	-0.30*	-0.10	0.30
48	2.50	-0.56*	1.00*	1.90**	0.30*	0.00	0.30
51	1.00	-0.50	0.30	-0.40	-0.10	0.60**	-0.20
55	0.80	-0.20	0.30	0.90	-0.20	0.10	-0.70*
57	5.10	-1.27**	3.40*	2.50**	0.10	-0.30*	0.40*
SE	**2.6**	**0.3**	**0.5**	**0.5**	**0.1**	**0.1**	0.2
Testers							
ZM523	10.24**	-0.43**	0.50**	0.50**	0.30**	0.10*	0.20*
Suwan-1	-10.24**	0.43**	-0.50**	-0.50**	-0.30**	-0.10*	-0.20*
SE	**0.9**	**0.1**	**0.2**	**0.2**	**0.1**	**0**	0.1

*, ** Data is significant at P ≤ 0.05 and P ≤ 0.01, respectively. ‡Downy mildew rating scores (% of diseased plants over a total number of plants in plot), $^\maltese$Scores for grain texture (1= flint and 5=dent type) and for ear and plant aspect (1=good and 5=worst).

Table 3.4 Specific combining ability estimates of 36 topcrosses for downy mildew rating scores, grain yield and secondary traits over three environments.

`Topcross	Downy mildew	Grain yield	Anthesis Date	Silking date	Grain texture	Ear aspect	Plant aspect
	scores [†]	(t ha^{-1})	(days)	(days)	(score)[¥]	(score)[¥]	(score)[¥]
1 x ZM523	5.16	-0.82*	1.0	1.4*	-0.4*	0.0	0.1
1 x Suwan-1	-5.16	0.81*	-1.0	-1.4*	0.4*	0.0	-0.1
2 x ZM523	-5.93	0.14	0.9	0.5	0.0	0.0	0.3
2 x Suwan-1	5.94	-0.15	-0.9	-0.5	0.0	0.0	-0.3
3 x ZM523	-3.22	0.39	-0.5	-0.2	0.1	-0.1	-0.4
3 x Suwan-1	3.22	-0.40	0.5	0.2	-0.1	0.1	0.4
5 x ZM523	6.73*	-0.61	-0.3	-0.7	0.2	0.0	0.1
5 x Suwan-1	-6.72*	0.60	0.3	0.7	-0.2	0.0	-0.1
8 x ZM523	3.61	-0.01	0.6	0.6	0.0	-0.2	-0.2
8 x Suwan-1	-3.61	0.00	-0.6	-0.6	0.0	0.2	0.2
10 x ZM523	-2.73	-0.19	-0.1	0.3	0.0	0.1	-0.1
10 x Suwan-1	2.74	0.18	0.1	-0.3	0.0	-0.1	0.1
13 x ZM523	1.94	0.14	-0.2	0.0	-0.7**	-0.3	-0.2
13 x Suwan-1	-1.93	-0.15	0.2	0.0	0.7**	0.3	0.2
19 x ZM523	-2.70	0.67	0.4	0.0	0.0	-0.3	-0.3
19 x Suwan-1	2.71	-0.68	-0.4	0.0	0.0	0.3	0.3
25 x ZM523	0.15	-0.14	0.4	-0.4	0.2	-0.1	0.2
25 x Suwan-1	-0.15	0.13	-0.4	0.4	-0.2	0.1	-0.2
30 x ZM523	0.53	0.08	-0.8	-0.8	0.1	0.3	-0.1
30 x Suwan-1	-0.53	-0.09	0.8	0.8	-0.1	-0.3	0.1
32 x ZM523	-1.88	0.25	-1.6*	-0.7	0.0	-0.1	0.0
32 x Suwan-1	1.88	-0.26	1.6*	0.7	0.0	0.1	0.0
42 x ZM523	-1.82	0.50	1'0	0.2	0.2	-0.2	0.1
42 x Suwan-1	1.83	-0.51	-1.0	-0.2	-0.2	0.2	-0.1
45 x ZM523	-2.51	-0.95*	-0.7	-0.9	-0.6*	0.2	0.1
45 xSuwan-1	2.51	0.94*	0.7	0.9	0.6*	-0.2	-0.1
47 x ZM523	2.01	-0.36	0.4	0.7	0.2	0.2	0.2
47 x Suwan-1	-2.01	0.35	-0.4	-0.7	-0.2	-0.2	-0.2
48 x ZM523	0.99	-0.71	0.1	0.2	-0.1	0.0	0.3
48 x Suwan-1	-0.98	0.71	-0.1	-0.2	0.1	0.0	-0.3
51 x ZM523	0.93	0.56	0.1	0.5	0.2	0.1	0.5
51 x Suwan-1	-0.93	-0.57	-0.1	-0.5	-0.2	-0.1	-0.5
55 x ZM523	0.25	0.66	-0.9	-0.6	0.5*	0.3	-0.3
55 x Suwan-1	-0.25	-0.67	0.9	0.6	-0.5*	-0.3	0.3
57 x ZM523	-1.47	0.31	0.1	-0.1	0.1	0.0	-0.2
57 x Suwan-1	1.47	-0.32	-0.1	0.1	-0.1	0.0	0.2
SE	**3.7**	**0.4**	**0.7**	**0.7**	**0.2**	**0.2**	**0.3**

*, ** Data is significant at P ≤ 0.05 and P ≤ 0.01, respectively, ‡Downy mildew rating scores (% of diseased plants over a total number of plants in plot), ¥Scores for grain texture (1= flint and 5=dent type) and for ear and plant aspect (1=good and 5=worst).

3.3.4 Standard heterosis, heterotic orientation and heterotic groups for grain yield

There were 20 topcrosses that had grain yield superior to the trial mean, 19 to the check hybrid mean, 12 to the best hybrid check, the same number to Suwan-1 (T-S) and 14 to ZM523 (T-Z) (Table 3.5). Based on specific combining ability data, for grain yield (Table 3.4), lines were classified into two groups S and Z depending on the direction of the SCA estimate. Group Z consisted of seven lines that showed negative SCA estimates in crosses with tester ZM523 (1, 5, 10, 25, 45, 47 and 48); while the 10 lines in S – group displayed negative SCA effects in crosses with tester Suwan-1 (2; 3, 13, 19, 30, 32, 42, 51, 55 and 57). Line 8 showed 0.00 and – 0.01 SCA effects for grain yield in crosses with S and Z testers, respectively, showing a slight inclination towards the Z tester group. However, based on its negative SCA effects in crosses with the ZM523 for the number of ears per plant (Table 3.4), which is a grain yield component, it was included in the Z group (Table 3.6).

Based on heterosis with the respective testers, the lines were divided into three groups S, Z and SZ. Lines 2, 30 and 42 displayed positive heterosis with both testers for grain yield hence they were allocated to SZ-group; lines 8, 10, 25, 45, 48 exhibited positive heterosis with Suwan-1 and were therefore classified in Z-group; and line19 exhibited positive heterosis with ZM523 and was fitted in S-group. Line 42 displayed the highest level of heterosis with Suwan-1 (32%) and ZM523 (29%) and out-performed all standard check hybrids, which qualified it as a potential candidate for further testing. The remaining nine lines did not show positive heterosis with any tester (Table 3.7). However, although these lines displayed negative heterosis with both testers, they were more negative with one tester than with the other, hence their inclination towards either the Z or the S tester could be established. Consequently, the lines 1, 5, 13 and 47 were more inclined to Z while the lines 3, 32, 51, 55 and 57 were inclined towards the S tester.

In general, results in Tables 3.6 and 3.7 indicate consistency of heterotic classification of the lines using the SCA and heterosis data.

Table 3.5 Percent grain yield (t ha^{-1}) of the 36 topcrosses relative to the trial mean, best check hybrid, mean of hybrids and testers across three environments during 2006 and 2007

Name	Type[†]	Relative Yield to trial mean	Relative Yield to best check hybrid	Relative Yield to mean of check hybrids	Relative Yield to mean of Suwan-1	Relative Yield to mean of ZM523
		(%)	(%)	(%)	(%)	(%)
42 x Suwan-1	TC	149.5	135.0	148.7	132.0	139.5
42 x ZM523	TC	137.9	124.5	137.1	121.8	128.7
19 x ZM523	TC	137.1	123.8	136.3	121.0	127.9
45 x Suwan-1	TC	136.5	123.3	135.7	120.5	127.4
10 x Suwan-1	TC	135.4	122.3	134.6	119.6	126.4
8 x Suwan-1	TC	128.5	116.0	127.7	113.5	119.9
25 x Suwan-1	TC	122.1	110.3	121.4	107.8	114.0
2 x Suwan-1	TC	120.5	108.8	119.8	106.4	112.4
30 x Suwan-1	TC	116.9	105.5	116.2	103.2	109.0
48 x Suwan-1	TC	114.6	103.5	114.0	101.2	107.0
2 x ZM523	TC	114.1	103.0	113.4	100.7	106.5
19 x Suwan-1	TC	113.3	102.3	112.6	100.0	105.7
Suwan-1	SOPV	113.3	102.3	112.6	100.0	105.7
13 x Suwan-1	TC	110.8	100.0	110.1	97.8	103.4
(23 x19) x 45	CH	110.8	100.0	110.1	97.8	103.4
30 x ZM523	TC	109.4	98.8	108.7	96.6	102.1
ZM523	SOPV	107.2	96.8	106.5	94.6	100.0
1 x Suwan-1	TC	106.3	96.0	105.7	93.9	99.2
55 x ZM523	TC	105.8	95.5	105.2	93.4	98.7
5 x Suwan-1	TC	104.1	94.0	103.5	91.9	97.2
25 x ZM523	TC	103.8	93.8	103.2	91.7	96.9
45 x ZM523	TC	103.0	93.0	102.4	91.0	96.1
10 x ZM523	TC	100.2	90.5	99.7	88.5	93.5
QS77-07	CH	98.3	88.8	97.7	86.8	91.7
47 x Suwan-1	TC	97.8	88.3	97.2	86.3	91.2
(21 x 23) x P66	CH	97.8	88.3	97.2	86.3	91.2
55 x Suwan-1	TC	96.9	87.5	96.4	85.6	90.4
(66 x 23) x (45 x 21)	CH	95.5	86.3	95.0	84.4	89.2
3 x Suwan-1	TC	91.7	82.8	91.1	80.9	85.5
8 x ZM523	TC	91.1	82.3	90.6	80.4	85.0
3 x ZM523	TC	90.8	82.0	90.3	80.2	84.8
32 x Suwan-1	TC	88.1	79.5	87.5	77.8	82.2
32 x ZM523	TC	87.0	78.5	86.4	76.8	81.1
13 x ZM523	TC	84.7	76.5	84.2	74.8	79.1
51 x ZM523	TC	80.6	72.8	80.1	71.2	75.2
51 x Suwan-1	TC	66.5	60.0	66.1	58.7	62.0
57 x Suwan-1	TC	63.7	57.5	63.3	56.2	59.4
57 x ZM523	TC	62.9	56.8	62.5	55.5	58.7
1 x ZM523	TC	62.3	56.3	61.9	55.0	58.1
48 x ZM523	TC	57.6	52.0	57.3	50.9	53.8
5 x ZM523	TC	43.2	39.0	43.0	38.1	40.3
47 x ZM523	TC	42.7	38.5	42.4	37.7	39.8
Mean		**3.6**				
Best hybrid men			**4.0**			
Check mean				**3.6**		
Suwan-1 mean					**4.1**	
ZM523 mean						**3.9**

[†]TC= Topcross, SOPV= open-pollinated-synthetic variety, CH= hybrid check.

Table 3.6 Heterotic grouping of lines based on SCA effects for grain yield in line x tester crosses

Line	Pedigree	SCA effects		Heterotic grouping	
		ZM523	Suwan-1	Z	S
1	(P501SRC0/P502SRC0)FS-12-1-1-3	-0.82	0.81	x	
2	(P501SRC0/P502SRC0)FS-12-3-1-1	0.14	-0.15		x
3	(P501SRC0/P502SRC0)FS-14-1-2-2	0.39	-0.40		x
5	(P501SRC0/P502SRC0)FS-31-1-3-1	-0.61	0.60	x	
8	DRAC$_1$F$_1$FS-13-1-3-1	-0.01	0.00	x	
10	DRAC$_1$F$_1$FS-15-2-1-1	-0.19	0.18	x	
13	INTB$_1$C$_1$F$_1$FS-12-1-4-1	0.14	-0.15		x
19	INTB$_1$C$_1$F$_1$FS-30-1-1-4	0.67	-0.68		x
25	Suwan8075DMR-28-2-1-1	-0.14	0.13	x	
30	TSEGRIMB$_2$-3-1-1-1	0.08	-0.09		x
32	TSEGRIMB$_2$-3-1-5-1	0.25	-0.26		x
42	ZM421-96-1-2-1	0.50	-0.51		x
45	ZM521-3-2-1-1	-0.95	0.94	x	
47	ZM521-11-1-3-1	-0.36	0.35	x	
48	ZM521-20-1-1-1	-0.71	0.71	x	
51	ZM521-29-2-1-4	0.56	-0.57		x
55	ZM621-8-1-2-1	0.66	-0.67		x
57	ZM621-15-3-1-2	0.31	-0.32		x

Table 3.7 Heterotic grouping of lines based on heterosis with relative testers

Line	Pedigree	Standard Heterosis (%)		Heterotic grouping		
		ZM523	Suwan-1	Z	S	Z / S
1	(P501SRC0/P502SRC0)FS-12-1-1-3	-41.9	-6.1	x		
2	(P501SRC0/P502SRC0)FS-12-3-1-1	6.5	6.4			x
3	(P501SRC0/P502SRC0)FS-14-1-2-2	-15.3	-19.1		x	
5	(P501SRC0/P502SRC0)FS-31-1-3-1	-59.7	-8.1	x		
8	DRAC$_1$F$_1$FS-13-1-3-1	-15.0	13.5	x		
10	DRAC$_1$F$_1$FS-15-2-1-1	-6.5	19.6	x		
13	INTB$_1$C$_1$F$_1$FS-12-1-4-1	-20.9	-2.2	x		
19	INTB$_1$C$_1$F$_1$FS-30-1-1-4	27.9	0.0		x	
25	Suwan8075DMR-28-2-1-1	-3.1	7.8	x		
30	TSEGRIMB$_2$-3-1-1-1	2.1	3.2			x
32	TSEGRIMB$_2$-3-1-5-1	-18.9	-22.3		x	
42	ZM421-96-1-2-1	28.7	32.0			x
45	ZM521-3-2-1-1	-3.9	20.5	x		
47	ZM521-11-1-3-1	-60.2	-13.7	x		
48	ZM521-20-1-1-1	-46.3	1.2	x		
51	ZM521-29-2-1-4	-24.8	-41.3		x	
55	ZM621-8-1-2-1	-1.3	-14.4		x	
57	ZM621-15-3-1-2	-41.3	-43.8		x	

3.4 Discussion

3.4.1 Variation among topcrosses and relative yield

The observation that topcrosses involving tester Suwan-1 performed better than those involving tester ZM523 in the tropical-lowland environments for most traits seem to suggest that Suwan-1 was more adapted to this environment compared to ZM523, especially under DM conditions. Contribution of each tester to grain yield in topcrosses was demonstrated by the number of top-10 topcrosses yielding between 15% to 50% more grain than the trial mean (Table 3.5). Eight were crosses to tester Suwan-1 and only two to ZM523, further demonstrating the superiority of Suwan-1 over ZM523 in the tropical lowland environments. Seventeen topcrosses with DM scores below the trial mean (by 19% to 100%, data not shown) were Suwan-1 related, showing its superiority for DM resistance *inter se*. These results confirmed the findings of Kim *et al.* (2003) that Suwan-1 in topcrosses contributed to resistance *inter se*. Ajala *et al*. (2003) also found Suwan-1 to be resistant to DM. There were significant relationships of yield data and its components with the downy mildew scores indicating that downy mildew had a significant influence on yield, hence all new varieties should have some acceptable baseline resistance to the disease. Yield is a function of its components, that is, large number of plants, larger number of ears and low percentage incidence of DM. This is consistent with reports of significant yield losses of up to 90%, resulting from DM attack (Bonde, 1982; CIMMYT-MaizeDoctor, 2010).

3.4.2 Combining ability effects

The genetic variation due to the lines (GCA_L) and testers (GCA_T) were significantly different across the three environments for DM resistance, grain yield and all other traits except plant aspect for lines and plant and ear aspects for testers. This implied that the additive effects were important for these traits; hence selection procedures such as recurrent selection for GCA in the base populations could be effective to improve these traits. Conversely, non-significance of the SCA effects suggested that the non-additive gene effects were not important in determining resistance to downy mildew, grain yield and other traits. These findings, especially for DM resistance and yield components are consistent with previous studies, although most of the studies reported significant non-additive gene action for DMR in addition to additive gene action for the traits (Jinahyon, 1973; Frederiksen and Ullpstup,

1975; Orángel and Borges, 1987; Kim *et al*., 2003). However, for grain yield, the significance of both GCA and SCA is consistent with reports in the literature. Significance of SCA for yield indicated that the trait could be improved through development of hybrids between complementary inbred lines. Further, significance of environment x cross interaction effects for grain yield, environment x tester interactions for DM scores and environment x line x tester for DM scores, yield and yield components implied that topcrosses had different interactions with environments. Therefore, hybrids should be tested widely to identify the best hybrids for different ecologies. However, the non-significance of the line x environment interaction effects for DM seemed to suggests that lines had similar interaction with environments, but the testers reacted differently in different environments hence they were mainly responsible for explaining the differential responses of topcrosses across environments. Nevertheless, these results were consistent with earlier reports of significant genotype by environment interactions for DM resistance and other traits in maize (Kim *et al*., 2003; Lee *et al*., 2003).

The GCA and SCA effects constitute the major criteria for rapid advancing of inbred lines and hybrids, respectively, in a breeding programme. The current study identified lines 2, 3, 19 and 42, that displayed significant negative GCA effects for DM incidence scores, which was desired because they contributed to resistance in their crosses. Kim et al. (2003) in Nigeria also observed lines with good GCA for DM. The same lines plus line 45, except line 3, also showed positive significant GCA estimates for grain yield, which was desired, further confirming their suitability for inclusion in the lowland maize breeding programme in Mozambique. These lines can be used directly for hybrid production and in the case of Mozambique where three-way hybrids are the preferred cultivars; these lines can be used as males and the single cross hybrids with high level of heterosis as females. Based on the GCA effects for DM resistance and grain yield, lines 2, 3, 19, 42, 45 and 57 are potential candidates for the future maize genetic improvement programmes focused on hybrids and synthetics development for the farmers in tropical lowland areas where DM is a significant constraint. The desirable GCA effects for the two traits also imply that simultaneous improvement for downy mildew resistance and grain yield can be achieved. Further, since earliness is an important drought escape trait in the tropical lowland environments in Southern Africa (Magorokosho *et al.,* 2003); lines 3, 30 and 45 which showed this trait are also valuable.

The contrasting GCA effects displayed by the two testers (ZM523 and Suwan-1) for DMR, grain yield and its components indicated that the testers were different. For DM, ZM523 showed higher susceptibility in the tropical lowland conditions that can be explained by the fact that it was bred under mid-altitude conditions in the absence of DM at CIMMYT, in Harare, Zimbabwe as opposed to Suwan-1 with a known DMR (Kim *et al.*, 2003). Tester Suwan-1 was able to discriminate lines on more traits based on GCA effects than tester ZM523 implying that Suwan-1 was an ideal tester for the tropical environment under DM pressure as described by Russell (1961). In this regard, Suwan-1 or lines developed from this synthetic like KUI1414 from West Africa (Kim *et al.*, 2003) might be used as testers to develop hybrids for similar tropical lowland environments in Southern African. The observation that line 5 showed positive GCA value, while the cross 5 x Suwan-1 exhibited a negative SCA value for DM resistance, suggested that line 5 only showed resistance reaction *inter se* in cross combination with non-additive effects conferring resistance. The same explanation can be applied to line 1 for grain yield but not to line 45 that combined both additive and non-additive genes for grain yield. Line 45 exhibited a positive GCA value and cross 45 x Suwan-1 displayed positive SCA effects for grain yield.   Overall, this indicated that productive hybrids, which are high yielding and resistant to downy mildew, could be obtained not only by crossing good x good GCA lines for both traits but also by crossing bad x good GCA lines with complementary traits.

3.4.3 Heterotic orientation and heterotic grouping

The topcrosses measure the relative behaviour of the lines under evaluation (Narro, 2003). It was possible to identify some possible heterotic patterns among lines in the current study using the two broad genetic basis testers. Lines were assigned to two heterotic groups based on the magnitude of their SCA values in crosses with the testers, because lines in crosses displaying low magnitude SCA effects are assigned to the same heterotic group, whereas those displaying high magnitude SCA effects belong to different heterotic groups (Vasal *et al.,* 1992).   Thus, two heterotic groups were identified: Group Z constituted by ZM523 oriented lines (1, 5, 8, 10, 25, 45, 47 and 48) and group S comprising Suwan-1 oriented lines (2, 3, 13, 19, 30, 32, 42, 51, 55 and 57).

Lines 2, 30 and 42, which displayed positive heterosis with both testers, could produce good hybrids if crossed with inbred lines derived from both testers. Lines 8, 10, 25, 45, and 48,

which exhibited heterosis with tester Suwan-1 and line 19 with tester ZM523, could form high yielding hybrids with lines derived from the testers' respective heterotic groups. The remaining lines did not exhibit heterosis with any of the two testers (Table 3.7); suggesting that they might not form good hybrids with lines that are derived from both heterotic groups. Unless another heterotic tester is identified, these lines might not be useful for the lowland breeding programme in Mozambique. However, there is a danger of discarding good lines when a wrong tester is used. For this reason, these and other lines were used in a diallel to identify possible heterotic patterns among them (see Chapter 4). In theory the lines could be assigned to the group they were more inclined based on the direction of their SCA effects, but in practice, they would be discarded. In future, identifying more testers would enhance the chances of identifying hybrids with lager specific combining ability effects.

3.5 Conclusions

The following conclusions were drawn:

1. GCA effects were significant for downy mildew scores, suggesting that additive gene effects were responsible for controlling the resistance to downy mildew; thus selection could be effective to improve DM resistance in these lines;

2. The SCA effects were not significant indicating that, in general, the non-additive gene effects did not play a major role in conferring resistance to downy mildew in the topcrosses;

3. Both testers were able to discriminate the lines based on their GCA for downy mildew scores and grain yield, implying that the testers can be successfully used in the early stages of a breeding programme to evaluate lines for GCA effects, separate them into heterotic patterns or to create high yielding topcrosses. However, Suwan-1 tended to have a better discriminating power than ZM523 in these test environments;

4. On the basis of SCA data, ten lines were grouped into the S heterotic group while eight could be assigned to the Z heterotic group;

5. Based on heterosis, lines 2, 30 and 42, which displayed positive heterosis with both testers for grain yield, were allocated to SZ-group while lines 8, 10, 25, 45, 48, which exhibited positive heterosis with Suwan-1, were classified into the Z-group. Line19, which showed positive heterosis with ZM523 was assigned to the S-group;

6. The lines 42, 8, 25 and 30 that showed superior performance for grain yield and DMR have the potential to be used as parental materials in developing both DMR and high grain yield performing hybrids for further testing across the tropical lowland environments.

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CHAPTER 4

Gene Action for Grain Yield, Heterosis and Heterotic Grouping of Tropical Lowland Elite Maize inbreds

Abstract

Heterosis has contributed to maize yield improvement worldwide. It can therefore be exploited to enhance production of the staple food without creating pressure on land in sub-Saharan Africa. The objective of this study was to determine heterosis, heterotic grouping, heterotic patterns and gene action for grain yield among tropical lowland maize germplasm. Crosses from an 11 x 11 half-diallel were evaluated in a simple 6 x 10 α-lattice design over six environments in Mozambique. General combining ability and specific combining ability effects were highly significant for grain yield, but with greater contributions from SCA effects. This implied that genes with both additive and non-additive effects were significant for grain yield but the non-additive effects were predominant. Mid-parent heterosis ranged between -46.0% and 228.3%, and heterosis with testers, Z523 (Z) and Suwan-1 (S), ranged between -12.5% and 46.9%. Based on the heterosis data, three heterotic groups were identified as S, Z and SZ. Six lines that did not show positive heterosis when crossed with Suwan-1 were allocated to S group, and one that did not exhibit positive heterosis with ZM523 was placed in Z group; while two lines that displayed similar level of positive heterosis with both testers were classified in SZ group. Exceptional heterotic patterns, one between S and Z groups (LP19 x LP37), and five within the S group (LP20 x LP55, LP23 x LP55, LP20 x LP23, LP19 x LP23 and LP55 x FU26) were identified. While superiority of single crosses over the testers was confirmed, there was not any apparent advantage of single cross hybrids over top crosses. A top cross, LP55 x ZM523 was ranked first, while LP55 x Suwan-1 was ranked nine and four others displayed ≥110% relative yield. Results have implications for the breeding programmes and recommendations of hybrids in similar sub-Saharan ecosystems.

Keywords: gene action, grain yield, heterosis, heterotic grouping, heterotic patterns, maize hybrids, open-pollinated varieties, top cross varieties.

4.1 Introduction

The maize production trends worldwide showed increased yields over the years. In some countries, such as Mozambique, and many others in Sub-Saharan Africa and parts of Latin America and Asia, increased production has been achieved by expansion of maize growing areas. On the contrary, in many industrialized countries, yield increase is mainly attributed to advances in maize research and farmers' access to improved maize technology (Dowswell *et al*., 1996) in the form of hybrids and improved agronomic management. Duvick (2001) considered that the dramatic maize yield increases were observed since breeders started moving away from open-pollinated varieties (OPVs) to double-cross and later to single cross hybrids. It has long been demonstrated that such hybrids are more productive than open pollinated varieties. Most breeders and agronomists are unanimous in considering successful exploitation of heterosis and improved crop management practices as the most important aspect that contribute to maize yield improvement over the years (Duvick, 1992, 2001, 2005; Xia *et al*., 2004). Thus, promotion of hybrids is one way of improving maize grain yield without expanding on land area in Mozambique and other lowland tropical environments where there is already a lot of pressure on land with serious consequences on social and economic stability.

Heterosis is the quantitative measure of superiority of performance of F_1-hybrids over their parents (Stuber, 1994). Paschold *et al*. (2009) more precisely defined heterosis as the phenomenon that describes the superiority of performance of highly heterozygous F_1-hybrids in relation to the average (mid-parent) performance of their genetically distinct homozygous parents. The manifestation of heterosis in maize has been reported since the early 1900s based on the findings of Shull (Tollenaar *et al*., 2004). The level of heterosis manifestation in the F_1-hybrid is strongly associated with genetic diversity of the parental lines. Generally, crosses between inbred lines from groups with divergent genetic backgrounds are expected to exhibit high levels of heterosis than those among lines from genetically more related groups (Hallauer *et al*., 1988).

The heterotic group is defined as a group of related or unrelated genotypes, which display similar combining abilities when crossed with genotypes from other groups. A heterotic pattern is defined as a pair of heterotic groups that show high heterosis in their crosses (Carena and Hallauer, 2001). Classification of germplasm into different heterotic groups and

identification of heterotic patterns between groups, within any breeding programme is one way of exploiting hybrid vigour and efficiently managing the germplasm. For example, in combination or pedigree breeding, breeders would cross inbred lines from the same group and then inbreed to generate new lines quickly. Then, they would cross these lines with testers from the other groups to generate hybrids for testing and release. Currently, there are no defined heterotic groups for the lowland breeding programmes especially in east and Southern Africa, and in particular Mozambique.

There are several heterotic groups on maize breeding programmes worldwide. In temperate maize for example, heterotic groups were established empirically by relating the heterosis observed in crosses with the origin of the parents included in the crosses (Hallauer *et al*., 1988). Therefore, generalized cross patterns were established between the flint x dent type germplasm. According to this classification system, two groups of maize germplasm have been used in the USA Corn Belt. One is the Southern dent with tall plant stature, full season, bigger tassel and ears, and the other one is the north flint with short stature. Generally, the USA system is built around the B73 and Mo17 orientation even though there are other smaller groups.

Diallel analysis or molecular markers (Hallauer and Miranda, 1988) can also determine heterotic patterns. When inbred lines are crossed among each other, those with a positive SCA effect generally indicate that they belong to different heterotic groups, and lines in the same heterotic group tend to exhibit negative SCA effects when crossed together (Vasal *et al*., 1992). However, heterotic groups are generally broad classes that are arbitrary; there could be exceptions where materials in the same group show positive SCA and exceptional heterosis. Each breeding programme can create its own maize heterotic grouping system that is convenient for use by the programme. The system can be based on a broad classification like the one used by the International Maize and Wheat Improvement Center (CIMMYT) for the convenience of managing their various genetic backgrounds. There are also narrow systems, with five to nine heterotic groups, which have been used successfully in the national breeding programmes of Zimbabwe, South Africa and China (Gevers and Whythe, 1987; Olver, 1988; CIMMYT, 2001; Mickelson *et al.,* 2001; Zhang *et al*., 2002; Derera, 2005). The lack of an effective heterotic grouping system has influenced negatively the efficiency of breeding maize hybrids in Mozambique and other developing countries.

In Mozambique, the national maize improvement programme introduced hybrid development, a component aimed to create high yielding hybrids adapted to the major maize agro-ecological zones. Thus, a lot of inbred lines and crosses were created since the programme was established in 1975 (Bokde, 1980; Bias, 2000; Fato *et al*., 2005). However, some important aspects for a successful hybrid oriented programme, like general combining ability, specific combining ability, heterotic groups and patterns were not considered as priority. Knowledge of combining ability and gene action is crucial in devising an appropriate breeding strategy. Although it is known that the maize germplasm used in the programme is from different sources, there is a need to re-organize the germplasm and the diversity into manageable groups, since the breeding emphasis is shifting from population improvement towards hybrid development.

The need to improve maize yield without expansion of area, which creates other problems related to land availability cannot be over-emphasized. Despite increased demand for improved seed, especially hybrids in recent years (Fato *et al.*, 2004), the public national breeding programme released only one three-way cross hybrid in 2008 (Hluvukane). The few maize hybrid varieties available in the local market were introduced from neighbouring countries, such as Zimbabwe (SC513), South Africa (PAN67 and QS7707) and Malawi (MH18), but these were not bred for the lowland environment. In many cases, farmers in Mozambique grow these hybrids in areas where they are not adapted, resulting in low yields.

One of the major production constraints facing most farmers in Southern Africa is unavailability of improved seed. In Mozambique, for example, only about 30% of maize seed is improved, and only about 4% of that is hybrid seed. While there might be obvious advantages of promoting hybrids over the current open pollinated varieties, there is need to validate the yield advantage of hybrids under the tropical lowland conditions in the target environments. This has implication on the cost of seed in relation to the yield gain by growing hybrids in the small-scale commercial and especially the subsistence sector in developing countries. There is also need to test whether to move from OPVs to topcrosses before moving to the single crosses based on the successful model of the United States and other countries.

The objectives of this study were to determine: (i) gene action for grain yield and associated traits, (ii) heterosis (iii) heterotic grouping (iv) heterotic patterns among Mozambican tropical maize germplasm, and (v) relative performance of topcrosses to the conventional single cross hybrids. This will provide the initial step in devising a prolific and appropriate maize hybrid oriented breeding programme for the tropical lowland environment. The information generated will be useful for application in other similar environments in tropical lowlands.

4.2 Materials and Methods

4.2.1 Germplasm

Eleven tropical lowland adapted maize parents were employed to generate 55 F_1 hybrids in an 11 x 11 half-diallel mating scheme. Eight lines were elite white grain endosperm lines, developed by the Maize National Research Program at Instituto de Investigação agrarian de Moçambique (IIAM), in Mozambique. They were derived from adapted populations and some populations that were introduced from CIMMYT and International Institute of Tropical Agriculture (IITA) programmes in Zimbabwe and Nigeria, respectively. Two parents were open pollinated synthetic varieties (SOPVs), ZM523 and Suwan-1-synthetic, introduced from CIMMYT in Zimbabwe and IITA in Nigeria, respectively (Table 4.1). Both SOPVs are potential testers in lowland tropical environments and were designated Z and S testers. There were included in the diallel cross to determine heterotic orientation of the elite eight inbred lines, and to check the advantage of potential hybrids over the OPVs and topcrosses. Table 4.1 gives a summary of the features of the parents.

Table 4.1 Pedigree and origin of maize germplasm used to generate diallel crosses

	Lines	Pedigree	Source population	Maturity group[†]
1	LP02	Mex 8049-230-2-3-1-2-1-1-X-B	Mex 8049	Intermediate
2	LP19	Matuba-6-2-1-1-1-1-X-B	Matuba	Early
3	LP20	Matuba-19-1-3-1-1-1-X-B	Matuba	Intermediate
4	LP23	Tzi- 4	Tzi- 4	Intermediate
5	LP31	EV8749BC6- 130-1-1-1-1-1-X-B	EV8749	Early
6	LP37	Pop44-1-1-1-4-6-6-X-B	Population 44	Early
7	LP55	H8321/ 21-8-1-1-3-1-3-X-B	H8321	Intermediate
8	LP63	H8321/ 21-28-1-1-2-4-1-X-B	H8321	Early
9	FU26	Suwan-1-synt -26-2-4-1	Suwan-1-synt	Early
10	ZM523	Synthetic of A x B lines at CIMMYT	ZM523	Early
11	Suwan-1	n/a	Suwan-1	Early

[†]Early =Anthesis date (60 to 65) days;
Intermediate = anthesis date (65 to 70) days;
Late = anthesis date more than 70 days;
n/a = information not known.

4.2.2 Research sites and test environments

Fifty-five F_1 crosses, two open pollinated synthetics (ZM523 and Suwan-1) and three hybrid checks (PAN67, Olipa, Hluvukane) were evaluated at Umbeluzi (in 2007/08 and 2008/09 major seasons; Chókwè (during 2008 off-season and 2008/09 major season); Sussundenga and Angónia (in 2008/09 major season). A single cross hybrid N3 x MP72 was used as a check to replace PAN67 in all environments in 2008/09 evaluations. Characteristics of each the six experimental environments are given in Table 4.2.

4.2.3 Experimental design and field management

A 6 x 10 alpha lattice (0.1) design with two replications was used to evaluate the 60 treatments consisting of 55 crosses and five check varieties under the six environments. The inbred parents together with other lines were evaluated, separately, in a 5 x 5 alpha lattice design with three replications at Chokwe in 2008 and 2009, and at Umbeluzi in 2008. Two-row plots of 5 m long were used in all environments except Umbeluzi 2007/08 evaluation, where one-row plots were used. The spacing of 0.25 m between stations and 0.80 m between rows was used in all environments. Two to three seeds per station were hand planted in each plot and three weeks after plant emergence, the crop was thinned to one plant per station, resulting in a total plant population of about 50,000 plants ha^{-1}. Fertilizer NPK (12:24:12) and urea were applied at planting and as top-dressing at four and eight weeks after plant emergence, respectively, in all environments. The total amount of fertilizer applied was 170 kg ha^{-1} N- 33 kg ha^{-1} P- 44 kg ha^{-1} in all environments. The insecticide (Supermetrine) was applied twice in all environments to control stem borer. The first application was two to four weeks after emergence and the second six to eight weeks after emergence. Herbicides and supplementary manual weeding were used to control weeds. Data on yield and other agronomic traits was recorded in each environment on a whole plot basis. Anthesis date was determined as the number of days when 50% of plants in a plot were shedding pollen, silking date, when 50% of plants in a plot had emerged silks. The total number of ears in the plot was determined at harvest. Grain yield data was adjusted to 12.5% grain moisture content.

4.2.4 Statistical Analysis

All the quantitative data were subjected to analysis of variance using the PROC GLM procedures in SAS computer software version 9.1. All quantitative data were treated to a Griffing's (1956) method IV analysis using the Diallel-SAS05 programme (Zhang *et al.*, 2005) in SAS 9.1. The relative contribution of GCA and SCA effects to the total genetic effect for each trait was computed as percentage of GCA and SCA sum of squares over sum of squares of the F_1 crosses. The relative importance of GCA and SCA in determining progeny performance was also assessed by estimating the ratio between GCA (σ_g^2) and SCA (σ_s^2) variances according to Baker's (1978) formula: $2*\sigma_g^2*(2*\sigma_g^2 +\sigma_s^2)^{-1}$. Standard heterosis (SH) and mid-parent heterosis (MPH) were calculated as SH = $((F_1- MT)/ MT) * 100$, where MT= Mean of S and Z testers, best hybrid or trial mean, F_1 = F_1 hybrid mean performance. Mid-parent heterosis (MPH) was determined as follows: MPH = $((F_1 – MP)/ MP) * 100$, where MP = $(P_1 + P_2)/2$ (mid parent mean), in which P_1 and P_2 are the means of the parent inbreds. Phenotypic correlations (r) were performed for grain yield ranking of genotypes over the six environments using the PROC CORR procedure in SAS.

Table 4.2 Sites and testing environments for single cross hybrids

Season	Site	Altitude (masl)	Latitude (degrees)	Longitude (degrees)	SADC Maize Mega-environments	Annual rainfall (mm)	Evapo- trans piration (mm)	Code	Environment designation
2007/08	Umbeluzi	12	26° 03'	32° 23'	lowland tropical dry	700	1500	E - 1	Rainfed plus supplemental irrigation
2008	Chókwè	34	24° 32'	33° 00'	lowland tropical dry	600	1700	E - 2	Irrigation
2008/09	Umbeluzi	12	26° 03'	32° 23'	lowland tropical dry	700	1500	E - 3	Rainfed plus supplemental irrigation
2008/09	Chókwè	34	24° 32'	33o 00'	lowland tropical dry	600	1700	E-4	Rainfed plus supplemental irrigation
2008/09	Sussundenga	635	19° 20'	33° 13'	Mid altitude humid hot	1100	1200	E - 5	Rainfed
2008/09	Angónia	1330	14°22'	34° 36'	Mid altitude humid hot	1200	1300	E - 6	Rainfed

masl = meters above sea level:

4.3 Results

4.3.1 Variation among F_1 crosses and gene action

Highly significant (P ≤ 0.01) variation was observed among environments, crosses, GCA and SCA, environment x crosses, environment x GCA, and environment x SCA for grain yield and all other traits (Table 4.3). The GCA sum of squares contribution to the crosses was significantly less than that of the SCA effects for grain yield, but they were predominant for the grain yield components (Table 4. 3).

Table 4.3 Mean squares of hybrids for grain yield and associated traits across environments

		Mean Square				
			Anthesis			Ears per
		Grain yield	date	Silking date		plot
Source	D.f	tha^{-1}	days	days	D.f	#
Env.	3	2.4543**	0.0709**	0.0851**	2	3.7011**
Rep (Env.)	4	0.1306	0.0092	0.0132	3	0.0341
Cross	54	0.0353**	0.0008**	0.0008**	54	0.0317**
GCA	10	0.0615**	0.0030**	0.0033**	10	0.0332**
SCA	44	0.0293**	0.0004**	0.0004**	44	0.0321**
Env. x Cross	162	0.0250**	0.0003**	0.0005**	108	0.0116**
Env. x GCA	50	0.0191	0.0000	0.0000	50	0.0000
Env. x SCA	220	0.0141	0.0001	0.0002	220	0.0024
Error	216	0.1247	0.011	0.0142	162	0.0739
Cross mean		3.51	61.2	57.49		24.51
R^2 (%)		80.49	91.88	90.68		92.21
CV (%)		20.37	0.68	0.81		5.39
GCA (SS) (%)		32.2	75.0	75.0		17.54
SCA (SS) (%)		67.8	25.0	25.0		82.46
$2*\sigma_g^2*(2*\sigma_g^2 + \sigma_s^2)^{-1}$		0.48	0.62	0.63		0.48

Data was transformed using Log10 (x + 1); *, ** Data is significant at P ≤ 0.05 and P ≤ 0.01, respectively.

4.3.2 Combining ability

Positive values for grain yield and number of plants per plot, and negative values for anthesis and silking dates are desirable. Positive significant GCA values were shown by lines LP23 (P ≤ 0.05) for grain yield and number of ears per plot and LP55 (P ≤ 0.01) for grain yield (Table 4.4). Highly significant and negative GCA values (P ≤ 0.01) for anthesis and silking date were exhibited by Suwan-1. Only LP37 x LP19 cross exhibited significant positive (desirable) SCA effect (P ≤ 0.05) for grain yield, while the other cross Suwan-1 x Fu26 exhibited negative SCA effects (data not shown). The cross LP55x LP23 had significant positive SCA for anthesis and silking dates, while Suwan-1 x FU26 displayed significant negative SCA for number of ear per plot.

Table 4.4 General combining ability estimates of parent inbred lines for grain yield and other traits over four environments.

Line	Name	Mean yield[†]	Grain yield	Anthesis date	Silking date	Ears per plot
		t ha^{-1}	tha^{-1}	days	days	#
1	LP02	1.3	-0.035	0.006*	**0.008***	0.022
2	LP19	1.8	-0.013	-0.005	-0.005	-0.005
3	LP20	1.4	0.015	-0.001	0.000	0.005
4	LP23	1.5	0.037*	0.017**	0.016**	0.049*
5	LP31	2.0	0.000	-0.001	0.001	-0.010
6	LP37	1.2	-0.004	-0.001	-0.002	0.008
7	LP55	1.3	0.057**	.0.000	0.002	0.029
8	LP63	1.8	-0.044*	0.001	0.000	-0.019
9	FU26	2.0	-0.009	-0.004	-0.005	-0.033
10	ZM523	3.2†	0.009	-0.003	-0.002	-0.026
11	Suwan-1	3.6†	-0.013	-0.009**	-0.012**	-0.02
SE			**0.022**	**0.003**	**0.004**	**0.024**

*, ** Data is significant at P ≤ 0.05 and P ≤ 0.01, respectively.
†Grain yield data of parental lines evaluated in three environments separately from diallel crosses. †Grain yield of testers evaluated together with diallel crosses.

4.3.4 Standard heterosis and mid-parent heterosis

Twenty-four F_1 crosses had grain yield superior to the trial mean; 16 to the best commercial check hybrid (QS7707 released with name of Olipa in Mozambique); 35 to the mean of all checks; 41 to the synthetic ZM523 and 22 to Suwan-1 (Table 4.5). Mid-parent heterosis estimates were computed using grain yield means from 11 parents evaluated over three environments (Table 4.4). The average mid-parent heterosis estimates ranged from - 46.7% (FU26 x Suwa-1) to 228.3% (LP20 x LP55). Among single crosses heterosis varied from 48.3% to 283.3% and for topcrosses from -46.7% and 107.7%. In general, there were 22 single cross hybrid combinations and one topcross that showed mid-parent heterosis exceeding 100% (Table 4.5).

Table 4.5 Mid-parent and standard heterosis for grain yield

Entry	Name	Type	MPH	RY to trail mean	RY to best Check	RY to check mean	RY to ZM523	RY to Suwan-1
			(%)	(%)	(%)	(%)	(%)	(%)
54	9x11	SC	-46.7	42.5	39.0	44.8	46.3	41.0
7	1x8	SC	51.4	66.4	61.0	70.0	72.4	64.1
42	6x8	SC	74.8	74.2	68.2	78.3	81.0	71.7
1	1x2	SC	74.8	76.4	70.1	80.5	83.3	73.8
37	5x8	SC	48.3	78.9	72.4	83.2	86.0	76.2
44	6x10	TC	27.0	79.9	73.4	84.3	87.2	77.2
32	4x9	SC	79.2	88.5	81.3	93.3	96.6	85.5
4	1x5	SC	91.8	88.9	81.6	93.7	96.9	85.9
6	1x7	SC	140.5	89.2	81.9	94.1	97.3	86.2
53	9x10	TC	22.0	89.9	82.6	94.8	98.1	86.9
18	2x10	TC	27.6	90.7	83.3	95.6	98.9	87.6
19	2x11	TC	17.8	90.7	83.3	95.6	98.9	87.6
35	5x6	SC	103.3	91.4	83.9	96.3	99.7	88.3
24	3x8	SC	105.2	91.7	84.2	96.7	100.1	88.6
16	2x8	SC	83.6	92.8	85.2	97.8	101.2	89.7
51	8x10	TC	30.3	92.8	85.2	97.8	101.2	89.7
25	3x9	SC	97.3	93.1	85.6	98.2	101.6	90.0
21	3x5	SC	98.2	93.5	85.9	98.6	102.0	90.4
3	1x4	SC	137.4	94.9	87.2	100.1	103.6	91.7
2	1x3	SC	151.9	95.3	87.5	100.5	104.0	92.1
5	1x6	SC	174.7	97.8	89.8	103.1	106.7	94.5
39	5x10	TC	33.2	98.1	90.1	103.5	107.1	94.8
13	2x5	SC	86.4	98.9	90.8	104.2	107.8	95.5
22	3x6	SC	171.3	98.9	90.8	104.2	107.8	95.5
26	3x10	TC	51.7	98.9	90.8	104.2	107.8	95.5
27	3x11	TC	39.1	98.9	90.8	104.2	107.8	95.5
46	7x8	SC	125.6	99.2	91.1	104.6	108.2	95.9
17	2x9	SC	87.6	99.6	91.5	105.0	108.6	96.2
8	1x9	SC	115.5	99.9	91.8	105.4	109.0	96.6
11	2x3	SC	126.8	101.0	92.8	106.5	110.2	97.6
45	6x11	TC	47.7	101.7	93.4	107.3	111.0	98.3
50	8x9	SC	92.4	102.4	94.1	108.0	111.7	99.0
34	4x11	TC	41.3	103.5	95.1	109.1	112.9	100.0
31	4x8	SC	122.4	104.2	95.7	109.9	113.7	100.7
41	6x7	SC	196.9	106.0	97.4	111.8	115.6	102.4
52	8x11	TC	38.4	106.7	98.0	112.5	116.4	103.1
15	2x7	SC	145.3	107.4	98.7	113.3	117.2	103.8
55	10x11	TC	11.2	108.1	99.3	114.0	118.0	104.5
36	5x7	SC	134.4	108.9	100.0	114.8	118.8	105.2
33	4x10	TC	62.7	109.6	100.6	115.5	119.5	105.9
43	6x9	SC	144.4	109.9	101.0	115.9	119.9	106.2
29	4x6	SC	185.8	110.3	101.3	116.3	120.3	106.6
9	1x10	TC	73.1	111.4	102.3	117.4	121.5	107.6
10	1x11	TC	59.0	111.7	102.6	117.8	121.9	107.9
40	5x11	TC	40.8	112.1	102.9	118.2	122.3	108.3
28	4x5	SC	129.3	113.1	103.9	119.3	123.4	109.3
49	7x11	TC	65.9	116.7	107.2	123.1	127.3	112.8
38	5x9	SC	110.1	117.1	107.5	123.4	127.7	113.1
47	7x9	SC	154.1	118.1	108.5	124.6	128.9	114.1
12	2x4	SC	158.4	120.6	110.8	127.2	131.6	116.6
30	4x7	SC	202.6	121.3	111.4	127.9	132.4	117.2
20	3x4	SC	198.1	121.7	111.8	128.3	132.8	117.6
23	3x7	SC	228.3	124.6	114.4	131.3	135.9	120.4
14	2x6	SC	212.3	132.1	121.3	139.2	144.1	127.6
48	7x10	TC	107.7	133.8	122.9	141.1	146.0	129.3
56	ZM523	SOPV		91.7	84.2	96.7	100.0	88.6
57	Suwan-1	SOPV		103.5	95.1	109.1	112.9	100.0
58	PAN67	TWC		87.2	80.1	91.9	95.1	84.2
59	Olipa	QPM-TWC		108.9	100.0	114.8	118.8	105.2
60	Hluvukane	TWC		83.0	76.2	87.5	90.5	80.2
Mean yield		**(t/ha)**		**3.5**	**3.8**	**3.3**	**3.2**	**3.6**

MPH, mid-parent heterosis; SOPV=open-pollinated synthetic variety; TWC, three way cross; QPM, quality protein maize; Standard heterosis was calculated as relative yields (RY) to trial men, best check, mean of checks and ZM523 and Suwan-1 means; SC, single cross and TC, topcross.

4.3.5 Heterotic grouping and heterotic patterns

Only a few crosses showed significant SCA effects therefore heterotic groups were assigned based on better parent heterosis, which is yield (%) relative to the tester varieties, ZM523 (Z) and Suwan-1 (S). Both varieties ZM523 and Suwan-1 were included in the diallel to confirm their heterotic divergence and to help discriminate the nine lines into different heterotic groups. The cross between the two testers displayed heterosis of 11.0%. Twenty-four crosses displayed relative yield ≥103%. Twenty-nine crosses did not show (relative yield <100%) any heterosis.The following lines did not display positive heterosis with tester S: LP02, LP19, LP20, LP23 and LP37. The line LP31 exhibited highly negative heterosis with Z tester. The lines LP37 and LP63 displayed similar levels of heterosis with both S and Z testers (Table 4.6). The heterotic patterns identified are listed for the top 10 hybrids (Table 4.7). Among the top-10 hybrid yielders, four crosses were between lines from S and Z- groups (LP55 x ZM523, LP19 x LP37, LP55 x FU26 and LP23 x LP31) and six between lines within S-group (Table 4.7).

Table 4.6 Heterotic grouping of the nine elite inbred lines based on better parent heterosis (%)

Lines		Pedigree	Testers		Heterotic grouping[†]
			ZM523 (Z)	Suwan-1 (S)	
1	LP02	Mex 8049-230-2-3-1-2-1-1-X-B	21.9	8.3	S
2	LP19	Matuba-6-2-1-1-1-1-X-B	0.0	-11.1	S
3	LP20	Matuba-19-1-3-1-1-1-X-B	9.4	-2.8	S
4	LP23	Tzi- 4	18.8	0.0	S
5	LP31	EV8749BC6- 130-1-1-1-1-1-X-B	6.3	8.3	S/Z
6	LP37	Pop44-1-1-1-4-6-6-X-B	-12.5	0.0	Z
7	LP55	H8321/ 21-8-1-1-3-1-3-X-B	46.9	13.9	S
8	LP63	H8321/ 21-28-1-1-2-4-1-X-B	3.1	2.8	S/Z
9	FU26	Suwan-1-synt -26-2-4-1	0.0	-58.3	S

[†]S= Suwan-1, Z= ZM523 and S/Z = Suwan-1/ ZM523 heterotic grouping.

4.3.6 Relative yield of single cross over topcross hybrids and open pollinated varieties

Among the top 10 hybrids, there were two top crosses and eight single crosses with superior grain yield to the trial mean with standard heterosis varying from 13.1% to 33.8%. In

addition, there were four top crosses among the top 16 varieties with 10% yield advantage over the trial mean (Table 4.7). The topcross between line LP55 and synthetic ZM523 (7 x 10) gave the highest grain yield among the top 10 varieties. It yielded up to 33.8% more than the trial mean; 22.9% to the best hybrid check; 41.1%, to the overall check mean and surpassed open pollinated synthetic varieties ZM523 and Suwan-1 by 46.0% and 29.3%, respectively. A cross between lines LP19 and LP37 (2 x 6) was superior among single crosses, surpassing the trial mean by 32.1%; the best hybrid check mean by 21.3%; the check mean by 39.2% and ZM523 and Suwan-1 variety means by 41.1% and 27.6%, respectively. Both the top crosses and single cross hybrids were generally superior to the open pollinated varieties Suwan-1 and ZM523, which did not feature in the top-16. Single crosses dominated the top 16 variety ranking because there were 10 single crosses versus six top crosses in the top 16 (Table 4.7). The top 16 ranking consisted of varieties with about 10% yield advantage over the trial mean, about 16.0% over the mean of checks, 20% over ZM523 and more than 6% better than Suwan-1.

Table 4.7 Top-16 maize hybrid yielders across four environments

Entry		Hybrid type[†]	Relative yield trial mean	Relative yield to best check hybrid mean	Relative yield to overall check mean	Relative yield to mean of ZM523	Relative yield to mean of Suwan-1
			(%)	(%)	(%)	(%)	(%)
Top 10 grain yield hybrids with SH ≥ 12% to trial mean							
LP55 x ZM523	7x10	TC	133.8	122.9	141.1	146.0	129.3
LP19 x LP37	2x6	SC	132.1	121.3	139.2	144.1	127.6
LP20 x LP55	3x7	SC	124.6	114.4	131.3	135.9	120.4
LP20 x LP23	3x4	SC	121.7	111.8	128.3	132.8	117.6
LP23 x LP55	4x7	SC	121.3	111.4	127.9	132.4	117.2
LP19 x LP23	2x4	SC	120.6	110.8	127.2	131.6	116.6
LP55 x FU26	7x9	SC	118.1	108.5	124.6	128.9	114.1
LP31 x FU26	5x9	SC	117.1	107.5	123.4	127.7	113.1
LP55 x Suwan-1	7x11	TC	116.7	107.2	123.1	127.3	112.8
LP23 x LP31	4x5	SC	113.1	103.9	119.3	123.4	109.3
Bottom hybrids with 10 to 12% heterosis to trial mean							
LP31x Suwan-1	5x11	TC	112.1	102.9	118.2	122.3	108.3
LP02 x Suwan-1	1x11	TC	111.7	102.6	117.8	121.9	107.9
LP02 x ZM523	1x10	TC	111.4	102.3	117.4	121.5	107.6
LP23 x LP37	4x6	SC	110.3	101.3	116.3	120.3	106.6
LP37 x FU26	6x9	SC	109.9	101.0	115.9	119.9	106.2
LP23 x ZM523	4x10	TC	109.6	100.6	115.5	119.5	105.9

[†] TC = Topcross hybrids, SC= single cross hybrid.

4.3.7 Rank of genotypes and relationships between test environments

The relative ranking of genotypes by the environments is summarised in Table 4.8, while the actual ranks of the genotypes are shown in Table 4.9. Most of the ranks between the environments were not significant; only three environments showed a significant and positive relationship for ranking the hybrids. However, these relationships were generally weak (r < 0.5). The actual ranks of genotypes within environments and the mean rank and its standard deviation reflect the level of stability of the genotypes over the six environments.

Table 4.8 Environment correlations for grain yield of 60 genotypes

Environment	Umbeluzi 2007/08	Chókwè 2008	Umbeluzi 2008/09	Chókwè 2008/09	Sussundenga 2008/09	Angónia 2008/09
Umbeluzi 2007/08		0.187	0.234	0.103	-0.005	0.298*
Chókwè 2008			0.112	0.364**	-0.039	0.341**
Umbeluzi 2008/09				0.297*	0.119	0.175
Chókwè 2008/09					0.01	0.299*
Sussundenga 2008/09						-0.061
Angónia 2008/09						

Table 4.9: Mean ranking and standard deviation of ranking of 60 genotypes for grain yield over six environments

Entry	Type	Umbeluzi 2008A	Chókwè 2008B	Umbeluzi 2009A	Chókwè 2009A	Sussundenga 2009A	Angónia 2009A	Average Rank	Standard deviation rank
LP20 x LP23	SC	9	12	13	7	15	7	10.5	15.9
LP02 x LP23	SC	1	9	15	3	59	2	14.8	19.8
LP20 x LP55	SC	4	1	12	15	33	29	15.7	22.3
LP55 x Suwan-1	TC	24	35	1	47	3	1	18.5	17.7
LP23 x Suwan-1	TC	21	2	18	1	60	9	18.7	21.2
LP23 x LP55	SC	3	7	57	13	32	4	19.3	4.1
LP19 x LP37	SC	41	27	27	4	5	13	19.3	15.3
LP02 x Suwan-1	TC	5	16	37	6	23	37	20.7	9.2
LP31 x FU26	SC	30	4	36	8	35	15	21.5	15.0
ZM523 x Suwan-1	TC	28	36	16	25	22	6	22.2	14.3
LP55 x FU26	SC	31	25	19	21	19	26	23.7	17.5
LP55 x ZM523	TC	47	28	43	2	8	14	23.7	26.6
LP19 x LP20	SC	6	49	14	38	29	8	24.0	13.0
Olipa	QMM-TWC	45	44	5	19	11	22	24.3	3.2
LP23 x ZM523	TC	35	38	17	12	34	10	24.3	14.3
LP63 x Suwan-1	TC	22	43	7	10	31	34	24.3	17.8
LP37 x LP55	SC	17	23	40	35	13	20	24.5	14.9
LP02 x LP37	SC	2	46	8	46	6	39	24.5	22.6
LP02 x LP31	SC	13	21	6	18	55	35	24.7	12.3
LP37 x FU26	SC	8	33	10	56	1	45	25.5	3.3
LP02 x ZM523	TC	27	42	23	17	42	3	25.7	9.6
LP23 x LP37	SC	34	26	4	22	50	19	25.7	14.8
LP19 x LP23	SC	32	59	3	5	2	56	25.8	13.0
LP23 x LP63	SC	23	5	22	40	43	24	26.5	18.3
LP20 x FU26	SC	29	8	29	27	38	30	26.8	10.0
LP23 x LP31	SC	39	32	41	9	12	28	26.8	22.2
LP23 x FU26	SC	15	17	31	29	40	32	27.3	12.7
LP20 x ZM523	TC	10	3	49	59	28	16	27.7	13.5
Suwan-1	SOPV	12	10	44	43	17	41	27.8	15.3
LP31 x Suwan-1	TC	53	6	46	44	9	11	28.2	13.8
LP19 x FU26	SC	7	58	21	52	4	27	28.2	21.3
LP63 x FU26	SC	36	39	11	11	25	54	29.3	9.6
LP20 x Suwan-1	TC	18	52	34	34	18	25	30.3	12.7
FU26 x ZM523	TC	42	13	30	28	39	31	30.3	21.9
LP19 x LP55	SC	48	14	53	26	10	38	31.5	16.4
LP19 x Suwan-1	TC	37	22	33	24	54	23	32.2	22.0
LP02 x LP55	SC	20	48	38	23	53	17	33.2	14.2
LP02 x LP20	SC	54	45	9	53	20	18	33.2	16.6
LP55 x LP63	SC	44	11	25	16	51	52	33.2	17.5
LP37 x Suwan-1	TC	19	37	56	33	16	40	33.3	21.6
LP19 x LP31	SC	52	20	24	36	27	47	34.3	10.7
LP31 x LP55	SC	60	51	50	20	21	5	34.5	20.3
LP37 x LP63	SC	26	30	2	39	56	55	34.7	22.4
LP20 x LP37	SC	33	18	54	32	24	53	35.8	5.9
LP20 x LP63	SC	46	40	26	50	7	57	37.7	14.9
ZM523	SOPV	50	19	28	48	44	44	38.8	18.1
PAN67/ N3 x MP72[†]	TWC/SC	11	53	52	54	45	21	39.2	5.0
LP19 x ZM523	TC	38	54	35	51	14	49	40.2	18.7
LP31 x ZM523	TC	56	31	59	41	47	12	41.0	19.8
LP31 x LP37	SC	57	15	45	55	30	50	42.0	13.5
LP19 x LP63	SC	43	41	47	42	37	42	42.0	17.0
Hluvukane	TWC	51	47	20	49	52	36	42.5	14.2
LP31 x LP63	SC	16	34	55	57	36	58	42.8	10.0
LP63 x ZM523	TC	59	57	39	31	26	46	43.0	14.6
LP02 x LP19	SC	40	56	42	14	57	51	43.7	10.3
LP20 x LP31	SC	55	29	51	37	46	48	44.3	12.4
LP37 x ZM523	TC	49	60	48	45	49	43	49.0	16.4
FU26 x Suwan-1	TC	25	50	60	60	58	60	51.8	18.9
LP02 x LP63	SC	58	55	58	58	48	59	56.0	16.7

[†] PAN67 was evaluated in 2008; N3 x MP72 was evaluated in 2009, SC = single cross, TC= topcross, SOPV = open pollinated synthetic variety, TWC= three way cross, QPM = quality protein maize.

4.4 Discussion

4.4.1 Gene action

The importance of both additive and non-additive gene action in controlling grain yield and its components was shown by the significance of both GCA and SCA effects for the traits. The relative contribution of GCA effects for grain yield and number of ears per plot was less than its contribution to anthesis and silking dates in crosses. This is consistent with other reports on grain yield and its components from studies on other germplasm (Derera, 2005; Gama *et al*., 2002). Based on the ratio between GCA and SCA variances for grain yield and number of ears per plot (0.5) it seems that non-additive effects were more important, with SCA accounting for 52% of the variance. However, based on the analysis of the sum of squares, SCA effects accounted for more than 67% of the variation in hybrids. This suggests a greater role of the genes with non-additive effects in determining overall grain yield and the number of ears per plot. The non-additive gene effects were reported to be more important for these same traits in other similar studies (Dhillon and Singh, 1977). However, other studies reported contrasting results where additive gene action was more important (Melani and Carena, 2005; Mickelson *et al*., 2001; Nass *et al*., 2002; Soengas *et al*., 2003). The different results from different studies can be attributed to the use of different germplasm sets and environments. In contrast, for anthesis and silking dates, the ratio of GCA to SCA variance was about 0.62 showing a greater preponderance of additive gene action. This was also supported by the larger sum of squares for GCA than SCA effects for the anthesis and silking dates. Therefore, non-additive effects for grain yield and its components can be exploited for development of hybrids and synthetic varieties from these lines. Preponderance of GCA effects for anthesis days and silking dates suggested that recurrent selection for GCA could be employed in the base populations from which lines were derived to obtain lines with early flowering dates.

4.4.2 Heterosis

The high values of MPH and SH confirmed superiority of single cross hybrids over the open pollinated synthetics and standard cultivars on the market, although a few topcrosses were as good as some single crosses. For example, topcross LP55 x ZM523's performance was similar to that of single cross LP19 x LP37 suggesting that topcrosses might be as good as

single cross hybrids. Pixley and Banziger (2001) reported contrasting results where conventional single cross hybrids produced an average of 20% more than topcross hybrids. Paterniani and Lonquist (1963) and Paterniani (1968) reported similar results with single cross heterosis values ranging from 4.0% to 136%. The lower MPH values for topcrosses compared to values of single crosses can be attributed to high levels of inbreeding depression, resulting in high heterosis in single crosses where both parents are inbred compared to top crosses where one of the parents is heterozygous. Silver and Miranda (2003) reported low heterosis in crosses involving synthetics which was attributed to the heterozygous nature of the synthetics. The exceptional topcrosses would be recommended for further testing in multi-environments trials in the lowland ecologies of Mozambique.

4.4.3 Heterotic grouping and patterns

Accurate characterization of line performance and line relationship to other germplasm is considered a major requirement for effective utilization of maize germplasm in breeding programmes. Based on the better-parent heterosis, (yield of inbreds relative to the testers ZM523 and Suwan-1), the nine inbred lines were classified into three heterotic groups S, Z and S/Z. A cross between the two testers (S x Z) displayed 11% heterosis suggesting that they were divergent as was expected. Heterosis has been used to classify inbred lines of maize into heterotic groups in other studies (Fan *et al*., 2009). The observation of higher levels of heterosis between the different lines and ZM523 (Z) than in crosses between the same lines with Suwan-1 (S) confirms the divergent heterotic orientation of lines in relation to the two testers. Based on this observation, most of the lines were fitted, therefore, in the S- group due to the high level of heterosis they exhibited with the opposite tester as explained by Vasal *et al*. (1992). Therefore, high levels of heterosis should be expected from lines belonging to S or S/Z group when crossed with the Z tester. Classification of the lines using SCA effects could not be done because only a few crosses had significant SCA effects. However, the use of heterosis alone was effective in achieving classification, but both heterosis and SCA effects are recommended to increase the precision of the classification (Menkir *et al*., 2004).

The classification of lines into the two major heterotic groups, S and Z, appeared to be related to the origin of the source population germplasm. Lines LP19 and LP20, which were selected from the same variety, Matuba, originating from DMR-SR population introduced

from IITA in the early 1980s, were classified into the S-group. Lines FU26 and LP23 selected from populations Suwan-1-synt and TZi-4, respectively, both populations originating from the IITA programme in Nigeria were classified into the S group. Line LP37, derived from the population 44 from the CIMMYT programme in Mexico, fell in the Z group of ZM523 tester, which is also from the CIMMYT maize programme in Zimbabwe. However, LP31, which was derived from the population EV8749 from CIMMYT, was classified in S/Z group because it showed similar heterosis performance with both testers though slightly higher heterotic with Suwan-1 (Table 4.7). The lines LP55 and LP63 were introduced at early generation from CIMMYT–Mexico. According to their pedigree, the two are sister lines and were recycled from the same hybrid. Line LP63 showed about 3% heterosis with both testers and was classified in S/Z- group. LP55 and LP02 also displayed higher heterosis with ZM523 than with Suwan-1 and were therefore classified into the S group. This was attributed to the fact that the two lines were also CIMMYT–Mexico population derivatives. The study confirmed reports by Vasal (1999) and Xia *et al*. (2005) that it was difficult to classify materials that originated from CIMMYT programme into distinct heterotic groups because the CIMMYT programme were mixing germplasm from several backgrounds as their emphasis was on population breeding (Mickelson *et al*., 2001). It is only recently that CIMMYT has started to focus on developing inbred lines for hybrid breeding programmes.

The observation of high heterotic patterns between S and Z groups (for example LP19 x LP37), and five within the S group (LP20 x LP55, LP23 x LP55, LP20 x LP23, LP19 x LP23 and LP55 x FU26) suggested the presence of heterotic patterns between the S and Z grouping and within the S group, respectively. The former indicated the efficacy of our grouping method using heterosis while the later indicated the existence of variation within the S group. Studies by Revilla *et al.* (2002), for example, also reported some heterotic patterns between lines from European flint-maize germplasm using both heterosis and SCA effects. Therefore, the use of both heterosis and SCA effects, if present and significant, could assist in the classification as suggested by Menkir *et al*. (2004). Lines such as LP19 were derived from the maize population DMR- SR from IITA, which was created by combining MSV resistant varieties with DMR sources introduced mainly from Asian tropical maize germplasm from Thailand. The observation of high level of heterosis between LP19 and LP37 was consistent with previous findings that materials from the Asian tropical maize germplasm, particularly Thailand, combined well with those from the CIMMYT program in Mexico (Mickelson *et al*., 2001). Population Suwan-1 is one example of an introduction from

Thailand that was used as a source of DM resistance at IITA; hence, it might have a relationship with LP19, while LP37 was derived from Population 44 from South America. Previous studies identified this population as ETO group and found that Suwan combined well with Tuxpeno group from South American maize germplasm like population 44 from CIMMYT maize programme (Mickelson *et al.,* 2001). It might be recommended to determine the genetic diversity between the lines used in the current study based on molecular markers.

4.4.4 Relative performance and advantage of single crosses over topcross varieties

The observation that crosses involving line LP23 were generally late compared to the other crosses suggests that LP23 was a late performer *inter se*. This was confirmed by its positive GCA effect for time to maturity. In contrast, LP19, LP55 and the Z and S testers were early performers as indicated by their negative GCA effects. However, the fact that GCA effects were predominant for both anthesis and silking dates indicates that earliness could be improved through selection. Improvement of earliness through selection has been reported in the literature (Moreno-Gonzalez *et al*., 1997).

The fact that hybrids did not show a clear advantage over the best topcrosses that were ranked first and ninth among the top 16 genotypes with at least 110% relative yield, suggests that a decision has to be made whether to deploy topcrosses or single cross hybrids. The best topcrosses can immediately be forwarded for further testing in multi-location environments towards release as non-conventional commercial hybrids, which can provide seed companies and farmers with a lower cost alternative. In producing topcrosses, the female or seed parent is a variety with some level of vigour, which results in higher seed yield per hectare than the inbred seed parents in the single cross seed production. This has implications on seed production costs usually borne by the farmers. In Mozambique, for example, the price of one kilogram of three-way or double cross hybrids is between 2 to 3 USA dollars, which might be beyond the reach of most subsistence farmers who live on less than a dollar per day. However, where farmers or emergent seed companies can afford to buy seeds of three-way and double-cross hybrids, which yield higher than topcrosses they can be availed to them for higher productivity. Following Cordova *et al*. (2001) procedures, the selected single cross hybrids from the top 10 can be used as female parents in developing three-way cross hybrids. These can be achieved by crossing them to the inbred

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lines with good GCA like LP55 from this study, and lines 2, 19, 42 and 45, which were identified in another study as potential high yielders and with high levels of resistance to downy mildew disease. On the other hand, the lines making up the top 10 hybrids can be used to create synthetic OPVs targeted for small-scale farmers with fewer resources in tropical lowland areas.   Indeed, most farmers in these environments, particularly in Mozambique, are still using more landraces and open pollinated varieties than hybrids, hence introduction of the topcrosses and three way crosses can be economic and sustainable for the "resource- poor" farmers as proposed by Pixley and Banziger (2001).

4.4.5 Relative ranking of hybrids across environments

Relative ranking of genotypes across environments can be used as a measure of yield stability, where small values of mean rank and its standard deviation represents superior performance and large values inferior performance. Analyzing the ranking of all genotypes over the six environments revealed that the environments did not rank the genotypes in the same way. It was explained by weak correlations among some environments for genotype ranking. For example, a topcross LP23 x Suwan-1 ranked among top five hybrids across the six environments, and it ranked among the top-10 in Angónia and Chókwè in both seasons; occupying 18th and 21st places at Umbeluzi in 2008/9 and 2007/08 seasons, respectively. This was attributed to the stability of the cross. However, the same cross (LP23 x Suwan-1) ranked last (60th) in Sussundenga, demonstrating either lack of adaptation or could have arisen from error of measurement. The observation of the lowest standard deviation for ranks of the cross A LP37 x FU26 implied that the cross was the most stable, and had yield comparable to the standard hybrid check "Olipa" (Table 4.9). Olipa is a QPM three-way cross hybrid from South Africa, which displayed general stability during extensive evaluations and hence, was released in Mozambique in 2008. In general, there were high potential single cross or topcross hybrids for grain yield superiority and stability across environments.   These potential crosses can be tested in more environments in Mozambique and in the Southern Africa region through the regional maize network for confirming their superiority and stability that was detected in this study.

4.5 Conclusions

From this study the following conclusions were drawn:

1. Both additive and non-additive effects were significant for determining grain yield and number of ears per plot, but the non-additive was predominant as indicated by the GCA to SCA variance ratios and relative sum of squares; thus yield could be improved by developing hybrids between complementary lines and between lines and populations ZM523 and Suwan-1;

2. Additive effects were more important for anthesis and silking dates. The early anthesis and silking dates would be preferred, hence the GCA variance can be exploited in selection to improve these lines;

3. There is genetic variation among the lines, and heterosis between the lines and the testers, which allowed assigning the lines in three heterotic groups S, Z and S/Z based on their heterosis levels with Suwan-1 and ZM523. This finding will contribute to the better management of this germplasm in tropical lowland programmes.

4. The superiority of performance and yield stability of both single cross and topcross hybrids was observed. This result can influence positively the seed systems for the tropical environments, where the use of conventional hybrids is limited by the high price of seed. Thus, the high yielding topcrosses can be further evaluated with a view to introduce them in the lowland environments;

5. Clear heterotic patterns, one between S and Z groups (LP19 x LP37), and five within the S group (LP20 x LP55, LP23 x LP55, LP20 x LP23, LP19 x LP23 and LP55 x FU26) were identified and will be incorporated in the breeding programme in Mozambique.

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CHAPTER 5

Generation Mean Analysis of Downy mildew Resistance in African Tropical Lowland Elite Maize Inbred lines

Abstract

Downy mildew (DM) can be effectively controlled by deploying resistant varieties in tropical lowland environments. Therefore, gene effects that control maize resistance to downy mildew were determined in two maize populations. Two DM resistant lines, DRAC and Suwan-L1, were crossed to LP67 (susceptible) and the F_1 was advanced to F_2 and crossed to both parents. The parents, F_1, F_2 and backcross progenies were then evaluated at two sites, in Mozambique. A generation mean analysis was performed in SAS. There were significant differences (P≤0.05) between sites and among generations for DM resistance in both populations. However, the generation x site interaction effects were not significant. The F_1 generation did not differ from the resistant parents in both populations suggesting existence of complete dominance. Resistance to DM was controlled by additive, dominance, additive x additive, and dominance x dominance effects in the population LP67 x DRAC. While for the population LP67 x Suwan-L1, the additive and dominance x dominance effects were significant for DM resistance. The estimate of minimum number of genes or factors affecting maize resistance to DM ranged from one to eight. Large additive gene effects (62%) in LP67 x Suwan-L1 suggests that DM resistance could be improved through selection. The significant contribution of genes with additive effects was relatively small (20%) in LP67 x DRAC indicating that DM resistance was largely (80%) under the influence of dominance and epistasis. In addition, heterosis, which averaged 47% in both populations, can be exploited to enhance resistance in hybrids.

Keywords: downy mildew disease, epistatic effects, gene effects, generation mean analysis, maize

5.1 Introduction

Maize downy mildew, which is caused by *Peronosclerospora sorghi* (Weston and Uppal*)* Shaw, is one of the most important maize diseases in the tropical and sub-tropical environments worldwide. It is prevalent in coastal lowland environments, especially in Africa and Asia. In Mozambique, for example, the disease is spreading to new areas causing high grain yield losses in farmers' fields, especially when susceptible varieties are grown. In Mozambique, 70% of the staple food maize is produced in the tropical lowland ($\leq$ 800 m.a.s.l) environment and is highly vulnerable to DM infection. Similar environments where DM can cause huge grain losses are found throughout the east and west coast in Africa, Asia, Latin America, and the United States.

Peronosclerospora sorghi is an obligate fungus, which infects maize through conidia from the early stages of the maize plant growth or by means of oospores through the seed and soil (Adenle and Cardwell, 2000; Thakur and Mathur, 2002). Conidia infection can result in both local and systemic infections in susceptible plants, which present discoloration of the upper leaf surface of the young plants and distorted inflorescence at the adult stage. *Peronosclerospora sorghi* is more serious in young plants, less than four weeks old, hence in general infected plants do not produce cobs resulting in devastating grain yield losses. Maize resistance has been reported to be associated with resistance to systemic infection by the pathogen (Bonman *et al*., 1983). Oospores are the sexual spores of DM pathogen, and constitute the primary source of inoculum in some areas and are a means of survival when the pathogen faces unfavourable climatic conditions. The oospores are capable of surviving and remaining infective in seeds with less than 10% grain moisture content for more than nine months in storage (Adenle and Cardwell, 2000). Therefore, the potential damage caused by DM can be severe with serious implications on food security if DM resistant varieties are not developed and deployed in the subsistence sector, especially in Sub-Saharan ecosystems.

The mode of resistance in maize associated with DM infection is not well known. Some previous studies for downy mildew resistance (DMR) in maize revealed the presence of both additive and non-additive gene effects, with preponderance of additive effects in determining disease reaction. Bocholt and Frederiksen (1972), for example, reported the presence of complete and incomplete dominance effects on the inheritance of resistance to DM on

maize, suggesting that only a few genes may be involved. Orángel and Borges (1987) reported intermediate disease reaction, suggesting a polygenic system for resistance to *P. sorghi* in maize. Jinahyon (1973), observed the same results in a study using open pollinated maize. However, Frederiksen and Ullstrup (1975), in their study found that resistance was dominant in some crosses and recessive in others. Many studies of resistance of maize for *P. sorghi* revealed that the resistance was controlled polygenically, but the phenotypic expression varied with the level of infection with some kind of threshold reaction. A high level of heritability (up to 70%) for the resistance has been reported (Nair *et al*., 2005), which also supports the preponderance of genes with additive effects for DM resistance in maize. However, the existence of different types of epistatic interactions in the expression of quantitative traits were referred as one of the major causes of over-estimation or under-estimation of heritability estimates, which results in additional bias in predicted genetic gains (Viana, 2000; Parvez *et al*., 2006).

The efficient exploitation of resistance sources for use in developing DM resistant maize varieties, particularly hybrids, requires a full understanding of the nature of gene action and inheritance associated with the expression of resistance in the potential breeding sources. Generation mean analysis is one of the powerful statistical methods used to determine gene effects including detection of all kinds of epistatic interactions (Viana, 2000; Parvez *et al*., 2006). Previous studies on the gene action of DM did not include models that are capable of separating the dominance effects from the three types of epistasis hence they were all confounded with dominance gene effects. In the generation mean analysis, several basic generations from a cross between two inbred lines differing in backgrounds for the trait of interest are used to study the genetic effects. This entails partitioning the epistasis into additive x additive, additive x dominance and dominance x dominance effects. The other methods such as the diallel are not capable of detecting the epistasis hence it is confounded with dominance. This includes determination of the number of genes involved in the resistance to DM in maize. Therefore, the present study was conducted to determine the types of gene effects controlling maize resistance to downy mildew, estimate the minimum number of genes involved in resistance and to determine heterosis for resistance in two maize crosses between susceptible and resistant lines. The inbred lines involved are crucial to maize breeding programmes that aim to emphasise downy mildew resistance in tropical lowland ecosystems.

5.2 Material and Methods

5.2.1 Germplasm

Two white grain endosperm, downy mildew resistant, S_5 inbred maize lines designated as DRAC (Resistant, R) and Suwan-L1 (R) were crossed to a common susceptible inbred parent LP67 to generate LP67 x DRAC and LP67 x Suwan-L1 F_1 populations. The lines DRAC and Suwan-L1 were derived from $DRAC_1F_1$ and Suwan 8075DMR populations introduced from the maize programmes of the International Maize and Wheat Improvement Center (CIMMYT) in Zimbabwe and the International Institute of Tropical Agriculture (IITA) in Nigeria, respectively. The line LP67 is also white grain endosperm, from the elite germplasm of the national maize research programme in Mozambique (Instituto de Investigação Agrária de Mozambique, IIAM). It was derived from population L.B. 82-223C_3 from the CIMMYT programme in Mexico. The three lines and their respective F_1 populations were advanced to F_2 by self-pollination and were simultaneously crossed to their two parents to generate backcross progenies. In this study, the LP67 (S) is designated P_1 parent, while the resistant parents DRAC (R) and Suwan-L1 (R) are designated P_2 in each cross; backcross to the first parent is designated BCP_1 and backcross to the second parent as BCP_2.

5.2.2 Field evaluation and experimental design

The six generations (P_1, P_2, F_1, F_2, BCP_1 and BCP_2) from both populations LP67 x DRAC and LP67 x Suwan-L1 were evaluated at Umbeluzi (26^0 03' S;$32^0$23' E and 12 metres above sea level, m.a.s.l) and Chókwè ($24^0$32' S; $32^0$00' E and 33masl) Research Stations in Mozambique, during the 2008/09 summer season. At each site, the trial was laid out as a randomized complete block design with two replications per population. A replication included one 5 m –row plot for P_1, P_2 and F_1 generations; six 5 m-row plots for the segregating F_2, BCP_1 and BCP_2 generations. The spacing within and between rows for all generations at both sites was 0.25 m and 0.80 m, respectively. The total number of plants evaluated for each generation at both sites is presented in Table 5.1. A compound NPK fertilizer (12%N: 24%P: 12K) was applied as basal at planting. Urea (46%N) was applied as top-dressing at four and eight weeks after plant emergence at both sites. The total amount of fertilizer was applied at the following rate: 170 kg N, 33 kg P and 44 kg of K ha^{-1} at both

sites. Supplementary irrigation was supplied at both sites during the crop growing cycle. Other standard cultural practices (treatments for insect control, manual weeding and harvesting) were followed at both sites. Downy mildew artificial infection was created at both sites using the method of spreader rows as suggested by Williams (1984) and Cardwell *et al.* (1994) and modified by Cardwell *et al*. (1997) and Denic (1996). Downy mildew incidence was scored by counting the number of infected plants in each plot.

Table 5.1 Number of plants per generation that were evaluated for DM resistance over two sites

		Generations and number of plants					
Site	Population	P_1	P_2	F_1	F_2	BCP_1	BCP_2
Umbeluzi	LP67 (S) x DRAC (R)	37	34	38	203	230	236
	LP67 (S) x SW-L1 (R)	37	38	39	229	243	243
Chókwè	LP67 (S) x DRAC (R)	36	34	40	208	207	239
	LP67 (S) x SW-L1 (R)	36	36	38	195	229	232

P_1= the first parent in each cross; P_2 = the second parent in each cross; F_1= (P_1 x P_2);
F2= selfing generation of F_1, BCP_1 and BCP_2 = crosses between P_1, P_2 and F_1, respectively.

5.2.3 Statistical Analyses

The mean values of DM scores for each generation were calculated, and variances were estimated across the replications, using Microsoft excel 2007 computer programme. The DM incidence data was subjected to the Log_{10} (x + 1) or SQR (x + 1) transformations for normalization before ANOVA was performed. Then the analysis of variance was performed following the general liner model (PROC GLM) procedure in SAS computer program version 9.1 as follows:

$$Y_{ijk} = m + r_i (S_k) + S_k + G_j + G \times S + \epsilon_{ijk}$$

Where:

Y_{ijk} = downy mildew mean score of jth generation evaluated in i^{th} replications over k^{th} sites;

m = grand mean (mid-parent value);

$r_i(S_k)$ = effect of replication nested within the s_k sites;

G_{j} = generation mean;

G x S = generation x site interaction effects.

$€_{ijk}$ = random experimental error.

Separation of means between generations was performed using the t-test (P≤ 0.05) in SAS as suggested by Kang (1994). The following model was used to perform a generation mean analysis in SAS as described by Kang (1994):

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

Where: m = mid-parent and intercept value;

α and β = matrix coefficients of generations;

a= pooled additive effects;

d= pooled dominance effects;

aa = pooled additive x additive (homozygote x homozygote) effects;

ad = pooled additive x dominance (homozygote x heterozygote) effects;

dd = pooled dominance x dominance (heterozygote x heterozygote) effects

The number of effective factors or genes (K) for downy mildew resistance on maize was estimated using the combination of three methods. The calculations were performed following method 1 (Wright, 1968), and methods II and III (Lande, 1981) as follows:

Method-I: $K_1 = (P_2 − P_1)^2 [1.5 − 2h)]/ 8* [VF_2 -0.25 (VP_1 + VP_2 + 2F_1)]$

Where: P_1 and P_2 = parental lines involved in the cross;

$h = P_1 / (P_2 - P_1)$;

VF_2, VP_1, VP_2 and VF_1 = variances of F_2, P_1, P_2 and F_1, generations, respectively;

Method-II: $K_2 = (P_1 − P_2)^2 / 8* [VF_2 -0.25 (VP_1 + VP_2 + 2VF_1)]$

Method-III: $K_3 = (P_1 − P_2)^2 / 8* [VF_2 - (VBCP_1 + VBCP_2)]$

Where: $VBCP_1$, $VBCP_2$ = variances of BCP_1 and BCP_2 generations, respectively.

In all the three methods it was assumed that segregating genes for resistance are all located in one parent, are not linked and have equal effects on resistance, and that epistatic effects and genotype x environment (G x E) interaction effects are absent (Wright, 1968). Average degree of dominance (ADD) at F_1 and F_2 generations was calculated according to the formulas:

For F_1: ADD = $(F_1 − MP) / (P_1 − MP)$

For F_2: ADD = $2 (F_2 − MP) / (P_1 − MP)$

Where MP = mid-parent value = $(P_1 + P_2)/2$

The coefficient of dominance (F) was calculated by the formula:

F = VBCP$_2$ – VBCP$_1$

Mid parent heterosis (MPH) was calculated by the formula:

MPH (%) = 100*(F$_1$ – MP) / MP

5.3 Results

5.3.1 Generation means and crop management

The means of DM scores at Chókwè were generally higher than at Umbeluzi (Table 5.2). The mean of susceptible parent was three times higher at Chókwè than Umbeluzi. The resistant parents for both populations also showed variation between the two sites.

Table 5.2 Downy mildew (DM) incidence for six generations over two sites

Site	Population	Generations and DM incidence means (%) [†]					
		P_1	P_2	F_1	F_2	BCP_1	BCP_2
Umbeluzi	LP67 (S) x DRAC (R)	13.5	9.0	2.4	11.6	23.9	21.8
	LP67 (S) x SW-L1 (R)	13.5	0.0	5.1	9.1	21.0	20.8
Chókwè	LP67 (S) x DRAC (R)	44.0	17.2	20.0	25.0	31.8	41.7
	LP67 (S) x SW-L1 (R)	44.0	14.3	13.6	35.2	34.5	34.9

[†]Downy mildew incidence (number of infected plants over total number of plants per plot), four weeks after emergence; P_1= the first parent in each cross; P_2 = the second parent in each cross; F_1= (P_1 x P_2); F_2= selfing generation of F_1, BCP_1 and BCP_2 = crosses between P_1, P_2 and F_1, respectively.

The ANOVA for both populations LP67 x DRAC and LP67 x Suwan-L1, showed highly significant differences between sites and among generations (P≤ 0.01) for both populations (Table 5.3). The generation x site interaction effects were not significantly different (P≥ 0.05) for DM scores in both populations.

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Table 5.3 Generation mean squares for downy mildew scores across two sites

Source of variation	LP67 x DRAC	LP67x SUWAN-L1
	Mean Square†	
Site	1.0045**	1.1616*
Replication/Site	0.2262	0.2400
Generations	0.2527*	0.6988**
Generation x Site	0.0706	0.0383
Error	0.2373	0.3500
Mean	21.82	20.48
R^2 (%)	82.13	79.17
CV (%)	19.13	30.82

†Data were transformed data using Log_{10} (x + 1), *, ** Data significant at $P \le 0.05$ and $P \le 0.01$, respectively.

For both populations no significant differences (P>0.05) were observed among backcrosses, susceptible parent (P_1) and F_2 generations (Table 5.4). The resistant parents (P_2) within each population were not significantly different from their respective F_1 progenies. However, the resistant parents and F_{1s} were significantly different from the other generations within each population.

Table 5.4 Generation means for downy mildew scores over two sites

LP67 x DRAC			LP67 x Suwan-L1	
Generation	Mean		Generation	Mean
BCP_2	31.8 A		P_1 (LP 67)	28.7 A
P_1 (LP 67)	28.7 A		BCP_2	27.8 A
BCP_1	27.9 A		BCP_1	27.7 A
F_2	18.3 A		F_2	22.1 A
P_2 (DRAC)	13.1 B		F_1	9.4 B
F_1	11.2 B		P_2 (Suwan-L1)	7.1 B
Parents mean	**20.9**		**Parents mean**	**17.9**
Population mean	**21.82**		**Population mean**	**20.48**

Means followed by the same letter are not significantly different (P >0.05).

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5.3.2 Genetic effects

For the population LP67 x DRAC, highly significant differences between sites and dominance x dominance effects (P ≤ 0.01), and significant differences (P ≤ 0.05) for additive and dominance effects and additive x additive epistatic interaction effects for DM score means were observed (Table 5.5). For population LP67 x Suwan-L1, highly significant differences were observed between environments, and for additive effects (P ≤ 0.01) and the dominance x dominance effects were significant (P ≤ 0.05). The dominance and additive x additive effects were not significant (P>0.05) for the LP67 x Suwan-1 population and additive x dominant effects were not significant (P≥0.05) for both populations.

Table 5.5 Mean squares of genetic effects for Downy mildew scores across sites

	LP67 x DRAC	LP67x SUWAN-L1
Source of variation	Mean Square†	Mean Square‡
Site	1582.10**	1.1616**
Replication/site	276.90	0.2400
Additive	486.41*	2.1321**
Dominance	537.94*	0.3175
Additive x Additive	356.13*	0.0672
Additive x Dominance	218.09	0.4060
Dominance x Dominance	760.50**	0.5271*
Error	8.80	0.3092
Mean	21.82	20.48
R^2 (%)	76.56	76.20
CV (%)	20.45	27.32

†Untransformed data used in GLM; ‡Transformed data using Log_{10} (x + 1) was subjected to GLM; *, ** Data significant at P≤ 0.05 and P≤ 0.01, respectively.

5.3.3 Relative contribution of genetic effects

Additive and dominance gene effects accounted for 43% of the total genetic variation in the population LP67 x DRAC (Table 5.6). Additive effects alone were responsible for 61% of the genetic variation in the population LP67 x Suwan-L1. The greatest contribution among

epistatic effects to the total genetic variation resulted from the dominance x dominance effects in both populations.

Table 5.6 Relative contribution of genetic effects (% sum of generation sum of squares) to downy mildew in each population

Source of variation	LP67 x DRAC	LP67x SUWAN-L1
	%	%
Additive	20.6	61.8
Dominance	22.8	9.2
Additive x Additive	15.1	2.0
Additive x Dominance	9.3	11.8
Dominance x Dominance	32.3	15.3

*, ** Data significant at P≤ 0.05 and P≤ 0.01, respectively

5.3.4 Genetic effect estimates

The midpoint was negative and not significant in the population LP67 x DRAC, but was positive and significant in the population LP67 x Suwan-L1 (Table 5.7). Compared to the non-additive effects, the additive effects had relatively low estimates in both populations but were significant. For both populations the effects were positive towards the susceptible parent. The highest estimates were observed from dominance effects followed by dominance x dominance epistatic effects. The epistatic effects were highly significant (P ≤ 0.01) for both populations and negative in magnitude toward to the resistant parents, while the dominance effects were only significant (P≤ 0.05) for the LP67 x DRAC. The additive x additive epistatic effects were also only significant (P ≤ 0.05) in population LP67 x DRAC. The additive x dominant epistatic effects were not significant (P > 0.05) in both populations.

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Table 5.7 Estimate of genetic effects (±standard errors) for downy mildew scores across two sites

Model	LP67 x DRAC	LP67x SUWAN-L1
	Genetic effects†	
Mean (m)	0.6±2.3	0.4±0.8
Additive (a)	0.8±0.3*	0.5±0.2**
Dominance (d)	15.8±5.5*	3.4±1.8
Additive x Additive (aa)	5.2±2.3*	0.6±0.8
Additive x Dominance (ad)	-2.0±1.5	-1.0±0.5
Dominance x Dominance (dd)	-12.0±3.4**	-2.7±1.1**

†Data transformed using Log_{10} (x+ 1), *, ** Data significant at P≤ 0.05 and P≤ 0.01, respectively.

5.3.5 Heterosis and average degree of dominance

In both populations, the level of mid-parent heterosis was almost equal in value (about 47%) and negative in both populations (Table 5.8). The level of dominance was less than one in both F_1 and F_2 generations in both populations. The coefficient of dominance showed different trends between the two populations, because it was less than zero in LP67 x DRAC and was greater than zero in LP67 x Suwan-L1 (Table 5.8).

Table 5.8 Mid-parent heterosis and average of degree of dominance for downy mildew resistance in two maize populations across two sites

Parameter	Populations	
	LP67 x DRAC	**LP67x SUWAN-L1**
Mid-parent value (%)	20.91	17.92
Mid-parent heterosis (%)	-46.47	-47.71
Average level of dominance (based on F_1)	-0.46	-0.48
Average level of dominance (based on F_2)	-0.25	0.47
Coefficient of dominance	-192.31	11.52

5.3.4 Minimum number of genes involved in Downy mildew resistance

The estimated number of genes ranged between one and eight depending on the method used (Table 5.9).

Table 5.9 Estimated minimum number of genes or factors involved in downy mildew resistance in two populations

Population	Minimum number of genes or factors		
	Method-I	Method- II	Method-III
LP67 x DRAC (S x R)	4.1	0.4	-4.0
LP67 x Suwan-L1 (S x R)	8.0	1.3	0.8

5.4 Discussion

5.4.1 Generation means

Results indicated clear significant differences between the resistant parents; DRAC and Suwan-L1 from the susceptible parent LP67 at both sites (Table 5.2), which is a major requirement for generation mean analysis. Different methods, which are arbitrary, have been used to classify genotypes according to resistance to downy mildews. According to Neeley (2001) a susceptible line or hybrid is one with incidence scores more than 50%, and resistant line or hybrid is one with incidence scores of less than 20% and intermediate one having scores between 20% and 50%. However, in this study generations with DM score means less than the population mean were considered resistant and those with DM scores greater than the population mean were considered as susceptible. Therefore, resistant parents DRAC and Suwan- L1 and F_1 crosses in both populations were grouped together in the resistant class. The susceptible parent LP67 backcrosses in both populations and F_2 populations were grouped in the susceptible class (Table 5.5). The lower downy mildew scores for Suwan-L1 suggest it was more resistant than DRAC at both sites.

5.4.2 Genetic effects and estimates

The study indicated that both additive and dominance gene effects were important for controlling DM resistance in both populations. Significance of additive gene effects is consistent with previous studies in different populations that revealed the importance of additive effects in controlling DM resistance in maize (Rifin, 1983; Agrama *et al*., 1999). Further, our study shows that epistatic gene effects had significant contribution to DM resistance. The highest contribution to DMR comes from dominance x dominance effects for LP67 x DRAC. The additive x additive interaction contributed significantly to the resistance in the LP67 x DRAC. However, several other previous studies reported the presence of non-additive gene effects for DM resistance on maize but without partitioning this component of the genetic variation (Frederiksen and Ullstup, 1975; Borges, 1987; Singburaudon and Renfo, 1982). The epistasis and dominance gene effects were confounded in these previous studies. Knowledge of presence of epistasis associated with downy mildew resistance in maize should help breeders to design appropriate selection method to exploit both additive and non-additive gene effects including favourable epistatic interaction effects. The type one interaction (additive x additive effects) can be fixed to develop inbred lines. According to Azizi *et al*. (2006) the other types of epistatic interactions can be effectively exploited by selecting lines that exhibit high levels of this trait in a cross with other inbred lines. The high levels of additive effects (62%) in LP67 x Suwan-L1 suggested that heritability could be high and that resistance could be improved by selection in that population. While low level of additive effects (20%) in LP67 x DRAC suggested that heritability was low and that selection might not be effective in improving DM resistance in this population. However, significant dominance effects especially in LP67 x DRAC, and heterosis effects reaching 47% in both populations could be exploited in developing hybrids with acceptable levels of resistance, which is supported by observation of complete resistance in F_1 hybrids.

5.4.3 Heterosis

A high level of heterosis, which was negative, was observed in both populations. This suggested that heterosis in both populations can be exploited depending on the predominant gene effects involved in resistance for downy mildew For example, the predominance of additive effects in population LP67 x Suwan-1 implied that the observed

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heterosis in this population can mainly be exploited through selection. Generally, the results indicated that heterosis could be exploited in hybrid development programmes in both populations. The levels of heterosis for DM resistance have been scarcely reported in the literature surveyed.

5.4.4 Number of genes associated with downy mildew resistance

Three methods were employed to estimate the minimum number of genes conditioning maize resistance to DM, which varied from one to eight depending on the method used (Table 5.10). However, these results might be biased because the underlying assumption of absence of epistasis was not observed. Clearly, epistasis mainly of dominance x dominance type was significant in both populations (Table 5.4). The other assumption of the absence of genotype x environment interaction effects, however, was observed because the generation x site interaction was not significant (P> 0.05). Some previous studies have reported that few or many genes controlled the inheritance (Bocholt and Frederiksen, 1972; Frederiksen and Ullstup, 1975; Lal and Sing, 1984). Jinahyon (1973) using conventional breeding found that many genes were responsible for DMR in maize. Gowda *et al.* (1995) and Agrama *et al.* (1999), using molecular markers to detect genes for resistance on sorghum and maize, respectively, found that two major genes were responsible for DMR on sorghum and one major gene and two minor genes were responsible for DMR in maize, respectively. Since, so far, our study appears to be the first that has used the generation means approach to study inheritance of DM resistance in maize; it will serve as an important base for future studies in this field.

5.4.5 Degree of dominance

The degree of dominance was investigated through estimates of average level of dominance at two generations (F_1 and F_2) and by determining a coefficient of dominance. The dominance levels between -0.25 and 0.47 were relatively low values indicating that most of the genes exhibited partial dominance. Previous studies have reported contrasting levels of dominance. Frederiksen and Ullstup (1975) reported resistance to be dominant in some crosses and recessive in others. Kaneko and Aday (1990), studying inheritance of Philippine downy mildew (*Peronosclerospora philippinensis* Weston), found that variation in

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inheritance depended on the epiphytotic levels. Low infection (<50%) was associated with complete dominance and high level of infection (>50%) was associated with partial dominance. At 50%, level of infestation the level of dominance was zero. The coefficient of dominance parameter (F) determines which of the parents carry more number of alleles with dominance effects (Chahal and Gosal, 2002). Our results found F< 0 for LP67 x DRAC population and F > 0 for a LP67 x Suwan-L1 population, suggesting that the resistant parent DRAC has more dominant alleles in favour of DM resistance (F= -192.31) than LP67. In sharp contrast results suggested that the susceptible parent LP67 had more dominant alleles for susceptibility (F = +11.52) than Suwan-L1. This is consistent with observation of non-significant dominance effects in the LP67 x Suwan-L1 populations while they were significant in the LP67 x DRAC population (see Tables 5.5 and 5.6). Belum *et al*. (1992) reported similar results in a study of *P. sorghi* inheritance in two advanced generations of sorghum using generation mean analysis procedure. A survey of the literature indicated that the coefficient of dominance for DM resistance has not been previously reported.

5.5 Conclusions

From this study the following conclusions were drawn:

1. Additive genetic effects were important for controlling DM disease resistance in both LP67 x DRAC and LP67 x Suwan-L1 populations;

2. Dominance genetic effects were also important for controlling disease resistance in population LP67 x DRAC, but not in LP67 x Suwan-L1;

3. Dominance x dominance epistatic effects were significant for DM resistance in both populations;

4. Additive x additive epistatic effects were significant for DM resistance in LP67 x DRAC, but were not significant in LP67 x Suwan-L1;

5. The additive x dominance epistasis was not significant in both populations;

6. Although one of the two underlying assumptions, of absence of epistasis was not observed determining the number of factors involved, the results suggest that one to eight genes or factors were probably involved in conferring the DM resistance in both populations;

7. Relatively high levels of additive effects (about 62%) in LP67 x Suwan-L1 suggests that DM resistance could be improved through selection;

8. Relatively small additive effects (about 20%) in LP67 x DRAC indicated that DM resistance was to a large extent under the influence of dominance (22%) and epistasis gene effects (58%), suggesting that selection might not be effective to improve the resistance;

9. Observation of significant dominance effects especially in the F_1 hybrids that displayed similar resistance with their resistant parents and exhibited relatively high levels of heterosis, averaging about 47% in both populations, suggests that heterosis can be exploited to enhance DM resistance in hybrids.

Overall, our results indicated that genes with non-additive effects (80%) control the resistance in DRAC, while the resistance in Suwan-L1 is largely under the influence of genes with additive effects (62%). These findings have serious implications on the effective use of these downy mildew resistance sources in breeding programs that aim to generate varieties with downy mildew resistance.

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CHAPTER 6

Overview of the research findings

6.1 Introduction

This chapter makes an overview of the study by summarizing the major objectives and highlighting the major findings. The implications of these findings and recommendations are discussed.

The specific objectives of the study were as follows:

1. To investigate farmers perceptions on maize varieties and production constraints and farmers' preferences on seed traits in two maize ecologies in Mozambique.
2. To determine combining ability for downy mildew resistance and yield among the new tropical lowland maize inbred lines in Mozambique using the line by tester mating design.
3. To determine gene action, the heterotic patterns and grouping of tropical lowland Mozambican elite maize germplasm.
4. To determine inheritance of downy mildew resistance by generation mean analysis of two maize populations created among elite and two new tropical maize lines.

6.2 Major Findings

6.2 .1 Farmers perception on maize varieties and constraints

- Farmers have limited knowledge about improved varieties, particularly hybrids and most of them predominantly use landraces.
- The following factors appeared to be major causes of low adoption rate for new varieties among small-holder farmers:
    - unavailability of basic input suppliers at community level,
    - lack of knowledge among farmers about improved varieties;
    - high prices of improved seed is not affordable to the small-holder farmers with low household incomes and face several socio- economic factors which impact negatively on their capacity to access improved seed and the supporting inputs.

- Maize grain yield under small-scale farmers growing systems are low due to the various factors referred to above and other production constraints.
- The major constraints for each environment included:
  - maize downy mildew, drought, cutworm and stem borer for lowland environments,
  - seed and fertilizer availability, ear rot, turcicum leaf blight and gray leaf spot diseases, and low soil fertility in the high altitude environments.
- Farmers selected the following traits as the most important for maize seed:
  - grain yield,
  - short maturing cycle,
  - white and "flint" grain type,
  - tolerance to drought and low soil fertility, and
  - resistance to the local diseases and post-harvest pests.

6.2 .2 Combining ability for downy mildew resistance and yield among the new tropical lowland maize inbred lines

- Results showed that the additive effects were more important for controlling the resistance to downy mildew than non-additive effects in this set of new inbreds.
- Both testers were able to discriminate the lines based on their GCA for downy mildew scores and grain yield, however, Suwan-1 was a more discriminating tester than ZM523 in these test environments.
- Based on SCA data ten lines were grouped with S tester, eight lines with the Z tester.
- Based on heterosis, lines 2, 30 and 42 were heterotic with both testers for grain yield and were allocated to SZ-group; lines 8, 10, 25, 45, 48 exhibited positive heterosis with Suwan-1 and were classified in Z-group, and line19 exhibited heterosis with ZM523 and was fitted in S-group.
- Based on correlation data between downy mildew incidence scores and grain yield and other yield related traits, it was evident that downy mildew disease was the most important factor for grain yield reduction.
- The lines 42, 8, 25 and 30 were superior for both yield and downy mildew resistance hence their topcrosses have potential for further testing in these environments.

6.2 .3 Gene action, heterotic patterns and grouping of lowland tropical Mozambican elite maize germplasm

- Results showed that there is potential for developing high yield and early maturing hybrids, especially for lowland environments and this germplasm can be easily managed due to identification of new heterotic groups.

- Although both additive and non-additive effects were significant, the non-additive effects were predominant in determining grain yield. They were also more important for anthesis and silking dates.

- There was high genetic variation among the lines and heterosis between them and respective Suwan-1 and ZM523 testers.

- Based on heterosis and reaction with the testers Z523 and Suwan-1, lines were assigned into S, Z and S/Z heterotic groupings.

- The superiority of performance and yield stability of both single cross and topcross hybrids was observed. This result can affect positively the seed systems for the tropical environments, where use of conventional hybrids is limited by the high price of seed. Thus, the high yielding topcrosses can be further evaluated to overcome the problem of price, thus make available more productive varieties to the resource-poor farmers.

- Exceptional heterotic patterns, one between S and Z groups (LP19 x LP37), and five within the S group (LP20 x LP55, LP23 x LP55, LP20 x LP23, LP19 x LP23 and LP55 x FU26) were identified and will be advanced in the breeding programme in Mozambique.

6.2.4 Inheritance of downy mildew resistance of two maize populations created among elite and two new tropical maize lines.

- The study showed that it is possible to develop downy mildew resistant cultivars hybrids, either lines or synthetics through both selection and hybridization breeding approaches.

- Additive, dominant and epistatic effects were involved in determining resistance to downy mildew in the elite maize lines. However, the results showed that the relative importance of each type of gene effect can vary from cross to cross:

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- additive effects were important for controlling downy mildew disease resistance in both LP67 x DRAC and LP67 x Suwan-L1 populations;

- dominance genetic effects were also important for controlling disease resistance in population LP67 x DRAC, but not in LP67 x Suwan-L1;

- dominance x dominance epistatic effects were significant for downy mildew resistance in both populations;

- additive x additive epistatic effects were significant for downy mildew resistance in LP67 x DRAC, but were not significant in LP67 x Suwan-L1;

- The results suggested that one to eight genes or factors were probably involved in conferring the downy mildew resistance in both populations;

- Observation of significant dominance effects especially in the F_1 hybrids that displayed similar resistance with their resistant parents and exhibited relatively high levels of heterosis, averaging about 47% in both populations, suggests that heterosis can be exploited to enhance downy mildew resistance in hybrids.

- Overall results indicated that genes with non-additive effects (80%) control the resistance in DRAC, while the resistance in Suwan-L1 is largely under the influence of genes with additive effects (62%). These findings have serious implications on the effective use of these downy mildew resistance sources in breeding programmes that aim to generate varieties with downy mildew resistance.

6.3 Implications for breeding and way forward

Maize is the major crop in the region and is grown under most diversified environments and production systems, by most heterogeneous universe of farmers. Increased yield especially under smallholder farmers growing environment requires combined approaches in breeding programmes and crop management as well as seed systems. More emphasis must be given to the participatory breeding to incorporate farmers' needs and preferences in the breeding objectives, thus, high level of acceptance of new varieties among farmers can be achieved. In Mozambique for example, farmers need high yield and early maturing varieties, which are tolerant to drought and low soil fertility, resistance to the major diseases (downy mildew, ear rot, turcicum leaf blight, grey leaf spot and maize streak virus) and pests (cutworm, stem borers and weevils). Generally, the new variety must be white and have flint endosperm. Results from this study showed that this farmers' "idiotype" variety could be

achieved by manipulating existing germplasm through conventional breeding. The farmers' involvement in breeding process at the early stage of genotype testing is crucial. To make sure that the ideal traits are captured.

The identified new heterotic groups, the predominance of both additive and non-additive effects, high heterosis among identified heterotic patterns for grain yield and downy mildew resistance on new and elite maize germplasm used in Mozambique, can be exploited to develop new varieties. Both selection and cross breeding methods can be employed. Indeed the knowledge about existence of epistatic effects involved in expression of downy mildew resistance will be useful for breeders to adopt the appropriate breeding methods that allow achievement of high genetic gain in short period.

The prevalent seed systems among small-holder farmers characterized by low level of adoption of improved seed particularly hybrids, due to the various constraints; among them, seed price, is the starting point for deciding the type of final product to be developed in Mozambique. The result in this study showed that non-conventional hybrids (topcrosses) were equally superior to the single crosses. Therefore, topcrosses can be developed and promoted among smallholder farmers as alternative of using conventional hybrids due to low production cost of seed. Farmers would then use the conventional single cross hybrids when their cash income level improved. The identified heterotic patterns would be subjected to further testing in multi-location environments to confirm their yield potential and stability.